Molecular Genetics Method of Assessing Photo-Periodism in spring and winter Wheat Seedlings

[1] Magash, A.I., [2]Abdullahi, Y. [3]V.K. Plotnikov, [4]N.B. [5]Bakaldina, J.B. and [6]Alekseenko

***Abstract*—**The research was conducted in the laboratory of molecular genetics, Krasnodar Agricultural Research Institute named after P.P. Lukyanenko to assess wheat varieties reaction to photo-periodism grown under light 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, studied and analyzed from four days old seedlings of winter and spring wheat varieties, and the findings for photo-periodism of wheat varieties: krasnodarskaya-39, Bezostaya-1 and Drujna revealed 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). at $P<0.05$.

***Keywords*—**Affinity chromatography, photo-periodism, isolation, phenol-detergent, Stress steadiness, Uracil-sepharose, wheat.

I. Introduction

ANALYSIS of the matrix RNA population has great practical perspective especially in crop production. The effect of light specter and light intensity, on the steady state of a particular mRNA which encodes specific protein of photo system and key enzymes of metabolic activities, or the stress shock protein in tropical crop, will permit agronomist to carry an effective cultivars screening for photoperiodism thus consequently improving the crop productivity.[7,5]

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. [1,4]. While 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 note that the variation in the steady state of cytoplasmic mRNA determines the adaptation of an organism to its peculiar habitat. [1, 2]. The regulation of gene expression at mRNA stability level will obviously continue to be an Important focus of biological research. Further studies of the mechanism and structural determination of mRNA decay should in time bring significant new insight. [10, 12, 11]

The main objective of this eperiment is to study the regulation of gene expression at the stability level of mRNA from a wheat seedling which was prior subjected to light stress; hence the following specific objectives were set:

- Isolation of total RNA preparation from spring and winter wheat seedlings using phenoldetergent method
- Isolation and analysis of poly (A)+ mRNA content from total preparation using the method of affinity chromatography on poly (U) sepharose
- Study the effects of light on poly (A)++ yield from spring and winter wheat seedlings.

II. Materials and method

A. Condition of raising the seedlings

Wheat cultivars Seleksia KNII were three times washed thoroughly with water to remove none viable 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 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 into a plastic container provided for this purpose. Each container was labeled prescribing the type of experiment and wheat variety used. The whole containers were

[1]Department of Crop Science, Kano University of Science and Technology, Wudil, Nigeria.

[2]Department of Biology, Kano University of Science and Technology, Wudil, Nigeria.

[3,4,5] and [6] Laboratory of Molecular Genetics, Krasnodar Agricultural Research Institute named after P.P. Lukyanenko.

kept in phytotrone for about 9 hours under bright light condition.
The seedlings were kept in the dark inside the thermostat Mini cold Lab. at 20^0c 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, (5 and 18 hours) cut and stored in chilled liquid Nitrogen.

B. 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, PH 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 0C. 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 4^0C . The pellets were collected by sedimentation after centrifugation washed with enough volume 3000 70% of ethernol and centrifuged for 10 minutes again. [13] 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. RNA samples can also be extracted using the TRIZOL method (INVITROGEN) from seedlings as described before[19, 18]

The supernatants that contain RNA were transferred to a glass cube and stored at -20 ^{0}C . mRNA 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. Absorbance ratio of 1.8 or greater indicated level of purity which correlated well with subsequent restriction endonuclease digestibility at RNA in the extract.

C. 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. [13]

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 conducted by passing warm water at 65 0C 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. [13] To determine the RNA period of half life (turnover) 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 determined at $P< 0.05$.

III. 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 other growing conditions. Dubcovsky (2005) [3] observed that in a short day condition the acceleration of flowering was noted in photoperiod sensitive winter varieties. Since vernalization requirement and photoperiod sensitivity are ancestral traits in Triticeae species he suggested that wheat was initially a short day–long day plant and that strong selection pressures during domestication and breeding resulted in the modification of this dual regulation.[3]

Many years research on critical trait in the adaptation of temperate grasses to cold winters revealed the fact that a long exposures to low temperatures (vernalization) accelerate flowering. The vernalization requirement in temperate grasses is mainly controlled by allelic variation at the vernalization genes VRN1 and VRN2 [18, 19, 6, 17]
In this study different cultivars of winter wheat were studied. The yield of poly(A)RNA correlated clearly with the light intensity. Seedlings raised at 4^0C gave various yield of poly (A) RNA depending on the level of cold-steadiness of each cultivars.(table.1) It was the objective of this investigation to study the variable yields of poly (A)++ RNA (second circle) from poly (A)+ RNA (first circle) that tightly depend on the plants' physiological state which is subjected to the influence of temperature and light stress condition.

The findings of the effect of light on winter wheat (as shown in table 1) indicate that, wheat cultivars distinguish themselves in terms of rate of their reaction to light (short and long day conditions). [15] All spring and winter cultivars responded differently to light. Variety Krasnodarskaya-39, Bezostaya-1

revealed a very low yield of poly A ++ RNA, while spring Drujna B is noted for its high yield of RNA, comparatively. This finding agreed with the photo-physiologist's empirical observations on the contrast reaction of winter and spring wheat cultivars. [7] 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 and this is 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 the period of ontogenesis initiation [5].

TABLE I
THE EFFECT OF LIGHT ON POLY A++ MRNA YIELD FROM WINTER AND SPRING WHEAT AT 20 0^C
Poly A++ mRNA, % for 5 and 18 hours

Variety		Darkness 18 hours	light 5 hours	Difference
Krasnodarskay 39	(winter)	75+-0.2	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

\+ = first circle of affinity chromatography for determining mRNA half life period of decay

++ = second circle of affinity chromatography for determining mRNA half life period of decay.

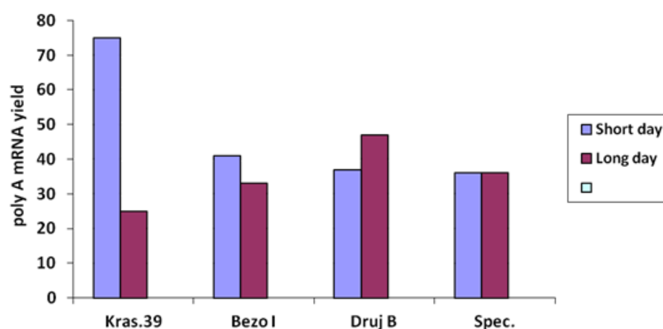

Fig. 1 The effect of light on Poly A mRNA from winter and spring wheat seedlings

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.[14]

The analysis of mRNA from the seedlings under light and other stress condition 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 proportional to plant growing conditions.

IV. CONCLUSION

The method of molecular genetics helps in reducing the period taken by conventional plant breeding 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 with high productivity, to less than one-third of normal duration. This research finding revealed the fact that Wheat 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.

REFERENCES

[1] Anne, S., Michael B. and Don W. (1989). Differential expression within a family of novel wound induced genes in potato: Molecular genetics, 219, 200-203

[2] Arbuzov, V.A. (1980). Osobennosti Metabolisma mRNA v opukholevih kletkah. Uspeh biohimiya 21, M. Nauk, , 79-80

[3] Dubcovsky, J., Chen, C. and Yan, L. 2005. Molecular characterization of the allelic variation at the VRN-H2 vernalization locus in barley. Mol. Breeding 15: 395–407 http://dx.doi.org/10.1007/s11032-005-0084-6

[4] Dolars, M., Luis L. and Aviln R. (1990). Expression of genes for cell protein in dividing and wounded tissue od Zea mays. Planta 180, 524-529. http://dx.doi.org/10.1007/BF02411450

[5] Gayle, L., Ferene, M., and Hamna C. (1989). Light regulated and organospecific expression of wheat cab gene in transgenic tobacco. Nature, V. 318, P.22-27

[6] Jensen, L.B., Andersen, J.R., Frei, U., Xing, Y.Z., Taylor, C., Holm, P.B. and Lubberstedt, T.L. 2005. QTL mapping of vernalization response in perennial ryegrass (Lolium perenne

[7] Kakoto H., Toshiaki M. and Takashi A. (1989). Regulatory role of Ca+ on the level of aamylase mRNA in rice seed. Plant physiology. Biochemistry, .27, 37, 349-354

[8] Khozhin, A.J., Doschanov, M.A. and Akachanov, A.U. (1976). Informosomes as a stored form of mRNA in wheat embryo. FEEBS Lett.. V.66, P.124-126 http://dx.doi.org/10.1016/0014-5793(76)80600-X

[9] Lanks, K.W. (1988). Modulator of the eukaryotic heat shock response. Exp. Cell. 185. 1-10

[10] Matthias, W. (1991). Hentze determinants and regulation of cytoplasmic mRNA stability in eukaryotic cells. Bioch. et Biophys., 1090, 281-292

[11] Mertbasov, M.P. (1990). Regulation of gene expression. Phys,biochem. Plant, 103, 264- 269

[12] Mullet, J., Klein, R.K. (1983). transcription and RNA stability are important determinants of higher plant chloroplast RNA level. EMBO journal, 6, 6, 1571-1579

[13] Poulson, R. (1973). Isolation, purification and fractionalization of RNA In the Ribonucleic acid. Berlin, 243-261

[14] Sachs, M.M. Hot, A.D.(1986). Alteration of gene expression during environmental stress in plant. Annu. Rev. plant Physiol., 37, 363-385
Salandra, J.J. Subjeet, J.(1983)/. Effect of glucose on protein synthesis and thermosensibity in chinese chamster ovary cell. J. biochem., 258, 20, 12091-12093

[15] Stout, E., Parenti, R. and Rusty, J.M. (1967). An increase in RNA polymerase activity after illumination of dark-grown maize seedling. Biochem/biophys. Res. Comm., 29, 3, 322-330 http://dx.doi.org/10.1016/0006-291X(67)90456-1

[16] hompson, D.M. and Meager, R.B.(1990). Transcription and posttranscriptional processes regulate expression of RNA encoding the small subunit of ribulose 1,5-biphosphate carboxilase in petunia and in soybean. Nuc. Acid, res., 18, 12, 3621-3629

[17] [17] VonZitzewitz, J., Szuˆ cs, P., Dubcovsky, J., Yan, L., Francia, E., Pecchioni, N., Casas, A., Chen, T.H.H., Hayes, P.M.and Skinner, J.S., 2005. Molecular and structural characterization of barley vernalization genes. Pl. Mol. Biol. 59: 449–467.

[18] Yan, L., Loukoianov, A., Blechl, A., Tranquilli, G., Ramakrishna, W., SanMiguel, P., Bennetzen, J.L., Echenique, V. and Dubcovsky, J. 2004b. The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. Science 303: 1640–1644. http://dx.doi.org/10.1126/science.1094305

[19] Yan, L., Loukoianov, A., Tranquilli, G., Helguera, M., Fahima, T. and Dubcovsky, J. 2003. Positional cloning of wheat vernalization gene VRN1. Proc. Natl. Acad. Sci. USA 100: 6263–6268. 480.