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THE EFFECT OF LIGHT, TEMPERATURE AND DESICCATION ON DIFFERENTIAL STABILITY OF mRNA, FROM SPRING AND WINTER WHEAT SEEDLINGS

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ABSTRACT
The research focused on working out a molecular method of assessing stress-steadiness and photo-periodism of wheat and Barley varieties Selekcia KN11 grown under stress conditions. A total RNA preparation was isolated from spring and winter seedlings using phenol-detergent method, and the isolated poly (A) RNA were recovered from the total RNA using Affinity chromatography with poly Uracil sepharose as an absorbent. One of the objectives of the study is to adapt mini preparation procedure for the effective and rapid isolation of poly (A) RNA, from total RNA preparation. Variable yields of poly (A) RNA were obtained from four days old seedlings of winter and spring wheat and barley varieties, Celekcia KN11 which were sown under various environmental condition, the poly (A) RNA was studied and analyzed and the findings for photo-periodism of wheat varieties: krasnodarskaya-39, Bezostaya-1 and Drujna showed that the former two varieties are highly light sensitive (long day) while the latter is moderately long day variety. Variety Spectra was found to be neutral to light (photo-periodism). On the other hand, the result of 3 varieties krasnodarskaya-39, Olimpiya and Bezostaya-1is consistent with the findings of conventional breeding for the assessment of wheat stress-steadines. The three varieties are all relatively resistant to drought in the following order: Krasnodarskaya-39 > Olimpiya > Bezostaya-1 at $P<0.05$.

Key words: Affinity chromatography, photo-periodism, isolation, phenol-detergent, Stress-steadiness, Uracil-sepharose, wheat

INTRODUCTION

Proteins play very important role in the life of every pro- and eukaryotic cell, they are the major constituents of cell's organelles, enzymes and hormones. They facilitate all biochemical and physiological process taking place in a living organism, which growth and development depend upon.(Anne *et.al*,1989 and Dolars *et. al.*,1990)

Messenger RNA serves as a template for polypeptide translation. In general, the rate of synthesis of a protein usually depends upon the concentration of RNA that encodes it, thus, in turn is determined by a balance between the rate of mRNA synthesis and its decay. A high unstable mRNA is a prerequisite for the rapid chain regulation of protein synthesis shortly after the transcription of a particular mRNA ceased. It's important to realize that the variation in the steady state of cytoplasmic mRNA determines the adaptation of an organism to its habitat.(Anne *et.a.l*,1989 and Arbuzov,1980)

The regulation of gene expression at mRNA stability level will obviously continue to be an important focus of biological research, while same general principles are already apparent. Further studies of the mechanism and structural determination of mRNA decay should in time bring significant new insight. (Mathias, 1991; Mullet and kllein, 1983; Mertbasov, 1990).

Analysis of the matrix population has great practical perspective especially in crop production. The effect of light specter and light intensity, temperature and moisture on the steady state of a particular mRNA which encodes specific protein of photo system and key enzymes of metabolic activities, or the heat shock protein in tropical crop, will permit agronomist to carry an effective cultivars screening for photoperiodism and stress-steadiness thus consequently will improve the crop productivity and give high yield.(Kakoto *et. Al.,*1989; Gayle *et. al*,1989 and Drummond *et.a.l*,1989)

The main aim of this research is to study the regulation of gene expression at the stability level of mRNA from a wheat seedling which was prior subjected to stress factors, hence the following objectives were set:

a. Isolation of total RNA preparation from spring and winter wheat seedlings using phenol-detergent method
b. Isolation and analysis of poly $(A)^+$ mRNA content from total preparation using the method of affinity chromatography on poly (U) sepharose
c. Study the effects of light, temperature and desiccation on poly $(A)^{++}$ yield from spring and winter wheat seedlings.
d. Working out a molecular method of evaluating stress-steadiness and photoperiodism of wheat and Barley varieties.

MATERIALS AND METHOD

Condition of raising the seedlings: Wheat cultivars Seleksia KNII were three times washed thoroughly with water to remove none germiable seeds and other impurities. Sowing of seeds was carried out on Yokovsona's table which is made up of seed trays; three quarter full of water covered with a wire-gauge and on the wire filter paper was placed whereon sowing was carried. A spacing of 10x19cm was made (an area within which seeds were sown). The sown seeds were then covered with humidified and perforated filter paper to facilitate germination and ventilation. To prevent light from inhibiting the seed's germination, some plastic containers were used to cover the seeds. The whole set was then placed in a controlled camera under 20 ^{0}c for three days to allow germination.

On the fourth day, the sprouted seeds were transferred to plastic containers. This was carried out by cutting out the sown seed area carefully without damaging rootlets and placing it in to a plastic container provided for this purpose. Each container was labeled prescribing the type of experiment and wheat variety used. The whole containers were kept in phytotron for about 9 hours under bright light condition.

The seedlings expected to be used for the study of light effect were kept in the dark inside the thermostat Mini cold Lab. at 20^{0}c for the next four days. And the control variants were taken at the fourth day while experimental variants were exposed to light and selected at given time interval, cut and stored in chilled liquid Nitrogen.

Seedling to be used for the study of drought resistance, were separated after the 9th hours lightening and each container was treated with the solution of 10% and 12% polyethylene glycol and kept for several hours. The cutting of the seedlings were carried for each container at a given interval of time 3, 4, 7, and 24 hours respectively, then the sample was stored in chilled liquid Nitrogen.

Isolation of total RNA phenol detergent method: The (stored in chilled liquid Nitrogen) seedlings were grounded to a fine state in a liquid Nitrogen using a mortar and pestle and defatted by buffer at ratio1: 5. The buffer solution of 0.2 M tris HCL, P^H 8.5, 0.05 M MgCL, 2% Sodium dodecyl sulphate was used. A chilled phenol / chloroform was added at 1:1 and mixed for 20 minutes at room temperature on a shaker Sh-4 premed (Poland). All the proteins were removed as SDS/ KOAC precipitates by centrifugation of the homogenates at 300g for 20 minutes on centrifuge K 23D MLW (Germany) at 4 ^{0}C. Supernatants were collected in a test tube of equal volume of phenol-chloroform and second deproteinization was repeated. The collected supernatant from second deproteinazation was mixed with 2.2 volume of absolute ethanol shaken and left for 2 hours.

After centrifugation at normal procedure precipitated nucleic acid were collected and dissolved in distilled water at 1ml per 1g of the precipitate and an equal volume of deproteinization solution was added (8m LiCl 8 urea and 4mM EDTA) this was kept for 16 hours at 40^0 . The pellets were collected by sedimentation after centrifugation washed with enough volume 3000 70% of ethernol and centrifuged for 10 minutes again. (Poulson,1973)

Spirit was used to wash the pellets and dried it in vacuum KPG-1M (Russia) and resuspended in 3.0 ml water and centrifuge for 30 minutes at 3000g. The supernatants that contain RNA were transferred to a glass cube and stored at -20 ^{0} C .

mRNA acid yield was calculated based on the observation that 1mg RNA in low salt absorbs 25 optical density (OD) at 260mm and the spectrophotometrical characteristic of RNA preparation was 260/280 = 2.0; 260/230=2.1. Absobance ratio of 1.8 or greater indicated level of purity which correlated well with subsequent restriction endonuclease digestibility at RNA in the extract.

Isolation of poly (A) RNA (Affinity chromatography method): Chromatograph was carried on wet chromatography 0.34 Hitach (Japan) that consist of thermostat, glass tube and pump, with affinity absorbent (Poly Uracil sepharose), which was sensitive to poly-adenine RNA. The chromatography was connected with circulation water in a water basin. The stored preparations of total RNA was mixed with an equal volume of buffer 40mM tris HCL pH 7.6 1 LiCl 0.2% SDS and shaken and then poured in to the glass column of chromatograph. Initially the glass tube was washed with this buffer to the time of no absorbance of optical density at 260 nm when the run down buffer was absorbed in the spectrophotometer. The same process was carried with the RNA preparation 2-3 times. (Poulson,1973)

All the none specific RNA were washed down, since they will not react with the poly (U) sepharose in the chromatography glass tube.

The release of poly (A) RNA was carried by passing warm water at 65 ^{0}C through the chromatography glass tube. All the content of the affinity tube was collected in a bottle and the concentration of Poly (A) RNA was determined at 260 NM on a spectrometer Specord m400. (Poulson,1973)

To determine the RNA period of half life (turn over) it is necessary to run the second circle of affinity chromatography of poly $(A)^+$ RNA from the first circle.The percentage of poly (A) RNA yield after second circle from the first circle taking first circle as 100% was determined. This shows the RNA stability. The experiment was repeated about 3 times and the value of experimental error was found to be $P< 0.05$

RESULTS AND DISCUSSION

The method of affinity chromatography was found to be an effective and simple scientific technique of studying matrix RNA turn over.

The principle of the method is releasing specific RNA from none specific RNA using a sensitive absorbent, and the percentage of the stable RNA which are resistant to turn over was determined by carrying a second circle of chromatography.

RNA turn over depends upon the physiological state of the source crop (as affected by different endo and exodermic factors). This study discovered that some of the factors that influence RNA turn over, include light, temperature of the plant and growing condition. In this study different cultivars of winter wheat and winter barley were studied. The yield of poly (A)RNA correlated clearly with the cold-steadiness. Seedlings raised at 4^0C gave various yield of poly (A) RNA depending on the level of cold-steadiness of each cultivars.(table.3)

It was the aim of the research to study the variable yield of poly $(A)^{++}$ RNA (second circle) from poly $(A)^{+}$ RNA (first circle) and the yield depend tightly on the physiological state of the plant, which is subjected to change under the influence of temperature, light and desiccation.

The findings of the effect of light on winter wheat and Barley cultivars (as shown in table 1) showed that wheat cultivars distinguish themselves in terms of rate of reaction to light. (Stout et.al,1967). All spring and winter cultivars responded differently to light. Variety Krasnodarskaya-39, Bezostaya-1 gave a low yield of poly A ++ RNA, while spring Drujna B gave a high yield of RNA comparatively. This finding agreed with the photo-physiologist empirical observations on the contrast reaction of winter and spring wheat cultivars. (Kakoto *et.el.,1989*). Therefore, the cultivar Drujna B has a distinguished sensitivity to light, on the other hand cultivar specter is known for its low sensitivity to light. This result has been confirmed in this study table-1.

Analysis of table-1 shows that light regulates ontogenesis (growth). That is why the growth winter cultivars is inhibited under equal day condition. Therefore, these cultivars are best sown when there is still long day condition at period of ontogenesis initiation (Gayle *et.al.,*1989).

Table 1: **The effect of light on Poly A^{++} mRNA yield from winter and spring wheat at 20 ^{0}C**

Variety	Poly A^{++} mRNA Darkness for 18 hours (a)	yield, % light for 5 hours (b)	Difference b-a
Krasnodarskaya 39 (Winter)	75±02	25±0.6	-50
Bezostaya I (winter)	41±1.1	33±0.8	-8
Drujna B (spring)	37±0.9	47±1.0	10
Spectr (spring)	36±0.9	36±0.8	0

Results are mean ± standard deviation, a figure that differ significantly at P< 0.05

Key: Poly A - poly adenine matrix RNA, + - a first circle of affinity chromatography to determine half life period of mRNA decay, ++ - a second circle of affinity chromatography to determine half life period of mRNA decay.

Plant reaction to light at the first growth stage determines vegetation period. Therefore all winter crop delay in vegetation period is brought about by short day conditions and on the other hand it is not noticeable during the short day condition. Depending upon the reaction of crop to light there exist early maturing and late maturing. The higher the reaction to light shown by a plant, the shorter is its vegetation period. Therefore, vegetation period of non-light sensitive is determined by their relation to light at the first life stage.

It was discovered that, the integral index of plant reaction to exogenic and endogenic factors is found to be correlated with plant growth intensity. (Sachs,1986)

An experiment was carried out to investigate if there is any relation between the variable yields of mRNA from wheat seedlings and intensive growth of the above ground biomass. It was discovered that these cultivars show different growth intensity, for instance variety Opal was recorded as the most vigorous with high above ground biomass, and it yielded 49% of mRNA higher than that of other two cultivars. Hupateca had lowest growth intensity and lowest yield of mRNA and Saratovskaya 29 (see table 2) gave moderate mRNA yield with moderate growth intensity comparatively. This shows that growth intensity is entirely dependent upon mRNA availability that encodes the growth regulatory proteins, thus mRNA stability determines plant growth.(Thompson and Meager,1990)

Table 2: Correlative relationships between Poly A^{++} mRNA yield from three spring winter wheat seedlings and the plant growth tempo

Variety	Biomass	Poly A^{+} mRNA yield, % of total mRNA	Poly A^{++} mRNA yield, % of Poly A^{+} mRNA yield
Opal	High	1.4±0.1	49±2.0
Saratovskaya 29	Moderate	2.1±0.2	39±1.1
Hupateca	low	2.1±0.2	32±1.0

Results are mean ± standard deviation, a figure that differ significantly at P< 0.05

Table 3: The response of Poly A^{++} mRNA yield from winter wheat seedlings, % to temperature

Variety	Temperature, ^{0}C			
	04	20	23	26
Krasnodarskaya 39	60±03	14±01	36±02	75±02
Olimpia	-	18±0.8	33±02	73±02
Bezostaya I	44±01	25±01	31±02	49±01

Results are mean ± standard deviation, a figure that differ significantly at P< 0.05

Table 4: The effect of desiccation on Poly A^{+} mRNA yield from winter wheat seedlings, grown at 20 and treated with 10% PEG

Variety	Treatments			
	0% PEG	10% PEG	Difference	Rank
Krasnodarskaya 39	37±06	94±2.1	57	1
Olimpia	5.0±0.2	30±0.8	25	2
Bezostaya I	30±0.6	35±0.7	5	3

Results are mean ± standard deviation, a figure that differ significantly at P< 0.05

The effect of temperature on mRNA stability as one of the most important exogenic factors that all life depends on, was observed on 3 wheat varieties (Table 3). Three winter wheat varieties were sown at various temperature level conditions of 4, 20, 23and 26^{0}C. The minimum RNA yield was observed on the whole 3 varieties at 20^{0}C and any declination from this temperature to either side resulted in an increase of mRNA yield. This shows that the seedlings performed well at 4, 23 and very well at 26^{0}C. The discovered result indicates that mRNA attained stability at stress temperature level, This clearly shows that a gradual decrease/increase in temperature makes organisms to adapt to unfavorable conditions.(Dolars et.al.,1990;Lanks,1983;Lanks,1988 Linquist,1986; Linquist,1981; Salandra and Subjeet,1983) Many years Research has revealed the fact that under a stress condition a new gene is born, thus Heat shock proteins,(Lanks,1983) which enable the other gene to be stabilized and prevent their turn over (decay). However, it's difficult to explain why a very low temperature of 4^{0}C stabilized the gene. This needs a further study and investigation but yet this remains a fact.

The best method of evaluating cultivars for drought tolerance and desiccation resistance is direct field observations, although the plant characteristics for drought tolerance depend upon all factors that affect bio-physiological processes in plant, thus enabling it to adjust and withstand these unfavourable conditions, It is postulated that plants resistance to stress conditions could be effectively assessed at molecular level (analyzing genes stability) than conventional method of field observations. (Pears, 1985 and Sign and Donald,1989).

The analysis of mRNA yield from 3 winter wheat that grown at 20^{0}C and subjected to desiccation within 5 hours show that, the higher yield of mRNA was obtained from var. Krasnodarskaya-39 with about 57% as compared with control treatment. Bezostaya-1 has the least yield of only 5% (table.4). Judging from this finding Krasnodarskaya-39 has high resistant to desiccation at physiological level, than Bezostaya-1with 5% stablised mRNA. This is in consistent with the report of conventional breeders on the field observation for evaluating winter wheat for desiccation and drought tolerance / resistance. (Anne et.al, 1989; Belenger *et.al,* 1986; William and Tanaka,1989 and Waker,1985)

On the other hand springs wheat cultivars Specter, Valeria and Budimir were treated under the same conditions and assessed for drought resistance and the findings were in agreement with the field observation of wheat breeders.

The analysis of mRNA from the seedlings under light, temperature and desiccation show that the period of mRNA half life decay, is determined by studying the period of mRNA turn over at second circle when compared with the first circle of affinity chromatography. The number of mRNA that remains after second circle shows the stability of genes in stress conditions. This indicates that the stable the gene, the effective its expression and directly proportions to plant growing conditions.

CONCLUSION

The method of molecular genetics helps in reducing the period taken by plant breeders to evaluate markers of resistance to stress conditions in plant or to introduce a new variety of plants resistant to diseases, pests and other unpleasant climatic factors that gives a promising yield, to less than one-third of normal duration. This research finding revealed the fact that

Wheat and Barley growth intensity and adaptability to stress conditions depend on the mRNA stability, which produces the corresponding proteins responsible for this physiological and biochemical readjustment. Therefore it's very important to study the measures of regulating mRNA stability and the effect of its turnover in genes expression.

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