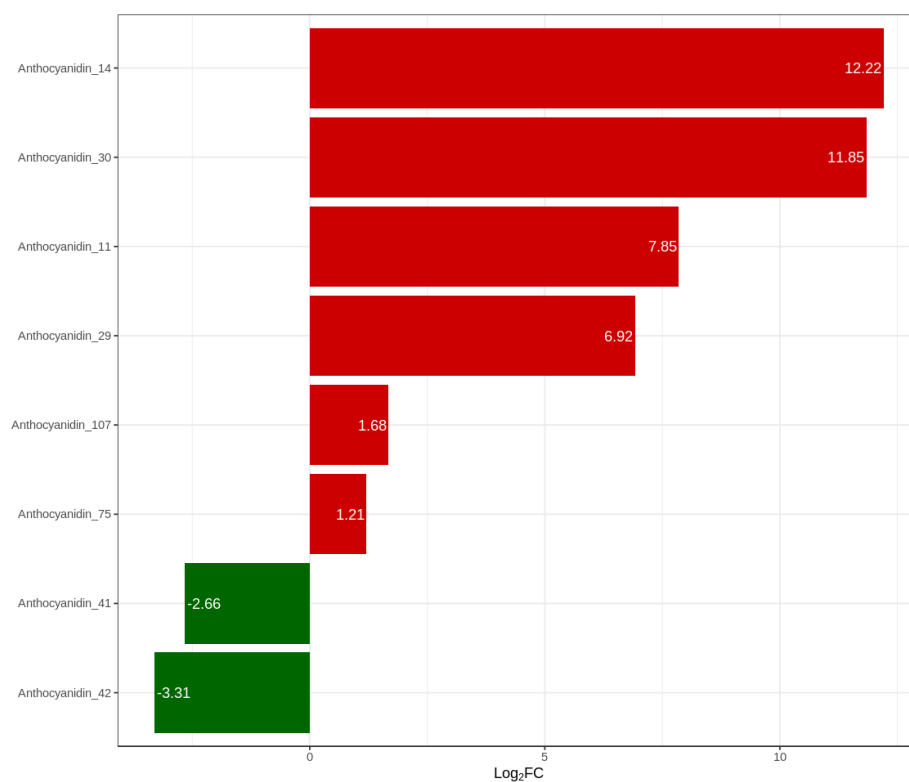


Supplement Table S1. List of 108 metabolites detected in roselle

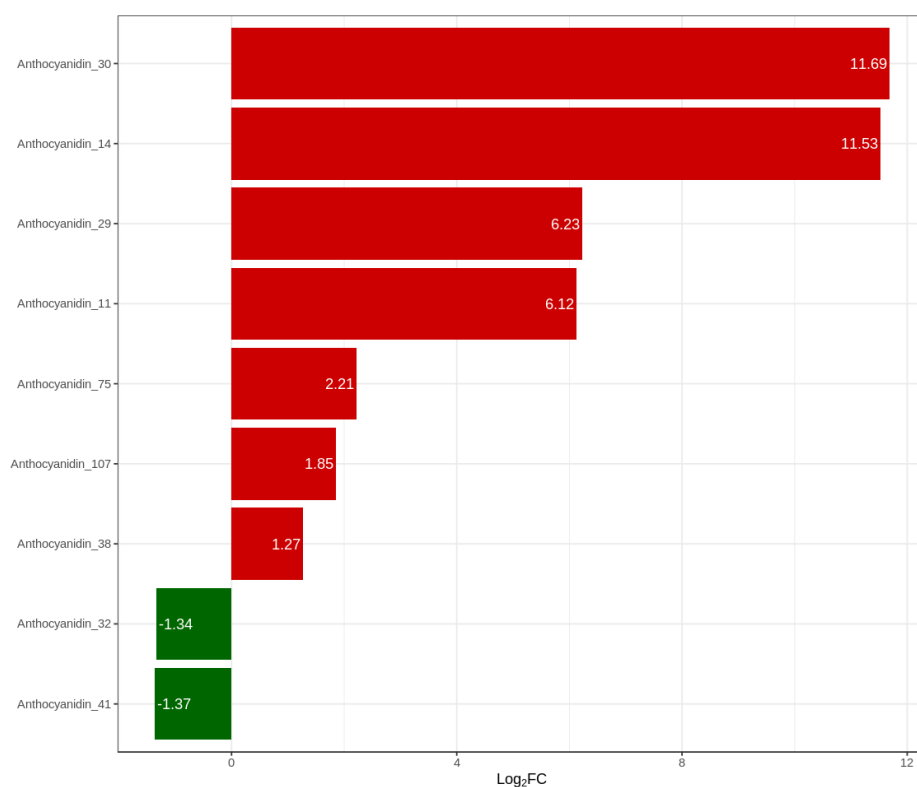
Index	Compounds	Class
Anthocyanidin_02	Cyanidin-3-(6"-caffeylsophoroside)-5-glucoside	Cyanidin
Anthocyanidin_03	Cyanidin-3,5,3'-O-triglucoside	Cyanidin
Anthocyanidin_04	Cyanidin-3,5-O-diglucoside	Cyanidin
Anthocyanidin_05	Cyanidin-3-O-(6-O-malonyl-beta-D-glucoside)	Cyanidin
Anthocyanidin_06	Cyanidin-3-O-(6-O-p-coumaroyl)-glucoside	Cyanidin
Anthocyanidin_07	Cyanidin-3-O-5-O-(6-O-coumaroyl)-diglucoside	Cyanidin
Anthocyanidin_08	Cyanidin-3-O-arabioside	Cyanidin
Anthocyanidin_09	Cyanidin-3-O-(6"-ferulylsophoroside)-5-glucoside	Cyanidin
Anthocyanidin_10	Cyanidin-3-O-galactoside	Cyanidin
Anthocyanidin_100	Petunidin-3-O-sambubioside	Petunidin
Anthocyanidin_101	Petunidin-3-O-sophoroside	Petunidin
Anthocyanidin_102	Petunidin-3-O-sambubioside-5-O-glucoside	Petunidin
Anthocyanidin_103	Procyanidin A1	Procyanidin
Anthocyanidin_104	Procyanidin A2	Procyanidin
Anthocyanidin_105	Procyanidin B1	Procyanidin
Anthocyanidin_106	Procyanidin B2	Procyanidin
Anthocyanidin_107	Procyanidin B3	Procyanidin
Anthocyanidin_108	Procyanidin C1	Procyanidin
Anthocyanidin_11	Cyanidin-3-O-glucoside	Cyanidin
Anthocyanidin_12	Cyanidin-3-O-rutinoside	Cyanidin
Anthocyanidin_13	Cyanidin-3-O-rutinoside-5-O-glucoside	Cyanidin
Anthocyanidin_14	Cyanidin-3-O-sambubioside	Cyanidin
Anthocyanidin_15	Cyanidin-3-O-sophoroside	Cyanidin
Anthocyanidin_16	Cyanidin-3-O-xyloside	Cyanidin
Anthocyanidin_17	Cyanidin-3-O-sambubioside-5-O-glucoside	Cyanidin
Anthocyanidin_18	Delphinidin	Delphinidin
Anthocyanidin_19	Delphinidin-3,5-O-diglucoside	Delphinidin
Anthocyanidin_20	Delphinidin-3-O-(6-O-acetyl)-glucoside	Delphinidin
Anthocyanidin_21	Delphinidin-3-O-(6-O-malonyl-beta-D-glucoside)	Delphinidin
Anthocyanidin_22	Delphinidin-3-O-(6-O-malonyl)-glucoside-3'-glucoside	Delphinidin
Anthocyanidin_23	Delphinidin-3-O-(6-O-p-coumaroyl)-glucoside	Delphinidin
Anthocyanidin_24	Delphinidin-3-O-5-O-(6-O-coumaroyl)-diglucoside	Delphinidin
Anthocyanidin_25	Delphinidin-3-O-arabioside	Delphinidin
Anthocyanidin_26	Delphinidin-3-O-galactoside	Delphinidin
Anthocyanidin_27	Delphinidin-3-O-glucoside	Delphinidin
Anthocyanidin_28	Delphinidin-3-O-rhamnoside	Delphinidin
Anthocyanidin_29	Delphinidin-3-O-rutinoside	Delphinidin
Anthocyanidin_30	Delphinidin-3-O-sambubioside	Delphinidin
Anthocyanidin_31	Delphinidin-3-O-sophoroside	Delphinidin
Anthocyanidin_32	Delphinidin-3-O-rutinoside-5-O-glucoside	Delphinidin
Anthocyanidin_33	Delphinidin-3-O-sambubioside-5-O-glucoside	Delphinidin

Anthocyanidin_34	Afzelin	flavonoid
Anthocyanidin_35	Chalcone	flavonoid
Anthocyanidin_36	Dihydrokaempferol	flavonoid
Anthocyanidin_37	Dihydromyricetin	flavonoid
Anthocyanidin_38	Kaempferol-3-O-rutinoside	flavonoid
Anthocyanidin_39	Naringenin	flavonoid
Anthocyanidin_40	Naringenin-7-O-glucoside	flavonoid
Anthocyanidin_41	Quercetin-3-O-glucoside	flavonoid
Anthocyanidin_42	Rutin	flavonoid
Anthocyanidin_43	Malvidin	Malvidin
Anthocyanidin_44	Malvidin-3,5-O-diglucoside	Malvidin
Anthocyanidin_45	Malvidin-3-O-(6"-acetylglucoside)-5-glucoside	Malvidin
Anthocyanidin_46	Malvidin-3-O-(6-O-malonyl-beta-D-glucoside)	Malvidin
Anthocyanidin_47	Malvidin-3-O-(6-O-p-coumaroyl)-glucoside	Malvidin
Anthocyanidin_48	Malvidin-3-O-5-O-(6-O-coumaroyl)-diglucoside	Malvidin
Anthocyanidin_49	Malvidin-3-O-arabinoside	Malvidin
Anthocyanidin_50	Malvidin-3-O-galactoside	Malvidin
Anthocyanidin_51	Malvidin-3-O-glucoside	Malvidin
Anthocyanidin_52	Malvidin-3-O-rutinoside	Malvidin
Anthocyanidin_53	Malvidin-3-O-sambubioside	Malvidin
Anthocyanidin_54	Malvidin-3-O-sophoroside	Malvidin
Anthocyanidin_55	Malvidin-3-O-sambubioside-5-O-glucoside	Malvidin
Anthocyanidin_56	Pelargonidin	Pelargonidin
Anthocyanidin_57	Pelargonidin-3-(6"-caffeylsophoroside)-5-glucoside	Pelargonidin
Anthocyanidin_58	Pelargonidin-3-sophoroside-5-glucoside	Pelargonidin
Anthocyanidin_59	Pelargonidin-3,5-O-diglucoside	Pelargonidin
Anthocyanidin_60	Pelargonidin-3-O-(6"-ferulylsambubioside)-5-O-(malonyl)-glucoside	Pelargonidin
Anthocyanidin_61	Pelargonidin-3-O-(6-O-malonyl-beta-D-glucoside)	Pelargonidin
Anthocyanidin_62	Pelargonidin-3-O-(6-O-p-coumaroyl)-glucoside	Pelargonidin
Anthocyanidin_63	Pelargonidin-3-O-[2-O-glucosyl-6-O-p-coumaroyl-glucoside]-5-O-glucoside	Pelargonidin
Anthocyanidin_64	Pelargonidin-3-O-5-O-(6-O-coumaroyl)-diglucoside	Pelargonidin
Anthocyanidin_65	Pelargonidin-3-O-[6-O-feruloyl-2-O-glucosyl-glucoside]-5-O-glucoside	Pelargonidin
Anthocyanidin_66	Pelargonidin-3-O-arabinoside	Pelargonidin
Anthocyanidin_67	Pelargonidin-3-O-galactoside	Pelargonidin
Anthocyanidin_68	Pelargonidin-3-O-glucoside	Pelargonidin
Anthocyanidin_69	Pelargonidin-3-O-rutinoside	Pelargonidin
Anthocyanidin_70	Pelargonidin-3-O-rutinoside-5-O-glucoside	Pelargonidin
Anthocyanidin_71	Pelargonidin-3-O-sambubioside	Pelargonidin
Anthocyanidin_72	Pelargonidin-3-O-sophoroside	Pelargonidin
Anthocyanidin_73	Pelargonidin-3-O-sophoroside-5-O-(malonyl)-glucoside	Pelargonidin
Anthocyanidin_74	Pelargonidin-3-O-sambubioside-5-O-glucoside	Pelargonidin
Anthocyanidin_75	Peonidin	Peonidin
Anthocyanidin_76	Peonidin-3-(caffeyl-glucosyl-glucoside)-5-glucoside	Peonidin
Anthocyanidin_77	Peonidin-3-sophoroside-5-glucoside	Peonidin

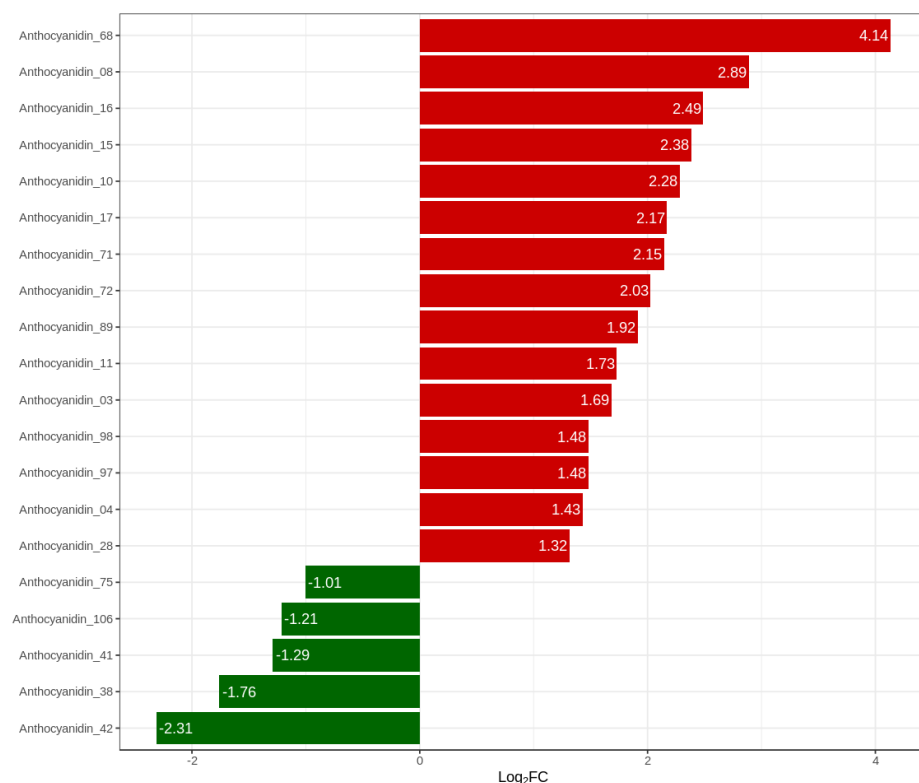
Anthocyanidin_78	Peonidin-3,5-O-diglucoside	Peonidin
Anthocyanidin_79	Peonidin-3-O-(6-O-malonyl-beta-D-glucoside)	Peonidin
Anthocyanidin_80	Peonidin-3-O-(6-O-p-coumaroyl)-glucoside	Peonidin
Anthocyanidin_81	Peonidin-3-O-5-O-(6-O-coumaroyl)-diglucoside	Peonidin
Anthocyanidin_82	Peonidin-3-O-arabinoside	Peonidin
Anthocyanidin_83	Peonidin-3-O-caffeoyl-feruloyl-sophoroside-5-glucoside	Peonidin
Anthocyanidin_84	Peonidin-3-O-(6"-ferulylsophoroside)-5-glucoside	Peonidin
Anthocyanidin_85	Peonidin-3-O-galactoside	Peonidin
Anthocyanidin_86	Peonidin-3-O-glucoside	Peonidin
Anthocyanidin_87	Peonidin-3-O-P-hydroxybenzoylsophoroside-5-glucoside	Peonidin
Anthocyanidin_88	Peonidin-3-O-rutinoside	Peonidin
Anthocyanidin_89	Peonidin-3-O-sambubioside	Peonidin
Anthocyanidin_90	Peonidin-3-O-sophoroside	Peonidin
Anthocyanidin_91	Peonidin-3-O-sambubioside-5-O-glucoside	Peonidin
Anthocyanidin_92	Petunidin-3,5-O-diglucoside	Petunidin
Anthocyanidin_93	Petunidin-3-O-(6-O-malonyl-beta-D-glucoside)	Petunidin
Anthocyanidin_94	Petunidin-3-O-(6-O-p-coumaroyl)-glucoside	Petunidin
Anthocyanidin_95	Petunidin-3-O-5-O-(6-O-coumaroyl)-diglucoside	Petunidin
Anthocyanidin_96	Petunidin-3-O-arabinoside	Petunidin
Anthocyanidin_97	Petunidin-3-O-galactoside	Petunidin
Anthocyanidin_98	Petunidin-3-O-glucoside	Petunidin
Anthocyanidin_99	Petunidin-3-O-rutinoside	Petunidin



(A)

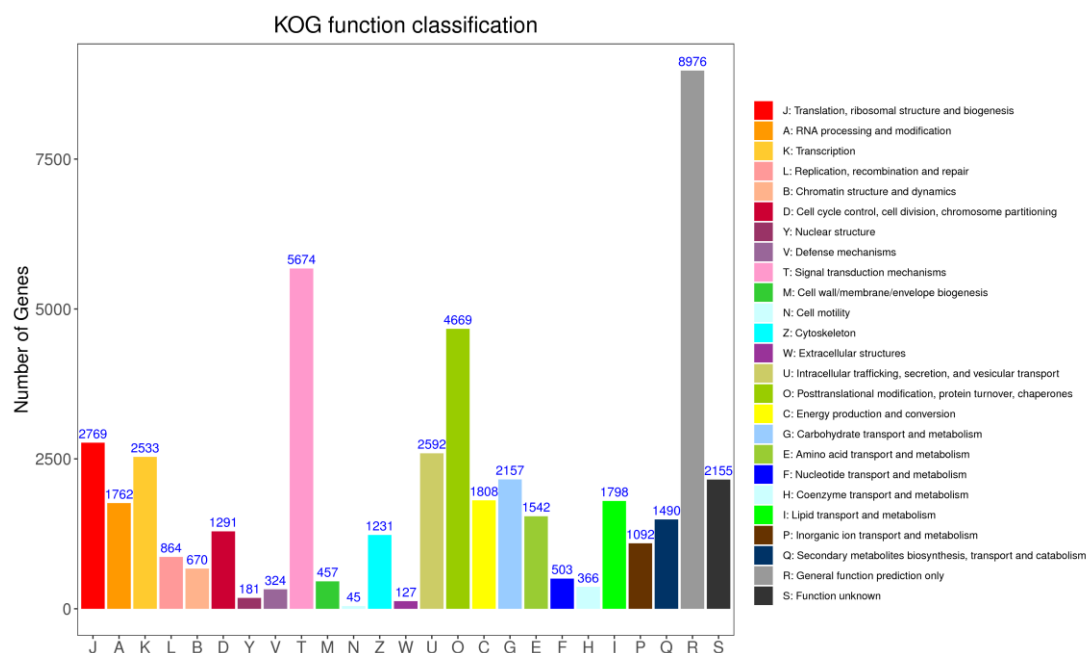


(B)

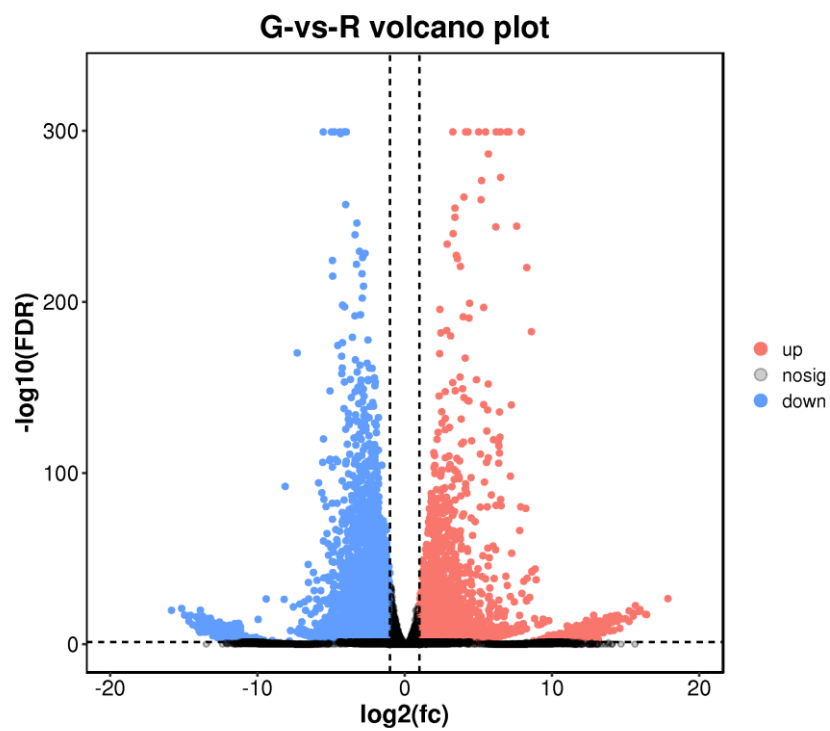


(C)

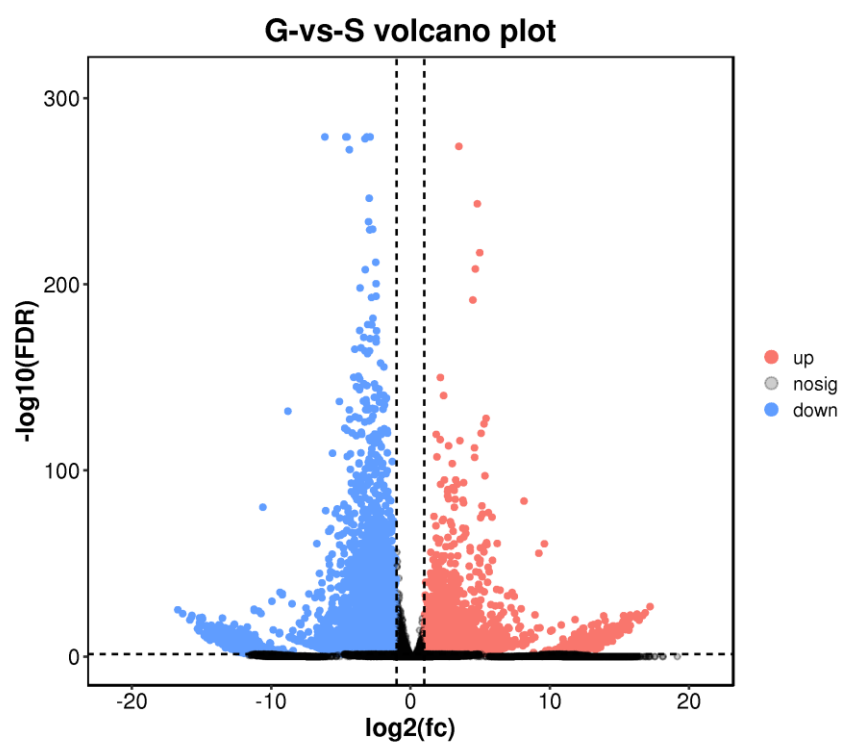
Supplement Figure S1. Differential metabolites bar chart. (A) Significantly different metabolite bar chart between G and R. (B) Significantly different metabolite bar chart between G and S. (C) Significantly different metabolite bar chart between S and R. R, FZ-72 (red calyx); G, Baitao K (green calyx); S, MG5 (stripped calyx)



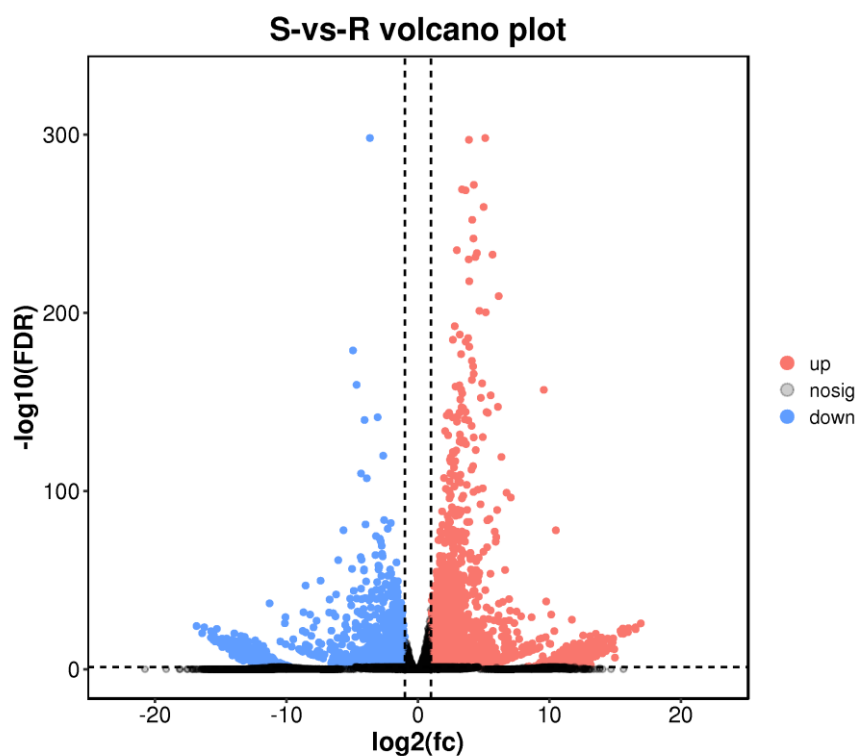
Supplement Figure S2. KOG functional classification statistics.



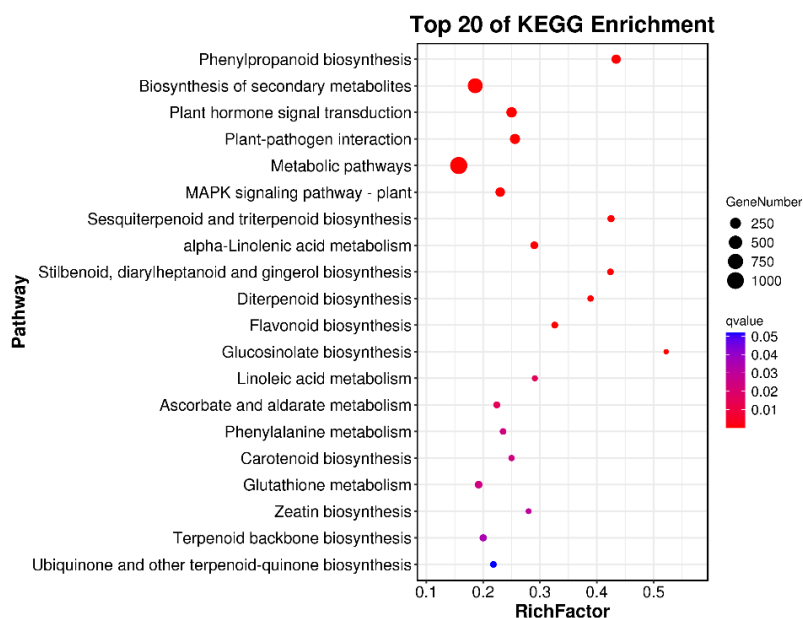
(A)

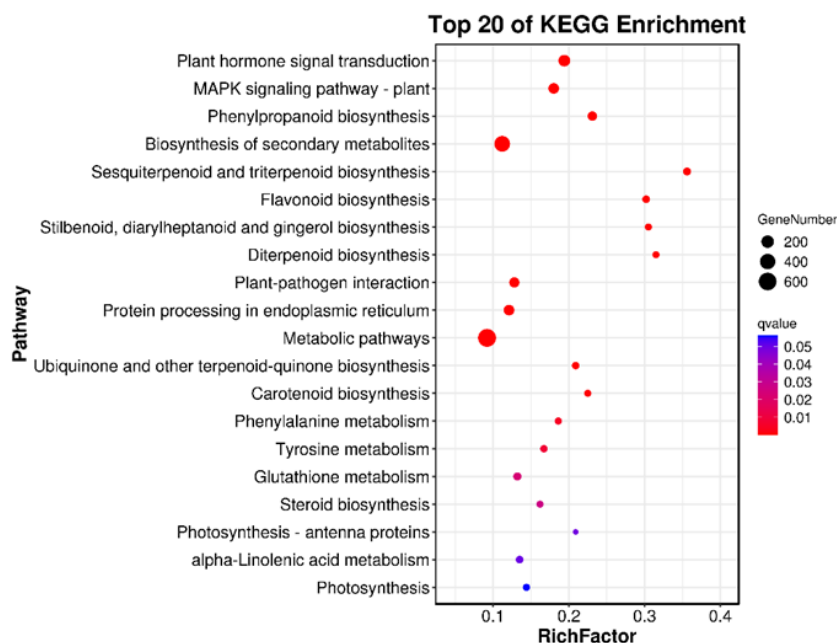


(B)



Supplement Figure S3. Volcano plots for different combinations. A larger absolute value of the abscissa indicates a greater difference between the two groups of genes; a larger value of the ordinate indicates a more significant difference in gene expression between groups and a more reliable result. R, FZ-72 (red calyx); G, Baitao K (green calyx); S, MG5 (stripped calyx)





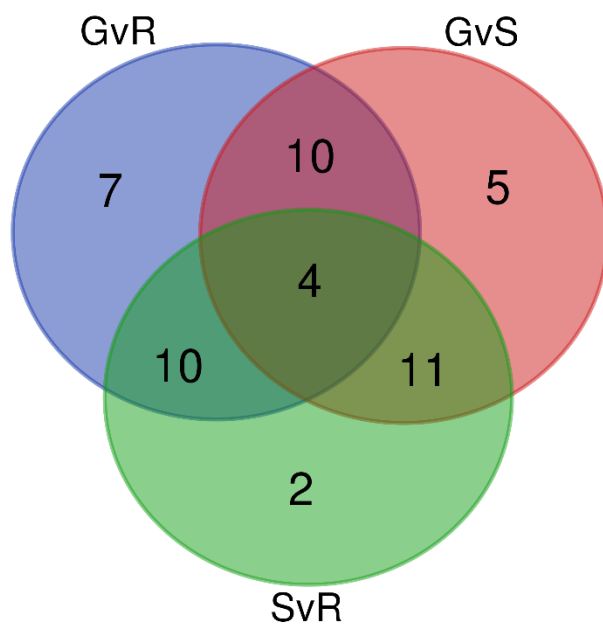
(B)

Supplement Figure S4. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment bubble plot. (A) GvsS differential gene enrichment bubble plot. (B) SvsR differential gene enrichment bubble plot. The top 20 pathways with the smallest Q values were used to construct the graph. The ordinate indicates the pathway and the abscissa indicates the enrichment factor. The size indicates the quantity. The redder the color, the smaller is the Q value. R, FZ-72 (red calyx); G, Baitao K (green calyx); S, MG5 (stripped calyx)

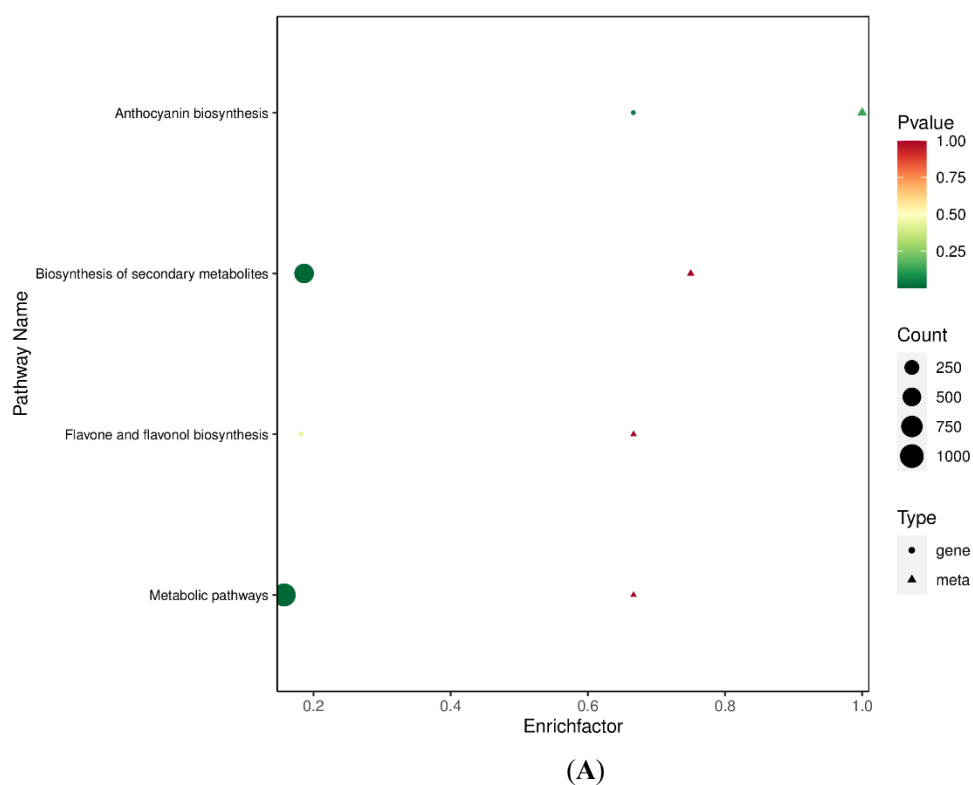
Supplement Table S2. Differentially expressed genes (DEGs) in the flavonoid synthesis pathway (ko00941) and anthocyanin synthesis pathway (ko00942) among the three combinations.

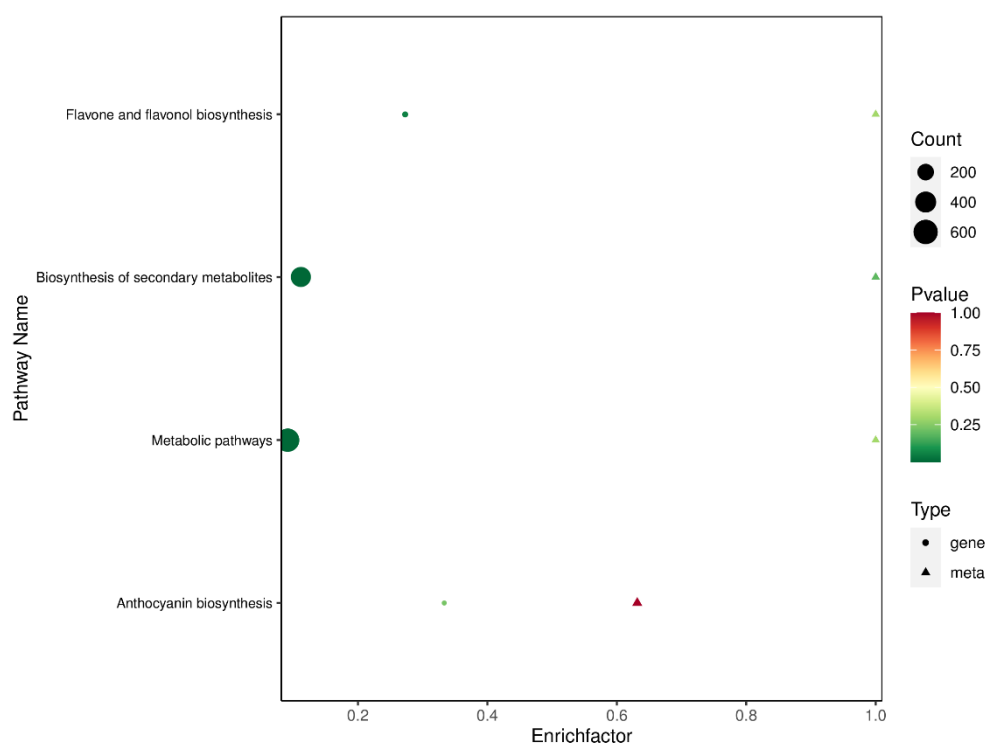
Unigene ID	short name	Full name
Unigene0029458	/	
Unigene0009721	ACT	adult cataract
Unigene0097172	ACT	adult cataract
Unigene0018909	AN3	ANGUSTIFOLIA 3,
Unigene0024761	ANR	anthocyanidin reductase
Unigene0009339	ANS	anthocyanidin synthase
Unigene0045492	At4g26220	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Unigene0014782	CCOAOMT1	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Unigene0074946	CCOAOMT1	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Unigene0006017	CCOAOMT5	caffeoyl-CoA O-methyltransferase 5
Unigene0065186	CCOAOMT5	caffeoyl-CoA O-methyltransferase 6
Unigene0074947	CCOMT	catechol-O-methyltransferase
Unigene0008117	CHI3	chitinase
Unigene0026462	CHS	chalcone synthase
Unigene0028712	CHS	chalcone synthase
Unigene0076313	CHS	chalcone synthase

Unigene0007849	CHS1	chalcone synthase 1
Unigene0022461	CHS1	chalcone synthase 1
Unigene0053976	CHS2	chalcone synthase 2
Unigene0053978	CHS4	chalcone synthase 4
Unigene0009044	CYP73A12	
Unigene0000986	CYP73A13	
Unigene0037625	CYP75A1	
Unigene0009121	CYP98A2	cytochrome P450 98A2
Unigene0043946	CYP98A2	cytochrome P450 98A3
Unigene0008836	DFR	dihydroflavonol 4-reductase
Unigene0095921	DFR	dihydroflavonol 5-reductase
Unigene0008826	FAOMT	anthocyanin O-methyltransferase
Unigene0008827	FAOMT	anthocyanin O-methyltransferase
Unigene0008828	FAOMT	anthocyanin O-methyltransferase
Unigene0008034	FLS	flavonol synthase
Unigene0001791	HST	homogentisate prenyltransferase
Unigene0008344	HST	homogentisate prenyltransferase
Unigene0016835	HST	homogentisate prenyltransferase
Unigene0026470	HST	homogentisate prenyltransferase
Unigene0029457	HST	homogentisate prenyltransferase
Unigene0035514	HST	homogentisate prenyltransferase
Unigene0035515	HST	homogentisate prenyltransferase
Unigene0045377	HST	homogentisate prenyltransferase
Unigene0052944	HST	homogentisate prenyltransferase
Unigene0080927	HST	homogentisate prenyltransferase
Unigene0083370	HST	homogentisate prenyltransferase
Unigene0101365	HST	homogentisate prenyltransferase
Unigene0007848	PKS5	protein kinase5
Unigene0007635	SAT	serine acetyltransferase
Unigene0047199	SAT	serine acetyltransferase
Unigene0105300	SAT	serine acetyltransferase
Unigene0030931	UFGT	anthocyanidin 3-O-glucosyltransferase 2
Unigene0088077	UFGT	anthocyanidin 3-O-glucosyltransferase 3



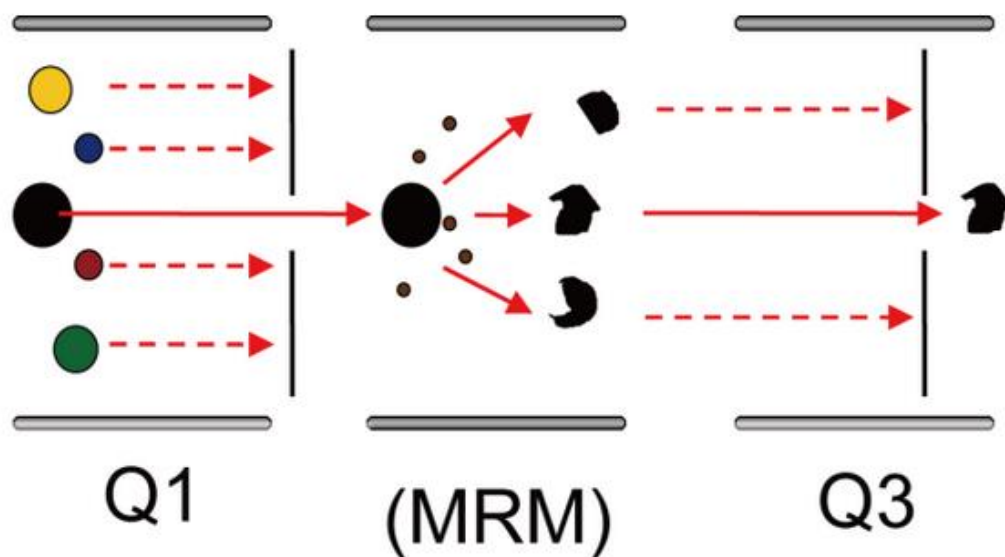
Supplement Figure S5. Venn diagram of significantly different genes in the flavonoid (ko00941) and anthocyanin (ko00942) synthesis pathways.



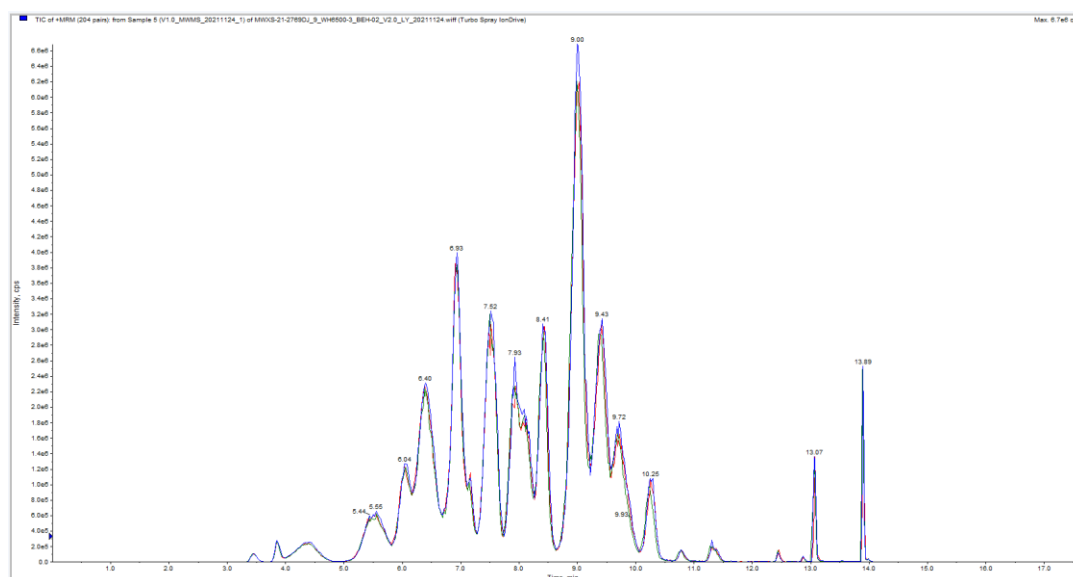


(B)

Supplement Figure S6. Bubble chart for the combined analysis of differentially expressed genes and differential metabolites. (A) Kyoto encyclopedia of genes and genomes (KEGG) enrichment bubble plot for G and S. (B KEGG) enrichment bubble plot for S and R. R, FZ-72 (red calyx); G, Baitao K (green calyx); S, MG5 (stripped calyx)



Supplement Figure S7. Multiple Reaction Monitoring (MRM) analysis of anthocyanin content in roselle calyx.



Supplement Figure S8. Chromatographic analysis of total ion flow.