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Effects of Salt Stress on Total RNA and Poly(A)$^+$RNA Contents in Tobacco Suspension Cultured Cells at the Different Time Course

Burçin Cokuysal, Hakan Cakici and Emre Erbas
Department of Soil Science, Faculty of Agriculture, Ege University, Bornova, Izmir-Turkey

Abstract: Suspension-cultured cells derived from pith cells of *Nicotiana tabacum* L. cv. Xanthi were used in experiments designed to determination of the effects of salt stress on total RNA and Poly(A)$^+$RNA contents at the different time course. Cultured cells were treated with 70 mM NaCl. Cells were harvested 7th day and 12th day of growing phase from control and stress treated media. In comparing stress and control, statistically differences in amount of total RNA and Poly(A)$^+$RNA were found. Also statistically differences were found depending on a time course. Total RNA was found 162.80 μg g^{-1} of cell fresh weight (CFW) at the 7th day control, 195.00 μg g^{-1} of CFW at the 7th day salt imposed, 135.40 μg g^{-1} of CFW at the 12th day control, 181.40 μg g^{-1} of CFW at the 12th day salt imposed cells as an average of replications. Poly (A)$^+$RNA was found respectively 3.13, 3.90, 2.41, 3.65 μg g^{-1} of CFW. All these data clearly indicate that the tobacco cells growing in salt stress conditions show distinct changes in the pattern of accumulation of total RNA and Poly(A)$^+$RNA for the synthesis of salt stress-specific proteins at the different time course.

Key words: *Nicotiana tabacum* L., stress conditions, cell culture, plant growth, RNA

INTRODUCTION

Extraction of high-quality RNA is necessary for making cDNA libraries, isolating genes by RT-PCR, or investigating gene expression profiles. The quality of the isolated RNA was consistently high as indicated by spectrophotometric readings and its separation on denaturing agarose gels. The yield and quality were suitable for RT-PCR and Northern blot hybridization[1].

Optimum plant growth and development require proper growing media conditions, including adequate water and nutrient supply. Depending on stressful environment plants are unable to express their full genetic potentia[2]. Stress genes are depressed to produce novel species of mRNA responsible for *de novo* synthesis of stress proteins. Posttranscriptional mechanisms including preferential translation of stress mRNA may also be involved. Stress proteins are transitory products. They disappear and their mRNA decay after removal of stress factors[3].

The adverse effect on the plant productivity from the excess of salinity is a worldwide problem[4]. Therefore, most of the researchers are focused on the control of the salt stress problems, determination of tolerance mechanism and finding new solutions at the molecular level in plants. Several investigators have shown the accumulation of RNA and Poly(A)$^+$RNA related with the synthesis of new proteins in cultured plant cells when subjected to stress condition[5-9]. For instance, a transient increase in calmodulin mRNA levels was observed in tomato cell suspension cells treated with 200 mM NaCl[10]. The mRNA level of the soybean is transiently increased by NaCl treatment[11]. Also the synthesis of such stress-induced proteins has been documented under salt stress in tobacco[12-16].

The aim of this study was to compare effects of salt stress and control on the amount of total RNA and poly(A)$^+$RNA and discussed the difference of RNA and poly(A)$^+$RNA accumulation between 7th and 12th day of the growing phase. Additionally, it is important to the construction of a cDNA library purifying with this poly(A)$^+$RNA for further studies.

MATERIALS AND METHODS

Medium nitrate-free were prepared[17]. Cells of XD line of tobacco derivated from pith cells *Nicotiana tabacum* L. cv. Xanthi, were grown on nitrate-less M-ID medium, adjusted to pH 6.2 with NaOH. Filter sterilized urea at a concentration of 80 mM was used as the sole nitrogen source supporting cell proliferation. The cultures were grown in the dark room on a gyrorotatory shaker (110 rpm)

Corresponding Author: Burçin Çokuysal, Department of Soil Science, Faculty of Agriculture, Ege University, A Blok, 35100, Bornova, Izmir-Turkey
Tel: +90 232 388 40 00/2651 Fax: +90 232 388 92 03

at 28°C. All the reagents were used analytical grade[18]. Cell samples were imposed with 70 mM NaCl for stress evaluation. Control and stressed cells were harvested by vacuum filtration on Whatman No.1 filter paper and the density of XD cells, total weight and necessary volume for the 1 g XD cells was calculated at 7th and 12th day of growing phase. Each 1 g of cell samples was kept at -80°C.

Total RNA was obtained using a conventional chloroform/phenol extraction from harvested cells[19,20]. The concentration of the RNA was determined by measuring the OD_{260} of an aliquot of the final preparation. A solution of RNA whose OD_{260} =1 app. Forty microgram of RNA mm^{-1}. Poly (A) RNA was purified from the preparation of total RNA and freed from contaminatingoligodeoxyribonucleotides by chromatography on oligo (dT)-cellulose using with 40 mM Tris. Cl (pH 7.6), 1 M LiCl, 2 mM EDTA, 0.2% SDS as a 2x loading buffer and 10 mM Tris.Cl (pH 7.5), 1 mM EDTA, 0.05% SDS as an elution buffer. Items of glassware were siliconized by soaking or rinsing in a 5% solution of dichlorodimethylsilane in chloroform and baked at 180°C for overnight[19,21]. The purity of RNA and poly $(A)^+$RNA and was estimated with ratio of between 260 and 280 nm (OD_{260}/OD_{280})[19,22].

All data points were based on a mean of five replications. Statistical calculations (ANOVA, LSD tests and confidence limits at 99%) were performed according to Mead and Curnow[23].

RESULTS AND DISCUSSION

The ratio of OD_{260} and OD_{280} was found 1.96-1.98 for all treatments. Pure RNA will exhibit OD_{260}/OD_{280} ratios of 2.0. However due to the variations between different starting materials and individual variation in performing the procedure to obtain RNA having OD_{260}/OD_{280} ratios ranging from 1.7-2.0[19,22]. Similar results have been reported to obtain OD_{260}/OD_{280} ratios greater than 1, and the OD_{260}/OD_{280} ratios were always between 1.8 and 2.0, indicating the absence of contaminants[24]. This result implies that the isolated RNA is pure, without any contaminants, and amenable to use for other downstream applications.

The response of two different conditions (control and salt stress) of tobacco cells, grown in suspension culture, to different time course (7th and 12th day of growth) was compared using the statistical method ANOVA (Table 1and 2).

On the time course basis, the amount of total RNA and Poly$(A)^+$RNA tented to decrease from 7th day to 12th day of growing period in both conditions. This result is associated with; the increasing density flask volume

Table 1: ANOVA statistics of compared two different time course and two different application on total RNA and Poly$(A)^+$RNA content of tobacco cells

	Calculated F-value	
Source of variation	Total RNA	Poly $(A)^+$RNA
Time Course (A)	444.422**	122.899**
Applications (B)	122.166**	28.797**
AxB interaction	13.840**	7.023**

$p<0.05$; ** $p<0.01$

Table 2: Effect of salt stress and time course on total RNA and Poly$(A)^+$RNA contents of tobacco cells

Treatments	Total RNA	Poly $(A)^+$RNA
7th Day Control	162.80 B,aa	3.13 B,aa
7th Day +70 mM NaCl	195.00 A,AA	3.90 A,AA
Difference	32.20	0.77
12th Day Control	135.40 b,bb	2.41 b,bb
12th Day +70 mM NaCl	181.40 a,BB	3.65 a,BB
Difference	46.00	1.24
LSD	7.66	10.272

Values in a column, followed by different letter(s) were statistically significantly different according to LSD test at $p<0.01$ (Total RNA) and $p<0.05$ (Poly $(A)^+$RNA)

depending on cell expansion and deaths because of senescence. The regeneration capability and genetic stability of suspension cells however decrease by the length of culture period[25]. The cells will continuously grow until one of the factors such as nutrient elements, mass of cells, becomes limiting causing cell grow to slow[26]. In addition, the amount of total RNA and Poly$(A)^+$RNA in stress imposed cells were found significantly higher than control cells at the 7th and 12th day of growing period. These results were considered as related with adaptation to stress conditions.

In the time course (A) 7th day was compared with 12th day of growing phase. In the applications (B) control cells were compared with stress imposed cells. Analysis of variance showed that total RNA and Poly$(A)^+$RNA contents were significantly influenced by different time course and stress conditions. Also interaction of these factors was found statistically significant. The significant interaction indicates that the response to stress depends on whether or not different time course.

The amount of the total RNA and poly $(A)^+$RNA were significantly higher in control cells at 7th day compared to 12th day of growing phase. Same results were observed in salt stress imposed cells (Table 2).

The highest total RNA and Poly $(A)^+$RNA was observed in NaCl treatment at the 7th day. In addition, a higher difference was found between 12th day control and 12th day +70 mM NaCl (46.00 for RNA, 1.24 for Poly $(A)^+$RNA) compared to between 7th day control and 7th day +70 mM NaCl (32.20 for RNA, 0.77 for Poly $(A)^+$RNA). Similar results have been reported to RNA was detected at low levels in non-stressed plants whereas its

accumulation was higher under salt stress in barley[9]. According to the results it may be concluded that, similarly as is observed in other plants[27-29]. Salt alters the accumulation of RNA and mRNA in plant cells[30].

In conclusion, tobacco cells were produced higher amount of RNA on media with salt imposed compared to control. Stress imposed cells were showed smaller amount of difference at 7th day compared to 12th day of growing stage. It has been proven that earlier growing stage, at the salt stress, is more sensitive. This reaction is related with salt tolerance mechanism. Salt-induced changes in the amount of several stress proteins. These proteins appeared to be associated with adaptation. Analysis of RNA, Poly(A)$^+$RNA related with genes whose expression is induced under salt stress is important to understand the mechanism of salt tolerance and possibly to use for breeding salt-tolerant plants. So it's obvious that certain RNA and Poly(A)$^+$RNA activity is necessary for survival of plants under salt stress.

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