Mining genes related to secondary metabolism in *Lycium*, traditional chinese medicine

Shao hua Zeng, Jian feng Zhou, Ying Wang
Wuhan Botanical Garden, the Chinese Academy of Sciences, Wuhan 430074, China

Introduction

Lycium chinense belonging to *Lycium* genus of the Solanaceae family is a crucial perennial shrubbery, traditional Chinese medicine. Several ancestral Chinese medicinal books had recorded its perfect medical and hygienical performance such as delaying senescence, fatigue-resistant, reinforcing kidney, elevating immunity and so on. Due to these advantages derived from many secondary metabolites for example LBP, lycine and β -carotene, *L*. chinense had served as a good material for exploiting hygienical products.

Up to now, the genomic information and gene expression patterns in *Lycium* have not been studied in details. And the secondary metabolism pathway in *Lycium* genus is not clear. Therefore, conventional methods, gene cloning and genetic transformation, is little or not useful for improving cultivars to elevate the contents of metabolites. To overcome the morass, a cDNA library was constructed in order to obtain enough EST sequences, which will be functionally annotated and categorized according to Arabidopsis. At the same time, a suppression subtractive hybridization library (SSH library) was also constructed to enrich the differential expression genes with respect to secondary metabolism and fruit development. Combinating the data of the two library, we expect to mine many genes related to secondary metabolism and fruit development.

Materials and methods

Plant materials

L. chinense plants were grown at greenhouse in Wuhan Botanical Garden, the Chinese Academy of Sciences. The young stage fresh leaves served as driver sample and red ripen fresh fruit served as tester sample were used for SSH library. And young stage fresh leaves were also used to construct cDNA library.

Total RNA isolation

About 50mg samples frozen in liquid nitrogen were powdered with mortal and pestle. And total RNA were isolated by 1ml TRIzol according to a protocol published in Invitrogen company website with minor modification.

cDNA and SSH Library construction

cDNA library was constructed according to BD SMART^{TM} cDNA Library construction Kit manufacturer's instructions. And SSH library was performed with young leaves and ripen fresh fruit according to PCR-Select cDNA Subtraction Kit (Clontech) manufacturer's instructions.

Results and discussions

Result 1 RNA quality

Table 1 Spectrophotometer analysis of the total RNA quality isolated from young leaf and ripen fruit of *L*. chinense

Sample	$A_{260/280}$	$A_{260/230}$
Young leaf	1.88	2.16
Ripen fruit	1.78	1.51

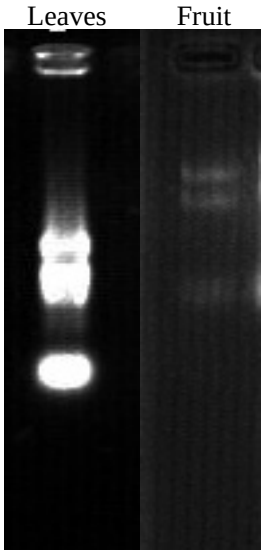

Figure 1 Electrophoresis analysis of total RNA isolated from leaves (left) and ripen fruit(right)

Since ripen fruit of *L.* chinense contain lots of polysaccharide, phenolic compounds and other secondary metabolites, which seriously affected the purification of total RNA and the efficiency of reverse transcriptase. In order to obtain high quality total RNA, TRIzol reagent were used twice to exclude the impurity. After that, the ratio of $A_{260/280}$ and $A_{260/230}$ of ripen fruit total RNA are adjacent to that of leaves total RNA.

Result 2 dscDNA quality

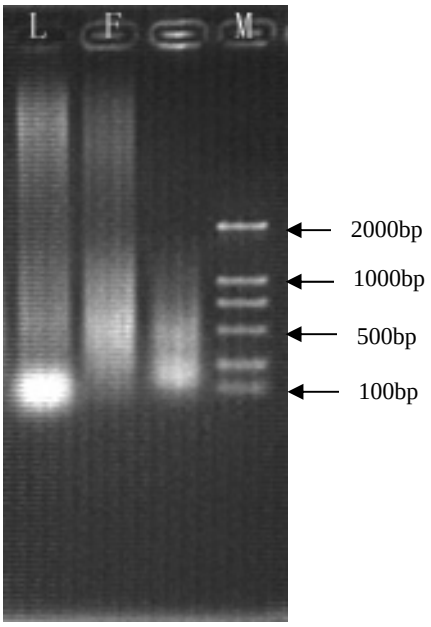

Figure 2 Electrophoresis analysis of dscDNA amplified after 30 cycles. L: young leaves, F: ripen fruit, M: DL2000

Figure 2 show that the dscDNA fragments derived from young leaves and ripen fruit display smear phenomenon. More results will be discussed in details in the future.