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植物生产层

柱花草总RNA提取方法比较 张倩茜,张伟丽,刘凤民,许修宏,庞丹丹

摘要:

以热研2号柱花草(Stylosanthes guianensis Reyan No.2)叶片为材料,利用紫外分光光度计和凝胶电泳法比较SDS 酚抽提法、CTAB法、Trizol试剂盒和柱式植物RNAout试剂盒法提取柱花草总RNA的质量和纯度。结果表明,改良CTAB法和柱式植物RNAout试剂盒法提取的RNA的OD260 nm/OD280 nm分别是1.85和1.93,OD260 nm/OD230 nm均大于2.0。凝胶电泳结果表明,改良CTAB法及柱式植物RNAout试剂盒法均有28S rRNA和18S rRNA两条清晰的条带,且无降解。其他两种方法获得的RNA品质较差,有降解和弥散现象。将改良CTAB法和柱式植物RNAout试剂盒法提取的RNA逆转录成CDNA,cDNA能扩增出一条清晰的β actin基因片段,进一步证明了改良CTAB法和柱式植物RNAout试剂盒法提取的总RNA具有很高的纯度,其中柱式植物RNAout试剂盒法的效果好于改良CTAB法。

关键词: 柱花草 总RNA 提取方法 RT PCR

Extraction methods of total RNA from Stylosanthes guianensis ZHANG Qian qian2, ZHANG Wei Ii, LIU Feng min, XU Xiu hong, PANG Dan dan

Abstract:

The modified SDS phenol extraction method, Modified CTAB method, common Trizol and Column Plant RNAout were used to extract total RNA in leaf of Stylosanthes guianensis Reyan No.2, respectively. The quality and quantity of total RNA from above mentioned methods were compared to select the better methods by UV spectrometer and gel electrophoresis. This study indicated that the value of OD260 nm/OD230 nm of RNA extracted by modified CTAB method and Column Plant RNAout method were higher than 2.0 and the value of OD260 nm/OD280 nm of RNA extracted by modified CTAB method and Column Plant RNAout were 1.85 and 1.93, respectively. Gel electrophoresis showed that RNA extracted by modified CTAB had clearer bands of 28S rRNA and 18S rRNA and they did not degrade, and that RNA extracted by Column Plant RNAout had two clearer bands of 28S rRNA and 18S rRNA and they did not degrade; however, RNA extracted by other two methods degraded and dispersed to some degrees. RNA extracted by modified CTAB method and Column Plant RNAout could be reversed to cDNA. The cDNA was amplified and one clear bands of g actin gene fragment was observed in agarose gel. These results further demonstrated that the quality and purity of the total RNA extracted by modified CTAB and Column Plant RNAout could be applied into molecular biology experiment, and quality and purity of the total RNA by Column Plant RNAout method were better than that by modified CTAB method.

Keywords: Stylosanthes guianensis total RNA extraction methods RT PCR

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