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CHARACTERISTICS OF THE *r*RNA SYNTHESIS TAKING PLACE AT LOW TEMPERATURES IN WHEAT CULTIVARS WITH VARYING DEGREES OF FROST HARDINESS

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Key Word Index—*Triticum aestivum*; Gramineae; winter and spring wheat; *r*RNA processing; *r*RNA precursors; critical temperature (LT_{50}); polyacrylamide gel electrophoresis; frost hardiness; hardening period.

Abstract—The characteristics and intensity of *r*RNA synthesis occurring at temperatures around freezing point were examined in connection with frost hardiness in wheat cultivars. The intensity of *r*RNA synthesis taking place near freezing point showed a close positive correlation with the critical temperature (LT_{50}) characteristic of the frost resistance of the varieties. The quantity of high MW precursors to *r*RNA appears to be heterogeneous in hardy and non hardy varieties at low temperatures and point primarily to an inhibition in the maturation processes of ribosomes in the final nuclease processing step in non-frosthardy varieties.

INTRODUCTION

The relation of water-soluble proteins to the development and maintenance of cold hardiness has been examined fairly thoroughly but relatively little specific information is available on the role played by the nucleic acids in frost hardiness and in the hardening period. The sparse examinations carried out in this field [1,2] only cover the determination of quantitative relationships. It was reported that an increase in RNA content precedes an increase in the cold hardiness of black locust [3]. The RNA and DNA contents of alfalfa plants were positively correlated with cold hardiness and the application of purines and pyrimidines make a genetically nonhardy variety very similar to a hardy variety [1]. Thus, there is a great need for the detailed analysis of the characteristics of nucleic acid synthesis in connection with frost hardiness. The characteristics and intensity of *r*RNA synthesis occurring at temperatures around freezing point were determined in wheat cultivars with varying degrees of frost hardiness.

RESULTS AND DISCUSSION

The examinations were carried out on 12 wheat varieties and the intensity of *r*RNA synthesis taking

Table 1. Incorporation of ^{32}Pi into 1.27×10^6 and 0.67×10^6 dalton *r*RNA at 3° in wheat cultivars with varying degrees of frost hardiness

Cultivars	Approximate specific activity†	Ratio of specific activity‡	LT_{50}
Cheyenne	13.0	1.92	−14.4
Bezostaya 1	10.8	2.00	−12.2
Mironovskaya 808	8.4	1.96	−12.0
Capelle	6.2	2.81	−11.0
Libellula	3.6	2.64	−11.0
Bánkuti 1201	5.8	2.08	−10.6
Berse	4.9	2.88	−9.0
Penjamo 62	2.0	3.37	−4.6
Lutescens 62	2.2	3.08	−4.4
Chinese Spring	2.5	3.00	−3.0
Siete Cerros	1.9	3.26	−2.8

†Cpm $\times 10^{-3}$/100 μg $1.27 + 0.67 \times 10^6$ MW *r*RNAs. ‡$1.27 + 1.37 \times 10^6$ MW *r*RNA/0.67×10^6 MW *r*RNA. Intact seedlings were labelled with ^{32}Pi for 24 hr. Nucleic acids were prepared and separated electrophoretically as described in Experimental. LT_{50} = critical temperature characteristic of the actual frost hardiness of the cultivars as described in Experimental.

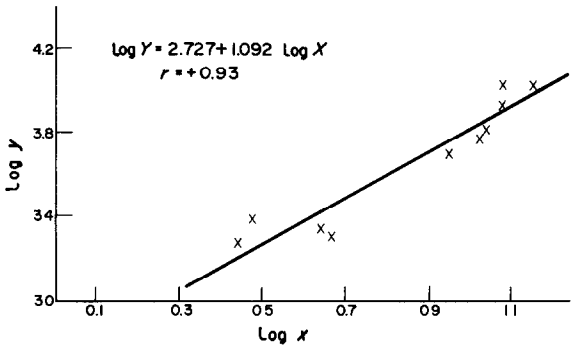

Fig. 1. The power function ($Y = aX^b$) existing between the critical temperature (x) and the degree of high MW cytoplasmic rRNA synthesis occurring at low temperature (y) converted to a linear regressive correlation on the basis of data presented in columns 1 and 3 of Table 1. The equation at the top of the figure expresses the direct equation derived by taking the logarithm of the power function. The $r = +0.93$ value in the binary correlation analysis is the value of the correlation coefficient, which points in this case to a close relation between the two variables. In other words, as the frost resistance of the varieties increases the degree of high MW rRNA synthesis occurring at low temperature also increases.

place in seedlings at low temperatures was examined at 3° using the incorporation of ^{32}Pi into rRNA.

In frost hardy varieties there was intensive rRNA synthesis at temperatures near freezing point (Table 1) and this showed a close positive correlation with the critical temperature (LT_{50}) characteristic of the frost resistance of the varieties (Fig. 1).

Further, the characteristics of rRNA synthesis taking place at low temperatures were investigated. For wheat varieties with good resistance two peaks usually appeared in the histograms and these coincided with the A absorbance peaks of the cytoplasmic rRNA present in the cells. By contrast, for spring varieties and for those with poor frost resistance, nother activity peak appeared prior to that of heavy rRNA (Fig. 2). This was found to have a MW of 1.37×10^6 and corresponded to an rRNA precursor [4].

Labelling experiments over a shorter period show (Fig. 3) that a high MW precursor to rRNA can be found both in hardy and non-hardy varieties at low temperatures, but they differ in quantity. In the frost hardy winter wheat Bánkuti 1201 the absorbance scan shows the two ribosomal RNA components with MW's of 1.27 and 0.68×10^6. The radioactivity scan of the RNA labelled with ^{32}Pi shows the components which were described above and two pre-rRNA components with MW's of 2.9 and 2.42×10^6, which may be leaf pre-rRNA [5]. In the non-hardy Siete Cerros spring wheat the leaf pre-rRNA with a MW of 2.9×10^6 is also well detectable. The 2.42×10^6 rRNA precursor, which is found in the hardy varieties is lacking in non-hardy spring wheat. But a component with a MW of 1.37×10^6 usually appears, which may be the immediate precursor of the mature ribosomal RNA [5]. The chloIoplast RNA does not become labelled at this stage.

The increase in the quantity of the 1.37×10^6 rRNA precursor points primarily to an inhibition in the maturation processes of the ribosimes [4], especially in the final nuclease processing step. In non-frost hardy

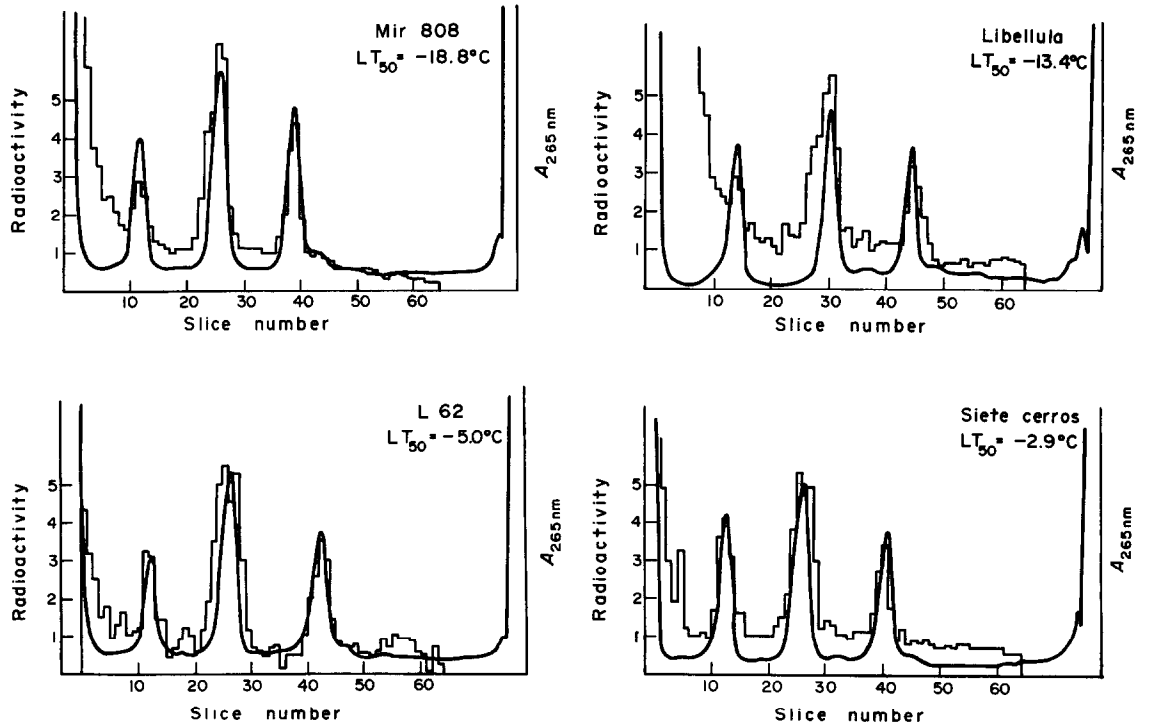

Fig. 2. rRNA synthesis at 3° in wheat cultivars with varying degrees of frost hardiness. Wheat seedlings were labelled with ^{32}Pi for 48 hr at 3° and the nucleic acid was fractionated by electrophoresis on 2.4% polyacrylamide gel, as described in Experimental. Continous line: A_{265}, histogram: ^{32}Pi, LT_{50}: critical temperature characteristic of the actual frost hardiness of the cultivars determined as described in Experimental.

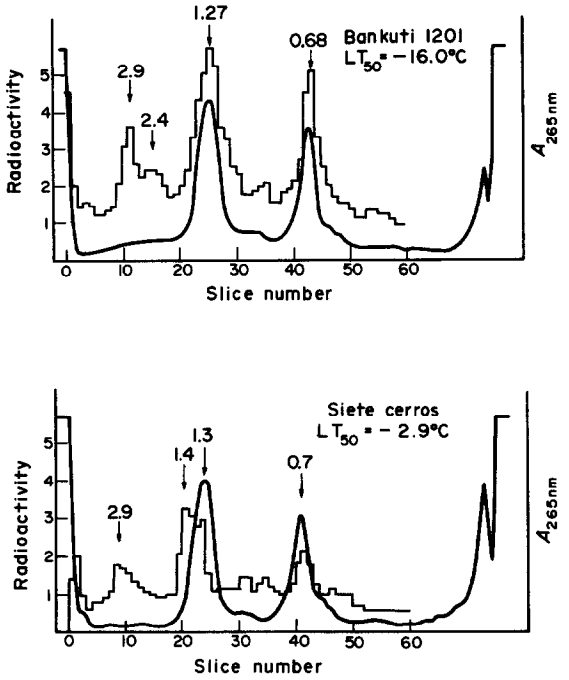

Fig. 3. Pre-RNA from the frost hardy winter wheat Bánkuti 1201 compared to that from the non-hardy spring wheat Siete Cerros. Intact seedlings were labelled with ^{32}Pi for 24 hr at 3°. The nucleic acid was extracted and DNA digested, as described in Experimental. Electrophoresis was carried out on 2.4% polyacrylamide gel and 40 μg RNA was applied to each gel. The size of the components indicated on the electrophoretograms was determined as described in Experimental. Continuous line: A_{265}, histogram: ^{32}Pi, LT_{50}: critical temperature characteristic of the actual frost hardiness of the cultivars determined as described in Experimental.

varieties the increase in the *r*RNA precursor level indicates that the disturbances caused by low temperatures do not act primarily through the ^{32}Pi uptake of the seedings but rather by inhibiting the maturation processes of the ribosomes. The simplest interpretation of the results is to assume that frost hardy wheat varieties are generally characterised by a lack of disturbance of *r*RNA synthesis and of the maturation processes of the ribosomes at low temperatures. This undisturbed processing could be realized in a higher synthesis of the heavy and light rRNA subunits. The relatively harmonious nature of this metabolism is destroyed as the frost hardiness of the varieties decreases and this then reaches expression in the lower quantity of *r*RNA synthesized.

EXPERIMENTAL

Growth and labelling of wheat (Ttriticum aestivum *L.*) *seedlings.* The expts were carried out on the winter wheat cultivars: Cheyenne, Mironovskaya 808, Bezostaya 1, Bánkuti 1201, Capelle, Berse and Libellula, and on the spring wheat cultivars: Lutescens 62, Penjamo 62, Siete Cerros and Chinese spring. The seeds were sterilized by washing in Br–H_2O and were germinated under sterile conditions on 1% agar containing 2% sucrose at room temp. for 48 hr in darkness. The intact seedlings were incubated with their root tips resting in 5 ml 1000-fold diluted Knop-soln per 50 seedlings, containing 500 μCi ^{32}Pi for 24 or 48 hr at 3°. RNA was extracted from the isolated seedlings.

The degree of frost hardness was characterised by the critical temperature (LT_{50}). In order to determine this value the seedlings were subjected to temperatures of −5, −10, −15, −18, −20 and −25° for 48 hr and the percentage survival was examined after 14 days cultivation at 15°. The value at which 50% of the seedlings survived was taken as the critical temperature (LT_{50}).

RNA assays. Total nucleic acid was prepared from wheat seedlings using the modified phenol procedure [7]. The purified nucleic acid was fractionated by electrophoresis on 2.4% polyacrylamide gel at 50 V (6mA/tube) for 3.5 hr [6]. After electrophoresis the gels were washed for 30 min in H_2O and scanned at 265 nm. Distribution of radioactivity was determined by freezing the gels in dry ice to the scanned length prior to cutting 1 mm slices, which were dried on filter paper (Whatman No. 1). Slices were counted in toluene with 0.5% PPO and 0.3% dimethyl POPOP. The size of the components on the gels was determined by co-electrophoresis with *r*RNAs from *Escherichia coli* (1.1×10^6 and 0.56×10^6) [6]. The quantities of *r*RNAs were calculated by measuring the area under the electrophoretogram curves. The 1.1×10^6 dalton *r*RNA extracted from *E. coli* was taken as the *r*RNA standard. The radioactivity of the rRNAs was obtained from the electrophoresis scans, taking only counts above the heterogeneous background [4].

Digestion of DNA. 20 μg/ml of electrophoretically purified DNAse and 40 μl/ml of M $MgCl_2$ were added to the RNA soln (100 μg/ml in 0.05 M Tris–HCl, pH 7.2) and incubated at 20° for 20 min. After incubation the soln was deproteinized by shaking for 15 min with an equal vol of a 24:1 mixture of $CHCl_3$ and 3-methylbutan-1-ol. The top aq layer was removed after centrifugation at 2500 *g* for 15 min and further deproteinized as described above. The DNA-free RNA was precipitated by the addition of two vol of EtOH.

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