

Function analysis of genes with SNP differences between high nicotine conversion line TS01 and Yunyan 85

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INTRODUCTION

Special aroma style "sakura red"(called "Zhushayan" locally) has been appearing in Yunnan tobacco-planting area in China for a long time, previous studied shown that Yunnan "Cherry red" tobacco has light aroma style, strong rice fragrance, which is contrast to smoke fragrance in "Cherry red" tobacco produced from other country. Here a "Cherry red" tobacco line TS01 was obtained from *Nicotiana tabacum* Yunyan85 through multi-year screening Yunnan province in China, in order to analyze the molecular mechanism of style formation of line TS01, the transcripts expressed in root and leaf of both TS01 and Yunyan 85 were sequenced by illumina technique. Function of genes with SNP differences between TS01 and Yunyan85 were analyzed and discussed.

METHODS AND RESULTS

➤ Materials:
Nicotiana tabacum Yunyan 85, Yunyan 87, K326 and TS01.

➤ Methods:
The epidermal leaf cell of flu-cue tobacco was observed by using The Hitachi TM3030Plus Tabletop SEM.

The nicotine and nornicotine was analyzed by using GC/MS technique.

The transcripts of root and leaf of both Yunyan 85 and TS01 was performed in Illumina platform.

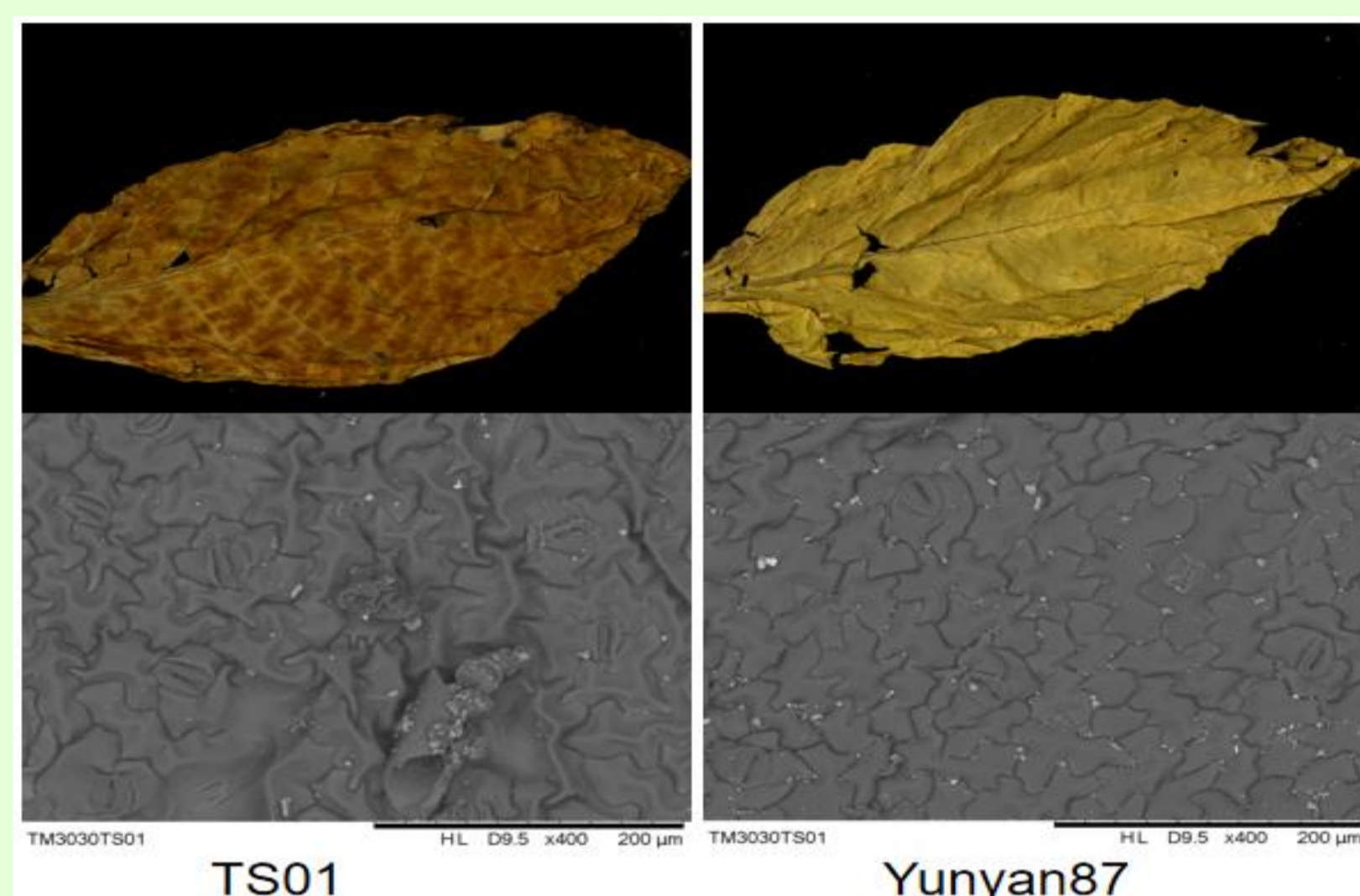


Fig.1 The comparison in leaf between TS01 and Yunyan 87

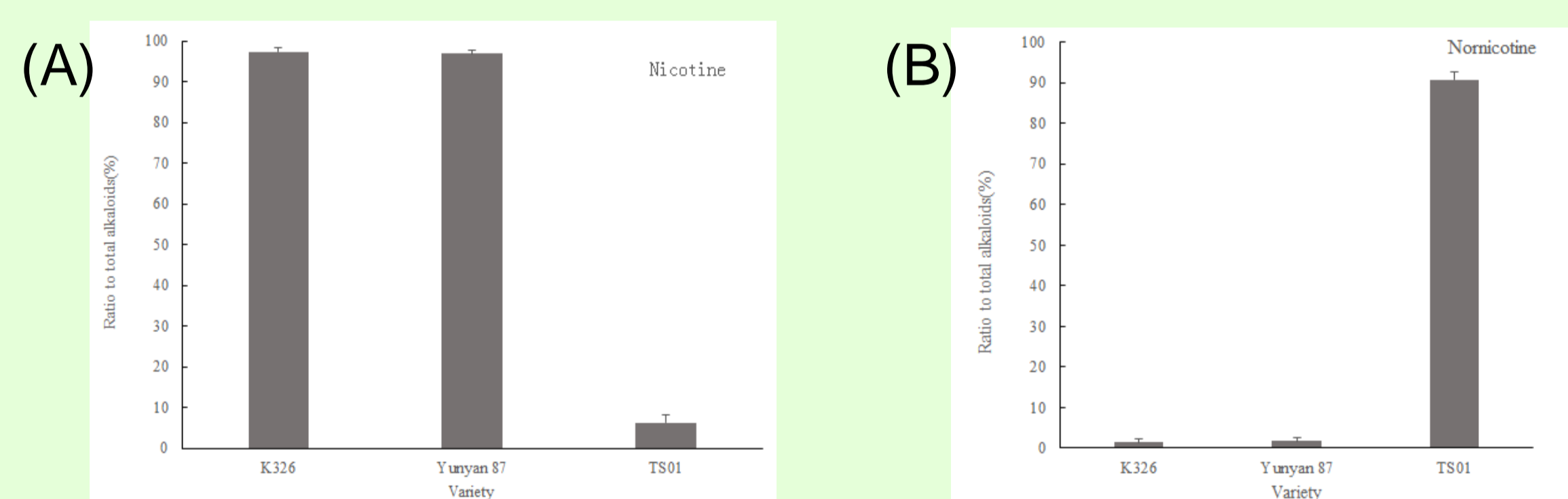


Fig. 2 The nicotine and nornicotine content in TS01, Yunyan 87 and K326 (A): nicotine (B): Nornicotine

Table 1 Statistics data for Illumina RNA-Seq analysis of leaf and root of TS01 and Yunyan 85

Samples	ID	Clean reads	Clean bases	GC Content	%≥Q30
TSL_1	TSL_1	35,801,747	10,738,023,172	43.35%	94.34%
TSL_2	TSL_2	37,008,661	11,100,499,918	43.21%	94.30%
TSL_3	TSL_3	37,817,295	11,342,693,566	43.01%	94.29%
TSR_1	TSR_1	35,676,542	10,700,289,406	42.76%	93.97%
TSR_2	TSR_2	37,155,696	11,144,256,542	42.64%	94.33%
TSR_3	TSR_3	36,402,691	10,917,827,820	42.40%	93.41%
YY85L-1	YY85L-1	35,916,814	10,772,712,810	43.19%	94.98%
YY85L-2	YY85L-2	35,887,949	10,763,919,272	42.98%	94.49%
YY85L-3	YY85L-3	35,259,265	10,575,606,230	43.25%	93.98%
YY85R-1	YY85R-1	36,828,107	11,046,098,186	42.50%	94.29%
YY85R-2	YY85R-2	35,963,435	10,786,357,320	42.38%	95.13%
YY85R-3	YY85R-3	35,987,934	10,793,658,532	42.53%	94.53%

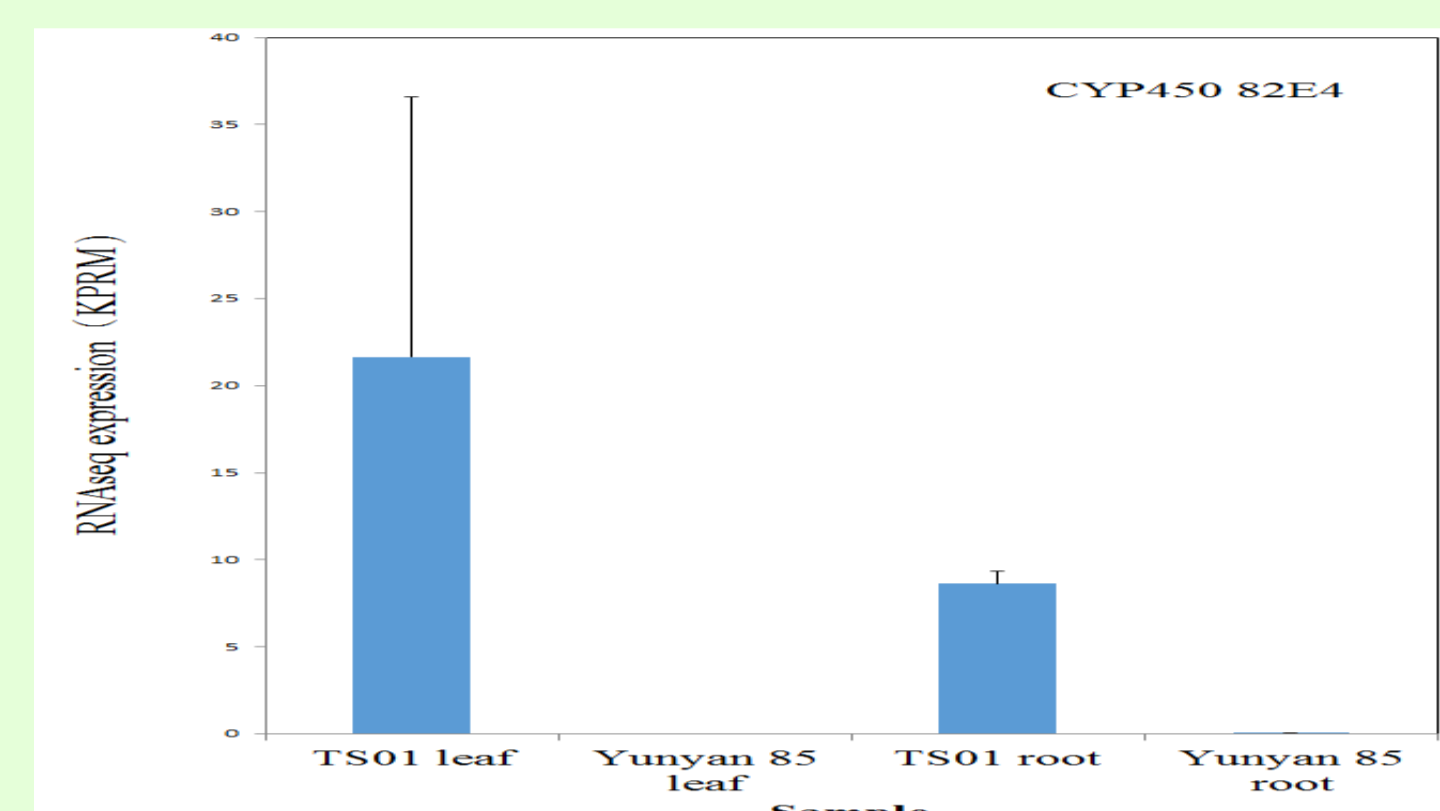


Fig. 3 The expression of Nicotine N-demethylase CYP450 82E4 in TS01 and Yunyan 85

Table 3 The annotation of 19 genes with more than 29 SNP site between TS01 and Yunyan 85

Gene ID	SNP sites	Annotation
gene_83024	30	U-box domain-containing protein 44-like
gene_11636	31	protein IQ-DOMAIN 32
gene_50340	31	probable sulfate transporter 3.3
gene_83111	31	large proline-rich protein BAG6 isoform X1
gene_17460	32	receptor protein kinase CLAVATA1-like
gene_1794	33	alkaline/neutral invertase CINV2-like
gene_83152	34	lysine-specific histone demethylase 1 homolog 3
gene_53306	35	1,4-alpha-glucan-branching enzyme-like
gene_83031	35	protein S-acyltransferase 21-like
gene_83033	36	protein NLP4-like
gene_10226	39	BTB/POZ domain-containing protein At2g30600-like isoform X2
gene_28067	39	la-related protein 1C-like
gene_39543	40	nudix hydrolase 25 isoform X1
gene_11638	41	auxin response factor 5
gene_83145	44	lysine-specific histone demethylase 1 homolog 3
gene_83037	45	epidermal growth factor receptor substrate 15-like 1
gene_50966	48	DEAD-box ATP-dependent RNA helicase 28-like
gene_11190	50	uncharacterized TPR repeat-containing protein At1g05150-like
gene_83039	77	glutamate receptor 3.3-like

Table 2 The statistics of SNP sites in gene between TS01 and Yunyan85

SNP sites in gene between TS01 and Yunyan85	Gene counting
3	252
4	112
5	62
6	40
7	29
8	27
9	22
10	12
11	15
12	15
13	14
14	10
15	7
16	5
17	8
18	3
19	6
20	2
21	4
22	2
23	6
24	1
25	4
26	4
27	2
30	1
31	3
32	1
33	1
34	1
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39	2
40	1
41	1
44	1
45	1
48	1
48	1
50	1
77	1

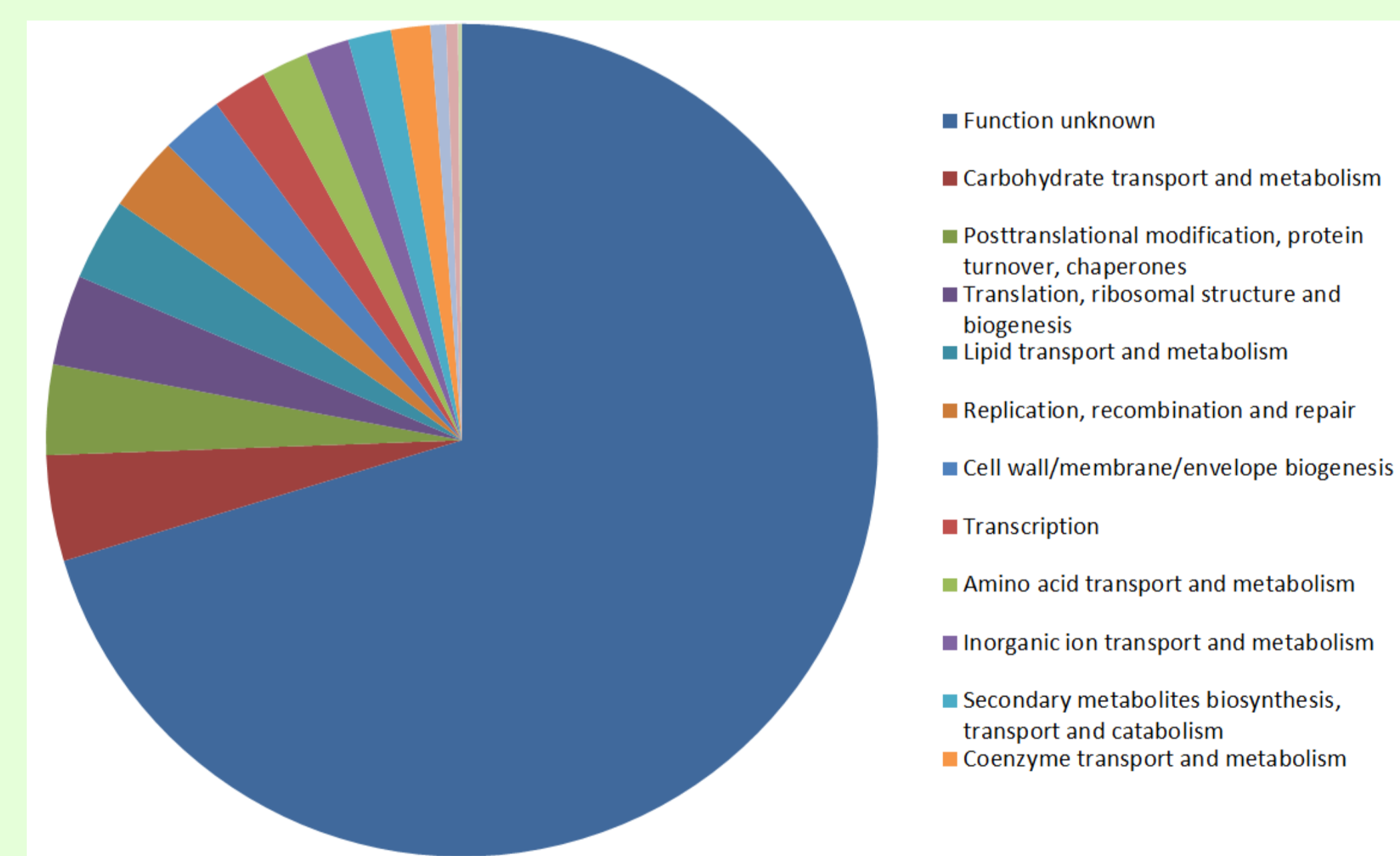


Fig. 4 Function analysis of 683 genes with SNP differences between TS01 and Yunyan85

CONCLUSIONS

- TS01 possessed up to 93% nicotine conversion rate, with very low nicotine content and high nornicotine, because Nicotine N-demethylase CYP450 82E4 was in high expression level in TS01 compared to Yunyan 85.
- Single nucleotide polymorphisms (SNPs) differences of genes between TS01 and Yunyan85 were obtained, 683 genes containing 3 or more SNP differential sites between TS01 and Yunyan85 were screened out.
- Genes with more than 40 SNP differential sites including nudix hydrolase 25 isoform X1, auxin response factor 5, lysine-specific histone demethylase 1 homolog 3, etc. Glutamate receptor possessed most SNP differential sites, up to 77 SNP sites between TS01 and Yunyan 85. Though function analysis of 683 genes with SNP differences between TS01 and Yunyan85, those genes were enriched in several pathways: carbohydrate transport and metabolism, posttranslational modification/protein turnover/chaperones, translation/ ribosomal structure and biogenesis etc..
- The sequence variation of genes involved in those pathways may lead to style formation of tobacco line TS01.