



The Development of Molecular Tools That Facilitate Analysis of Grain Filling in Rice

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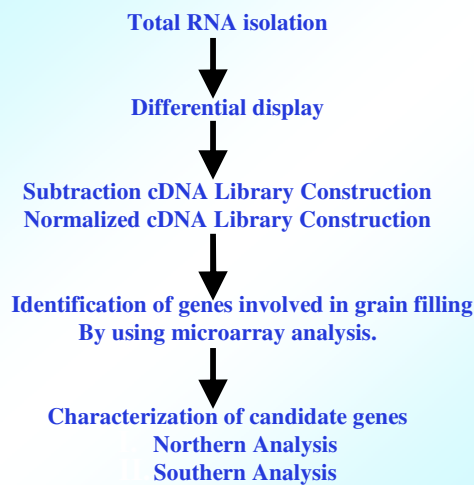
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ABSTRACT

Grain filling is an important multigenic agronomic trait in rice that is poorly understood. Abortion of the developing embryo or incompatible crosses that result in late embryo abortion adversely affect grain filling. It has been well established that environmental factor and plant hormones such as gibberellins play a role in grain filling. We are therefore using a genome wide gene expression profiling approach of DNA microarrays to understand the molecular basis of grain filling. We have identified five rice varieties that differ in their grain filling efficiency as starting material to facilitate the identification and cloning of genes involved in grain filling. We have used differential display as a means to identify important stages of grain development as well as to facilitate cloning of stage specific transcripts. Optimized protocols for RNA extraction and purification have been developed.

METHODS



RESULTS

I. RNA extraction

Table 1: Yield and Purity of RNA extracted from rice using different RNA extraction methods.

	Yield (µg/g)	Purity (A260/A280)
Qiagen Extraction Kit	12.5	1.60
NTES Method (1999)	24.5	1.91
Liu <i>et al.</i> (1998)	34.9	1.95
Gao <i>et al.</i> (2001)	88.4	1.85



Figure 1: RNA extraction from different methods (A) NTES method (B) Gao *et al.* method.

II. Differential Display

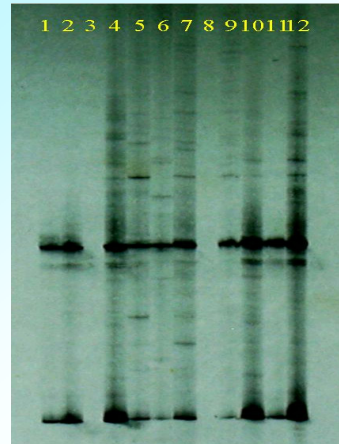


Figure 2: Differential display autoradiogram for variety MR 84.

Lanes 1-3: Panicle (week 1 to week 3),
Lane 4: Spikelet (week 3)
Lane 5: Panicle (week 4)
Lane 6: Spikelet (week 4),
Lane 7: Panicle (week 5),
Lane 8: Spikelet (week 5),
Lane 9: Panicle (week 6),
Lane 10: Spikelet (week 6),
Lane 11: Spikelet (week 7),
Lane 12: Spikelet (week 8).

DISCUSSION

The RNA extraction protocols tested included the guanidium thiocyanate method of López-Gómez and Gómez-Lim (1992) developed for fruits rich in polysaccharides. Guanidium thiocyanate method was not suitable for RNA isolation from the endosperm tissues of rice, because it could not remove starches effectively (data not shown). RNA isolated using Sodium Tris-EDTA-SDS (NTES) method yielded RNA of good quality; however it is contaminated by DNA. The Qiagen™ RNA extraction Kit is not suitable for extracting total RNA particularly from rice, which has high levels of starches (data not shown).

Differential display result showed no difference in transcriptional activities during the process of grain filling. Subtraction library and normalized library will be constructed to study the genes involved in grain filling.

CONCLUSION

The RNA extraction reported by Gao *et al.* (2001) was found to be the best method for total RNA isolation. Differential display approach did not show any significant differences in the transcriptional activities throughout the process of grain filling.

ACKNOWLEDGEMENT

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