دراسات وراثية جزيئية على تنشيط الإجهاد البيئي للعناصر الوراثية المتنقلة الرجعية فى حقيقيات النواة

رسالة مقدمة من

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قسم الوراثة
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MOLECULAR GENETIC STUDIES ON ENVIRONMENTAL STRESS ACTIVATION OF RETROTRANSPOSONS IN EUKARYOTES

By

MARWA MAHMOUD SHEHATA AHMED

B.Sc. Agric. Sc. (Genetics), Fac. Agric., Ain Shams University, 2007

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ABSTRACT

Marwa Mahmoud Shehata Ahmed: Molecular Genetic Studies on Environmental Stress Activation of Retrotransposons in Eukaryotes. Unpublished Ph.D. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2020.

Retrotransposons comprise the major part of eukaryotic genomes. They have the ability to replicate themselves through RNA intermediate via reverse transcription process. During normal development, these elements become quiescent, but they are stimulated by stresses. The availability of PCR-based techniques to detect the variation in retrotransposition rate due to salinity was tested. IRAP and SCoT markers were applied in two salinity-tolerant eukaryotic genomes: Yeast (*Saccharomyces cerevisiae* L.) and Barley (*Hordeum vulgare* L.). Semi-quantitative analysis was applied with only the two barley cultivars.

The DNA of the yeast strain EMCC-49 and two barley cultivars Giza-123 and Giza-2000 were extracted. Five IRAP primers with two combinations and nine SCoT primers were applied. The yeast strain was grown in the YPG media with 0.5 M, 1 M, 1.5 M NaCl and the control. The barley cultivars were irrigated with 0.25 M, 0.6 M NaCl or just distilled water as the control.

This research aimed to study the effect of salinity stress on the activation of retrotransposition. IRAP technique developed three markers in the yeast under the different levels of salinity. ScM1 IRAP primer showed a band with molecular size of 456 bp in the yeast under 0.5 and 1.5 M only. Another band with molecular size (MS) of 409 bp appeared under the control only. The third IRAP marker was shown by the ScM2 primer with MS of 1952 bp under the 0.5 M treatment. While, two IRAP markers appeared in barley due to salinity stress. The 5'LTR IRAP primer showed an 886 bp band in the barley cultivar Giza-2000 under the control condition only. Sukkula IRAP

primer displayed the second IRAP marker in the cultivar Giza-2000 of barley with MS of 330 bp under the 0.6 M only.

SCoT markers showed 17 markers response to salinity stress in yeast with MS ranged from 1911 to 271 bp with SCoT-31 and SCoT-26 primers, respectively. SCoT-26 primer gave the highest number of markers per SCoT primer (five different markers). In barley, 18 SCoT markers were detected under salinity stress. They MS were between 1762 (SCoT-26) and 281 bp (SCoT-7). SCoT-32 primer showed five markers in barley under salinity. The results showed that the high levels of salinity led to new retrotransposition.

In semi-quantitative analysis the banding patterns obtained with actin primer as housekeeping gene showed the same pattern in the control and all treatments in Giza 123 and Giza 2000 with no difference in the band intensity. With specific primer of *TY1B* gene (*reverse transcriptase* gene), in Giza 123 had low intensity in 0.25 M NaCl (T1) compared with the control. Giza 2000 showed that the intensity of band was more in 0.6 M NaCl.

This study confirmed that PCR techniques; like IRAP and SCoT can exhibit the activation of retrotransposition due to salinity stress. Good positive results were obtained and we recommend the using of these techniques for different molecular purposes due to their advantage; easy, fast, cheap and effectiveness.

Keywords: Retrotransposon, salinity, IRAP, SCoT techniques and semi-quantitative

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