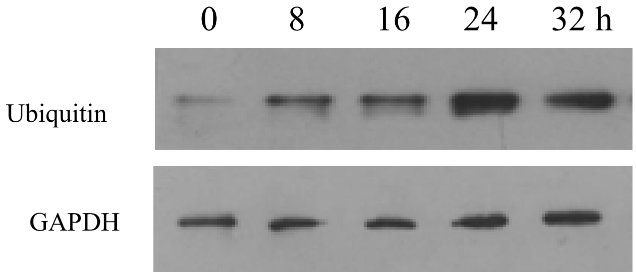
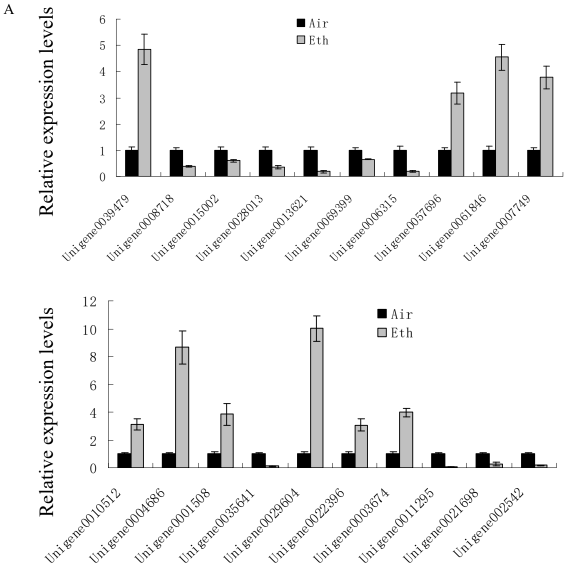


Figure S1 Effects of ethylene on the expression of ubiquitin in petunia corollas in protein level. The proteins were detected by western blot analysis using an anti-ubiquitin antibody or an anti-GAPDH antibody.

Figure S2 Venn diagram of annotation results against four protein databases. The number in each color block indicated the number of unigenes that can be annotated by single or multiple databases.



B

The representative values of qRT-PCR and transcriptome

candidate genes	qRT-PCR Eth/Air	transcriptome Eth/Air	candidate genes	qRT-PCR Eth/Air	transcriptome Eth/Air
Unigene0039479	4.843	14.236	Unigene0010512	3.107	5.417
Unigene0008718	0.396	0.138	Unigene0004686	8.654	34.944
Unigene0015002	0.596	0.224	Unigene0001508	3.843	3.661
Unigene0028013	0.351	0.077	Unigene0035641	0.103	0.050
Unigene0013621	0.178	0.075	Unigene0029604	10.026	22.727
Unigene0069399	0.655	0.025	Unigene0022396	3.079	3.961
Unigene0006315	0.193	0.042	Unigene0003674	3.981	3.022
Unigene0057696	3.175	4.791	Unigene0011295	0.075	0.025
Unigene0061846	4.547	3.022	Unigene0021698	0.268	0.047
Unigene0007749	3.775	2.351	Unigene002542	0.176	0.195

Figure S3 Confirmation of digital gene expression data by qRT-PCR. A. Expression of 20 random genes used in quantitative real-time PCR in corollas in response to 2 μl l^{-1} exogenous ethylene or air treatment 16 h. Relative expression levels are shown as fold change values. Data are presented as the mean ± SD (n = 3). Different letters mean significant difference at P=0. 05 level. B, The representative values of qRT-PCR and transcriptome (Eth/Air) of 20 candidate genes.

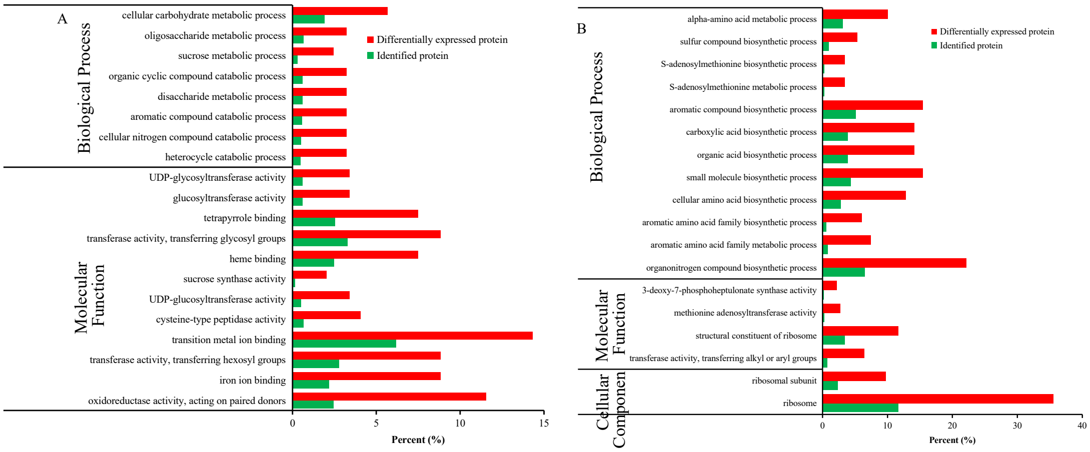
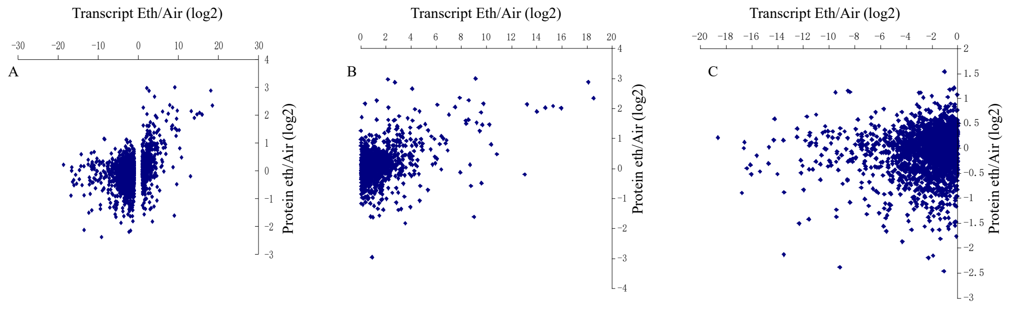
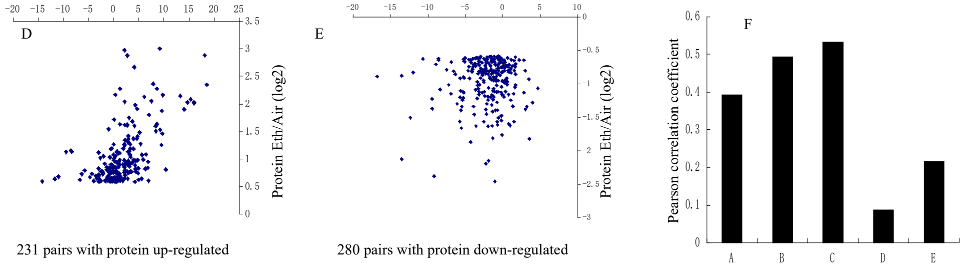


Figure S4 Functional enrichment analysis of differently expressed proteins. GO-based enrichment analysis of up-regulated (A) and down-regulated (B) proteins. The percent of differentially expressed proteins indicates the ratio of the maping proteins to all maping proteins. The percent of identified proteins indicates the ratio of the background proteins to all background proteins. The significance level was set at P <0.05 (Fischer's exact test). The data come from the SD Exc4 Sheet1-3.



2118 pairs with transcripts changed at p<0.05 1187 pairs with transcripts up-regulated at p<0.05 2388 pairs with transcripts down-regulated at p<0.05



231 pairs with protein up-regulated more than 1.5

280 pairs with protein down-regulated more than 0.67

Figure S5 Concordance between changes in the abundance of mRNA and its encoded protein. A–E, Correlation between protein and transcript fold-changes upon ethylene treatment for all transcripts transcript/protein pairs (A), significantly up-regulated transcripts (B), down-regulated transcripts (C), significantly up-regulated proteins (D) and significantly down-regulated proteins (E). F, Pearson correlations of the comparisons shown in A–E.

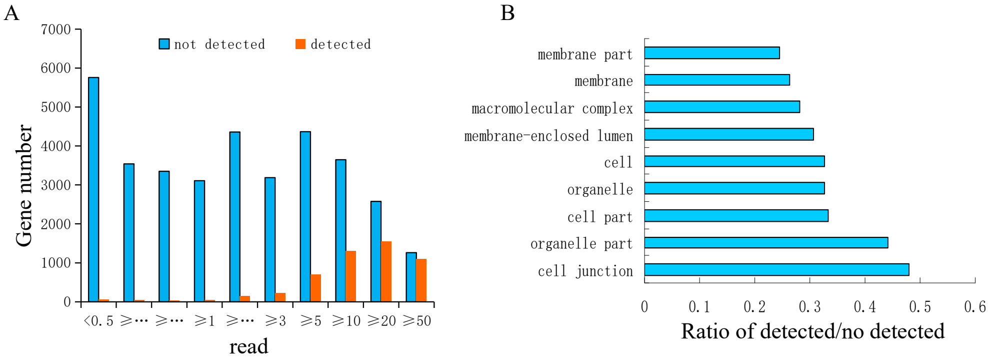


Figure S6 Detection of mRNAs and their cognate proteins. A, Dependence of protein detection on the number of reads of the corresponding transcript. B, Overrepresented gene ontology categories (cellular component) of detected and nondetected proteins.

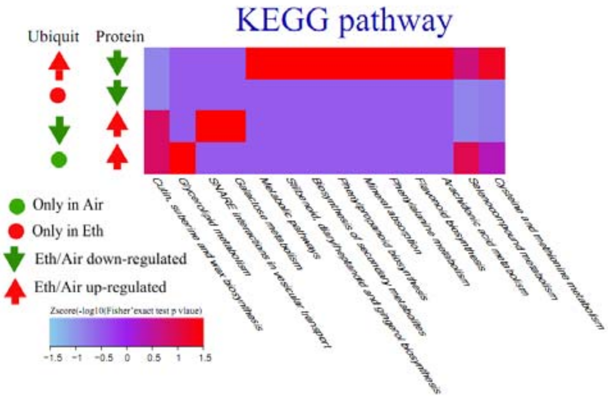


Figure S7 KEGG pathway enrichment heat map of proteins with opposite trends in protein and ubiquitination levels.

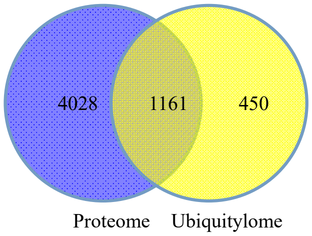


Figure S8 Venn diagram of proteomics and ubiquitinomic identification.

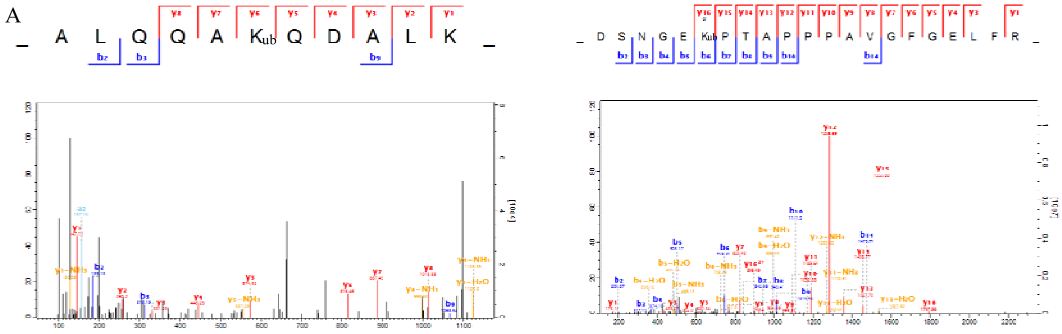


Figure S9 MS/MS spectra of ethylene receptor PhETR2 (Unigene0010512) (left) and ABC transporter B (Unigene0017904) (right) ubiquitination.

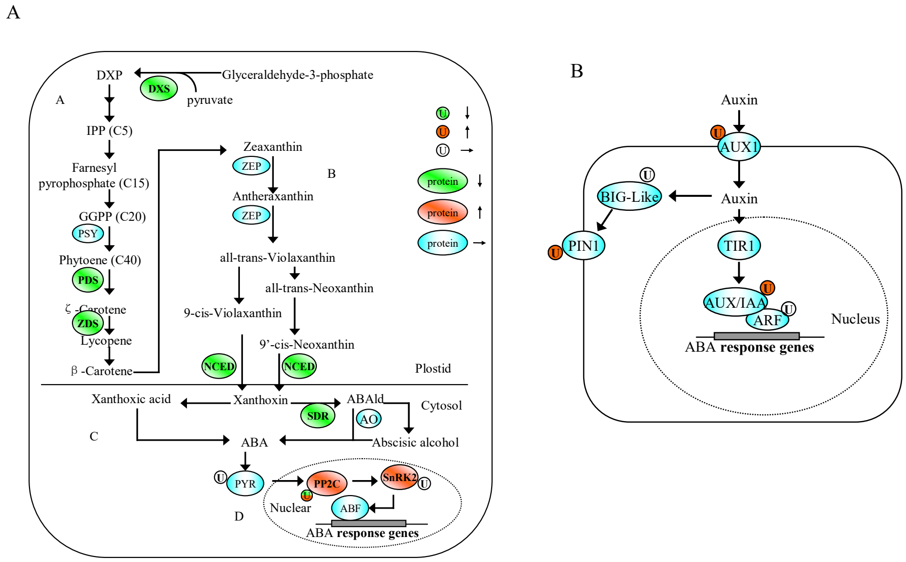


Figure S10 Effects of ethylene on the proteins engaged in ABA (A) and auxin (B) signaling transduction pathway in petunia. Differentially expressed proteins based on the statistical significance in this study are framed in oval boxes and differentially ubiquitination and phosphorylation of proteins are added round boxes. Red box indicates up-regulation, green box indicates up-regulation, and blue box indicates no significant change upon ethylene treatment. Abbreviations: ABA, abscisic acid; ABAld, abscisic aldehyde; AO, aldehyde oxidase; DXP, 1-deoxy-Dxylulose-5-phosphate; DXS, DXP synthase; GGPP, geranylgeranyl pyrophosphate; IPP, isopentenyl pyrophosphate; NCED, 9-cisepoxycarotenoid dioxygenase; PDS, phytoene desaturase; PSY, phytoene synthase; SDR, short-chain dehydrogenase/reductase; ZEP, zeaxanthin epoxidase.

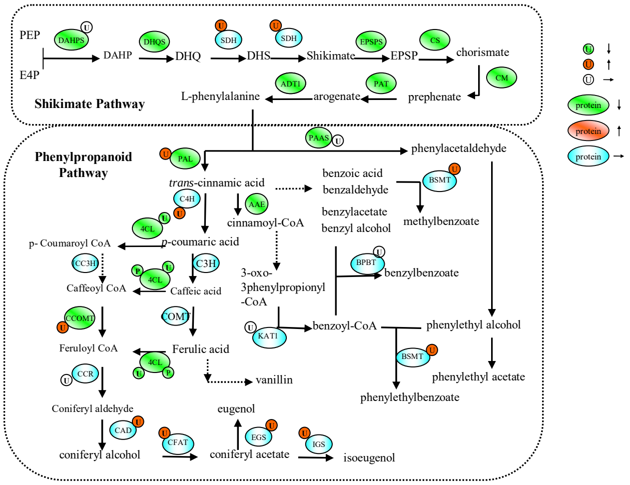


Figure S11 Effects of ethylene on floral scent biosynthesis in petunia. Differentially expressed proteins based on the statistical significance in this study are framed in oval boxes and differentially ubiquitination and phosphorylation of proteins are added round boxes. Red box indicates up-regulation, green box indicates up-regulation, and blue box indicates no significant change upon ethylene treatment. Abbreviations: AAE, Acyl-activating enzyme; ADT1, Arogenate Dehydratase1; BPBT, benzoyl-CoA:benzyl alcohol/2-phenylethanol benzoyltransferase; BSMT, S-adenosyl-L-Met: benzoic acid/salicylic acid carboxyl methyltransferase; C4H, cinnamate-4-hydroxylase; CAD, cinnamyl alcohol dehydrogenase; CCR, cinnamoyl-CoA reductase; CCOMT, caffeoyl-CoA O-methyl transferase; CFAT, coniferyl alcohol acetyltransferase; CM, chorismate mutase; CS, chorismate synthase; DAHP, 3-deoxy-D-arabino-heptulosonic acid 7-phosphate; DAHPS, 3-deoxy-d-arabino-heptulosonate-7-phosphate synthase; DHQ/SDH, 3-dehydroquinate dehydratase/shikimate 5-dehydrogenase; DHQS, 3-dehydroquinate synthase; E4P, D-erythrose-4-phosphate; EGS, eugenol synthase; EPSP, 5-enolpyruvateshikimate-3-phosphate; IGS, isoeugenol synthase; EPSPS, 5-Enolpyruvylshikimate 3-phosphate synthase; KAT, Ketoacyl CoA thiolase; PAAS, phenylacetaldehyde synthase; PAL, Phenylalanine ammonia-lyase; PAT, prephenate aminotransferase; PEP, phosphoenolpyruvate; SK, Shikimate kinase.

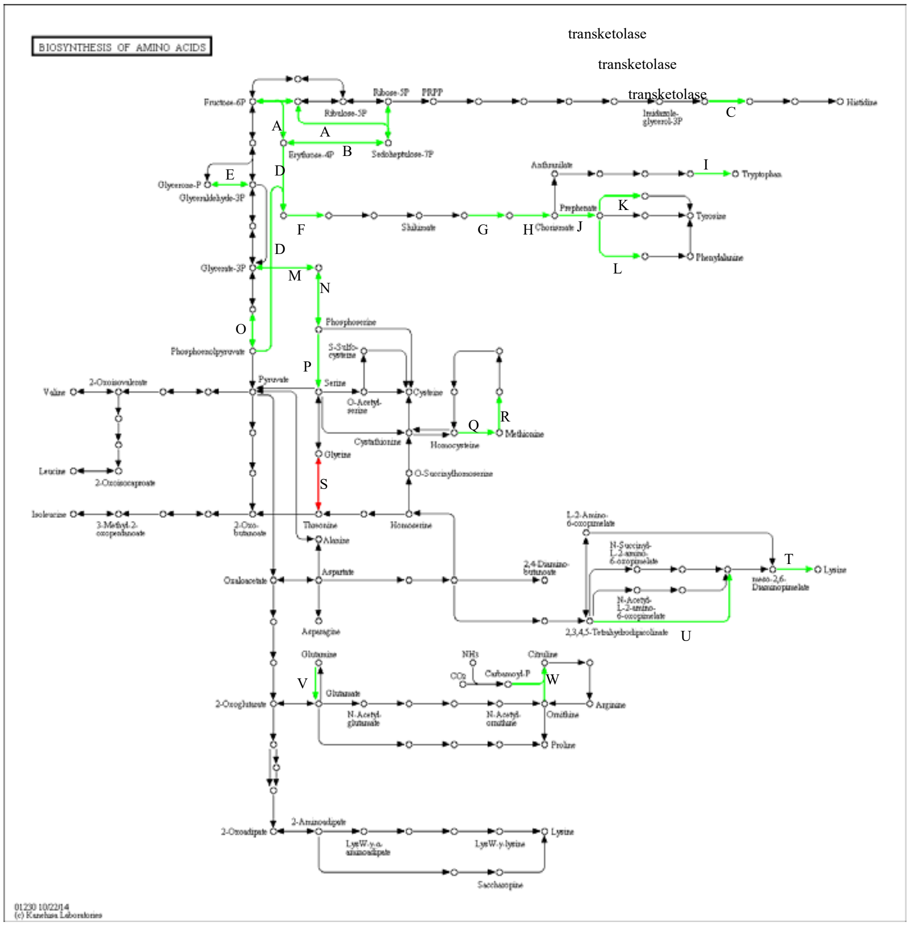


Figure S12 Effects of ethylene on amino acid biosynthesis pathway (KEGG: map01230) in petunia. The green line indicates down-regulation, and the red line indicates up-regulation in protein level upon ethylene treatment based on the statistical significance. A, ko:K00615 E2.2.1.1; transketolase [EC:2.2.1.1]. B, ko:K00616 E2.2.1.2; transaldolase [EC:2.2.1.2]. C, ko:K00817 hisC; histidinol-phosphate aminotransferase [EC:2.6.1.9]. D, ko:K01626 E2.5.1.54; 3-deoxy-7-phosphoheptulonate synthase [EC:2.5.1.54]. E, ko:K01803 TPI; triosephosphate isomerase (TIM) [EC:5.3.1.1]; F, ko:K01735 aroB; 3-dehydroquinate synthase [EC:4.2.3.4]. G, ko:K00800 aroA; 3-phosphoshikimate 1-carboxyvinyltransferase [EC:2.5.1.19]. H, ko:K01736 aroC; chorismate synthase [EC:4.2.3.5]. I, K01695 tryptophan synthase alpha chain [EC:4.2.1.20]. J, ko:K01850 E5.4.99.5; chorismate mutase [EC:5.4.99.5]. K, K00832 aromatic-amino-acid transaminase [EC:2.6.1.57]. L, K04518 pheA2 prephenate dehydratase [EC:4.2.1.51]. M, ko:K00058 serA; D-3-phosphoglycerate dehydrogenase [EC:1.1.1.95]. N, ko:K00831 serC; phosphoserine aminotransferase [EC:2.6.1.52]. O, ko:K01689 ENO; enolase [EC:4.2.1.11]. P, ko:K01079 serB; phosphoserine phosphatase

[EC:3.1.3.3]. Q, K00548 metH, MTR 5-methyltetrahydrofolate--homocysteine methyltransferase [EC:2.1.1.13]. R, ko:K00789 metK; S-adenosylmethionine synthetase [EC:2.5.1.6]. S, ko:K01620 ltaE; threonine aldolase [EC:4.1.2.5]. T, ko:K01586 lysA; diaminopimelate decarboxylase [EC:4.1.1.20]. U, K01206 LL-diaminopimelate aminotransferase [EC:2.6.1.83]. V, ko:K00264 GLT1; glutamate synthase (NADPH/NADH) [EC:1.4.1.13 1.4.1.14]. W, ko:K00611 OTC; ornithine carbamoyltransferase [EC:2.1.3.3].

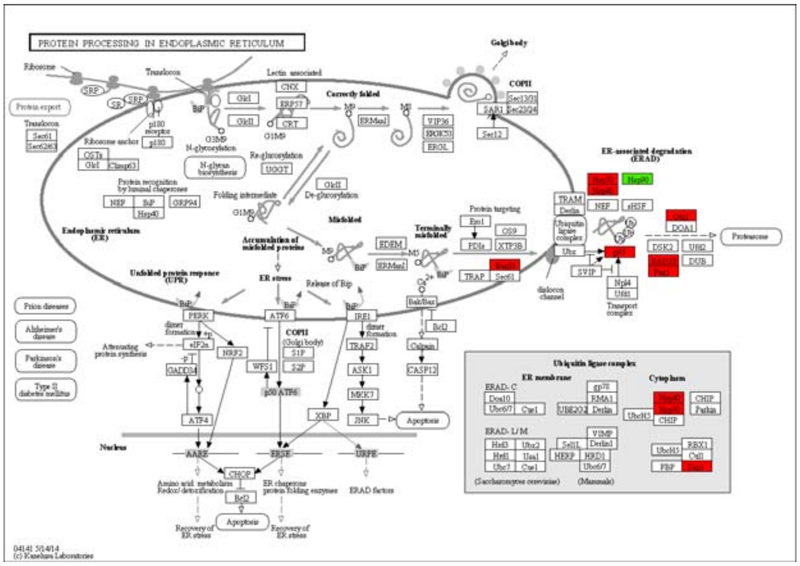


Figure S13 Effects of ethylene on ERAD (KEGG Pathway: ko04141) in petunia. Red box indicates up-regulation and green box indicates down-regulation in ubiquination level upon ethylene treatment based on the statistical significance in this study.

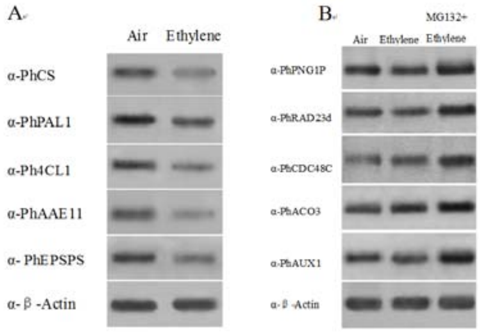


Figure S14 Confirmation of proteome and ubiquitylome data. A, Western blot analysis of petunia corollas after treatment with air or 2 μL L^{-1} ethylene for 16 h. B, Western blot analysis of petunia corollas after treatment with 80 μm MG132 for 4 h and 2 μL L^{-1} ethylene for 16 h. The representative results from three independent experiments were indicated.