GENE EXPRESSION OF δ1- AND δ3-CYCLINS IN ROOT MERISTEM CELLS OF PISUM SATIVUM L. UNDER CLINOROTATION.

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ABSTRACT

The data on the influence of altered gravity on plant cell proliferation are ambiguity [1, 3], although a delay of the cell cycle duration in comparison with the control was observed in the most cases. Evidently, the principal regulatory processes in a cell cycle occur in the G1-phase. Cyclins are important regulators in different phases of a cell cycle and have a high homology in plant and mammalian cells. δ-cyclins are specific for plants and control the presynthetic phase events and beginning of S-phase. Therefore, we firstly performed the study the influence of slow horizontal clinorotation on δ3- and δ1-cyclins genes expression in early events of pea root development.

1. INTRODUCTION

Cyclins are the important regulators of a cell cycle. There are several types of plant δ-cyclins (in particular δ1 and δ3) [2], which like to mammalian D-cyclins and have similar functions. Cyclin δ1 is principally expressed during G1, whereas cyclin δ3 is expressed later or over a longer period extending into S phase. As analogy of mammalian D-type cyclins, δ-type may act primarily as growth factor sensors, feeding information on the external environment of the cell into the cell cycle control system. Moreover, δ-type cyclins involved in plant cell proliferation and differentiation control in G1 [2].

The data on the influence of altered gravity on plant cell proliferation are ambiguity [1, 3], although a delay of the cell cycle duration in comparison with the control was observed in the most cases. That is explained by the extension of the cycle presynthetic phase [3]. Previous experiments showed also a delay of seeds germination under clinorotation [7]. As one of the causes in this delay can be the changes in cyclin expression. Therefore, a goal of our work was to determine δ1- and δ3-cyclins gene expression under clinorotation.

2. MATERIALS AND METHODS

Seeds of *Pisum sativum L.* cv. "Intensive" were germinated at 22±1°C in dark in the stationary conditions and under slow clinorotation (2 rpm). After 32 hours of seed germination, roots of 1,5 mm length were cut off and fixed in FAA (3,7% formaldehyde; 5% acetic acid, 50% ethanol) during 15 minutes. Then the apices were dehydrated in ethanol ascending concentration and embedded in paraffin. Paraffin-embedded roots sections (7 μm of thickness) were made on a sledge microtome REICHERT. Expression of cyclin genes and its localization were determined by the *in situ* hybridization method that has been adapted for pea. [4]. After hybridizitation and washing, the material was detected in NBT/BCIP (Nitro blue tetrazolium chloride/5-Bromo-4-chloro-3-indolyl phosphate, toluidine salt). Sections were hybridized with DIG-labelled cDNAs probes from *Arabidopsis* [2].

3. RESULTS AND DISSCUTION

An analysis of δ1-cyclin gene expression in pea root meristematic cells showed that transcripts of this gene were absent in the control (Fig.1, a). But under clinorotation, some accumulation of δ1-cyclin gene transcripts was observed (Fig.1, b). Transcripts of δ3-cyclin gene in the pea root meristematic cells in the control were not also revealed (Fig.2, a). It has been shown that δ3-cyclin gene expressed under clinorotation (Fig. 2, b). The δ3-cyclin transcript accumulation in the nuclei was well seen (Fig.1, b).

The obtained results on the absent of δ-cyclin transcripts in the control are suggested to testify the cyclin subunits degradation. Destruction or dissociation of cyclins is important for cell moving from one cycle phase to the next [6]. The increased level of δ-cyclin transcripts accumulation under clinorotation indicates the prolongation of the presynthetic phase in these conditions.

It is possible to assume that the presence of δ1- and δ3-cyclin gene transcripts in nuclei under clinorotation displays a delay of the G1-phase transition to the S-phase. These data can explain a delay of pea seed germination and proliferative activity under clinorotation that has been reported in a previous work [7]. Thus, we have evidenced the an influence of slow horizontal clinorotation on δ1- and δ3-cyclin gene expression in early events of pea root development. Our results agree with with the ideas about the role of

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δ-cyclins in the G1-phase events and their importance in a cell cycle in a whole [2]. It is known, that a mitotic index and a length of roots in altered gravity were lower compared to the control [3, 5]. The study of the DNA content in the nuclei of lentil seedlings cells showed that a cell cycle was modified in microgravity [1]. The prolongation of δ-cyclin gene expression time is evidence of really inluence of slow horizontal clinorotation on the presynthetic phase processes. These results suggest that accumulation of δ1- and δ3-cyclin gene transcripts in nuclei under clinorotation prevents cell progression to the S phase and may cause a delay of seed germination and following processes in a cell cycle.

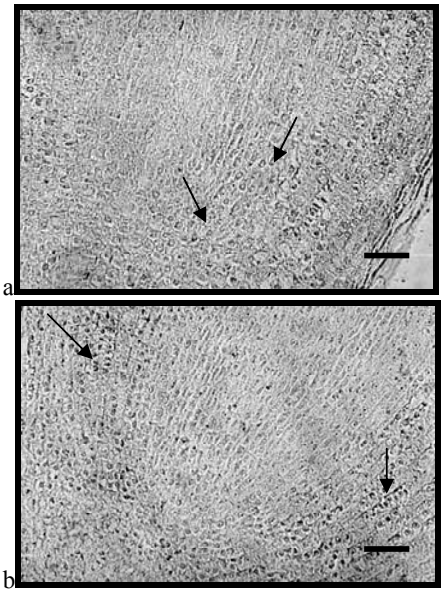
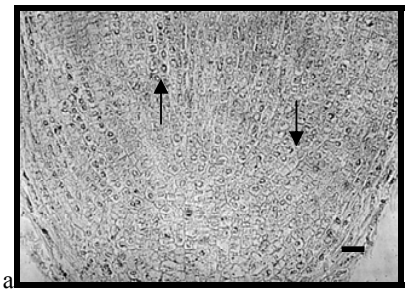


Fig.1. δ1-cyclin gene expression in pea root meristematic cells in the stationary conditions (a) and under clinorotation (b) by method *in situ* hybridization. Bar: 10μm. Arrows show the position of a cell labelled with δ1-cyclin



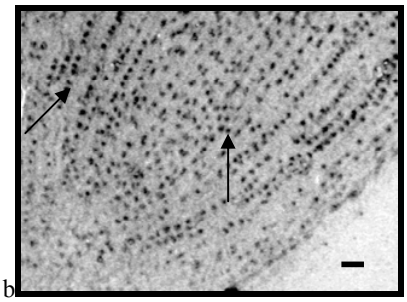


Fig.2. δ3-cyclin gene expression in pea root meristematic cells in the stationary conditions (a) and under clinorotation (b) by method *in situ* hybridization. Bar: 10μm. Arrows show the position of a cell labelled with δ3-cyclin.

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4. REFERENCES

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