The Effect of Galactose-Specific Mulberry Lectin (*Morus alba* L.) on Growth and Elongation of Wheat Coleoptiles

Eka Khurtsidze, and Mariam Gaidamashvili

***Abstract*—**The activity of endogenous auxins, growth inhibitors and lectin-like proteins were investigated in leaves and inflorescent of Georgian mulberry (*Morus alba var. Gruzia* L.) at different stages of ontogenesis. Highest activities (300-225%) of growth stimulating compounds were exposed in leaves and inflorescent during the growth phase. The amount of active substances were reduced considerably (180-150%) during florescence and fruitage. Positive correlation between the lectin-like protein quantity and stimulating activity of endogenous hormones was shown. Purified Gal-specific lectin of mulberry seeds (MNL) initiated the promotion of rapid elongation of wheat coleoptiles when applied the concentration of 50 μg/ml, which has 2.5 times higher affection in compare to that of endogenous auxin. The putative involvement of mulberry Gal-specific lectin in the processes of plant growth and development has been proposed. The potential use of endogenous plant lectins as the non-toxic growth-stimulating agents for agricultural application is considered.

***Keywords*—**Growth stimulation, lectins, *Morus Alba*, phytohormones.

I. Introduction

INFLUENCE of phytohormones on plant life cycle is one of the main interests of modern agriculture to control physiological and morphogenetic processes in plants. In addition to recognized plant phytohormones, recently described class of growth regulators include small signaling molecules of polypeptide nature. Plant phytohaemagglutinins or lectins are well documented to participate in multiple physiological activities based on selective binding to the carbohydrate structures. In this view, consideration of plant lectins as growth regulators tempts much speculation [1].

Lectins are found in a wide variety of living organisms. They interact with glycoconjugates and polysaccharides by binding to the specific carbohydrate residues [2]. Plant lectins have been reported to play significant role in various processes such as growth and development, differentiation, plant protection [3]. In previous works we proposed the possible physiological role of mulberry lectins in growth and development processes, based on the dynamic changes of lectin content and activity in different organs [4-5]. The regulatory potential of plant lectins are suggested in the works where was shown the expression of some lectin genes in meristemic tissues thus, proposing the direct implication of plant lectins in cell division and elongation processes [6]. In this respect plant lectins reveal the similar effects as do plant growth regulators, which play central role in growth and developmental processes in whole plants.

In the present paper we investigated the correlation between the activities of endogenous auxins and content of the lectins in growing leaves and florescent of mulberry at the different stages of ontogenesis from the view of their influence on plant growth and development.

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II. Materials And Methods

A. Materials

The leaves and florescent of mulberry (*Morus alba var. Gruzia* L.) were harvested in May-June. Plant tissues were freeze-dried and stored at -175^0C until use.

B. Methods

Endogenous auxins and growth inhibitors were separated by the solvent mixture of butyl/wine acid/water (40:12:28). Activity of auxins was studied according method of Keffel [7]. Mulberry leaves and florescent (5g each) were homogenized with 0.9% NaCl, 0.04 M K^+-phosphate buffer (pH 7.4) at the ratio of 1:10 (w/v), and filtered using Whatman CF/C filter. The filtrate was centrifuged at 5000 ×g for 15 minutes. Partial purification of the proteins was performed by successive precipitation in ammonium sulfate under 0-90% saturation. Suspension was centrifuged at 20 000 ×g for 20 minutes at 4^0C. Precipitate was dissolved in a K^+-phosphate buffer (pH 7.4) at the ratio of 1:10 (w/v) and centrifuged at 5000 ×g for 15 minutes. The supernatant was filtered through Whatman CF/C paper and millipore filters (0.22μ -0.45μ). Dialysis was performed by chromatography on a Sephadex G-10 column (50 × 2.7 cm) equilibrated with

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0.9% NaCl, 0.02 M K^+-phosphate buffer (pH 7.4). Gel-filtration was performed on Toyopearl HW-55 column (1.5×45 cm) equilibrated with the same buffer. Affinity purification of lectins was performed using chromatography in agarose-GalNAc column (6×0.8cm).

Purity of MAL was analyzed by PAGE in a 5-25% gradient polyacrylamide gel by the method of Davis et al [8]. Haemagglutination activity was detected by using of trypsin-treated 2% rabbit erythrocytes [9]. Specific agglutinating activity (SA) of MAL was calculated as the maximum dilution of 1 mg of protein, causing agglutination of rabbit erythrocytes. Protein concentration was estimated according Lowry method [10]. Growth stimulatory activity of the endogenous auxins and MAL was tested by using clippings of wheat coleoptile [11]. Elongation of the wheat coleoptiles in the presence of pure water was estimated as 100% of growth.

III. Results And Discussion

Activity of endogenous auxins in the leaves and inflorescence at the different stages of apical bud formation, pre-florescence, florescence and fruitage is shown in Fig.1. The activity of auxins changed significantly throughout the bud development. Activity has risen exponentially according to the bud development and achieves 250% to 300% during the first week of appearance of inflorescence and leaves respectively. At the stages of mature florescence and fruitage the activity of auxins has been reduced to 150% respectively. The highest activity of auxins was detected in apical leaves at the 7-th day after apical bud formation. After 33 days, along with the accomplishment of growth, leaves and inflorescence have been exposed to growth inhibitors and activity level was down to 95%.

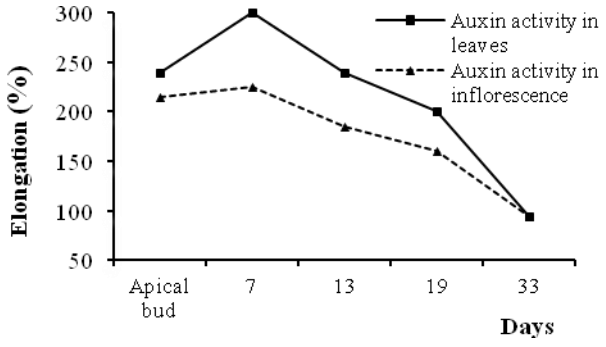

Fig.1 The activity of endogenous auxins in the apical buds of the leaves and inflorescence of *M.alba*.

The changes of the specific activity of *M. alba* lectins in leaves and inflorescence is shown in Fig.2. The specific activity of the lectins have been increased during the first week of apical bud development and reached maximum level of 256 000 ml/mg at 7-th day of formation in apical leaves. After 14-th day activity has dropped off in apical leaves. In opposite, inflorescence apical bud showed the high level of lectin specific activity (215 578 ml/mg) at the initial stages of development and following decrease to 8 µg/ml throughout the further development. By the end of growth lectin activity has been dramatically reduced. In both cases, the positive correlation between the changes in the activities of lectins and endogenous auxins was exposed. The obvious positive correlation was detected in the leave buds where activity of lectins and endogenous growth stimulators were high at the initial stages of bud development.

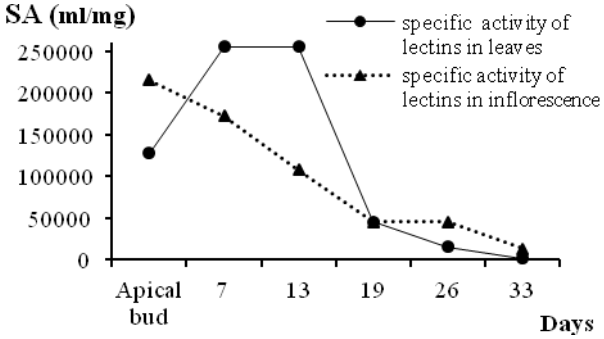

Fig.2 The changes of the specific activity (SA) of MAL in the apical buds of leaves and inflorescence of *M.alba.*

Consideration of the mulberry lectins as primary growth stimulators is very complex, however, the shown correlation between activities of lectins and endogenous growth stimulators tempt to speculate the regulatory role of endogenous lectins during the plant organogenesis.

In the further experiments influence of purified galactose specific mulberry lectin (MAL) on growth elongation was evaluated on the wheat coleoptiles. The results are shown in the Fig. 3. MAL promotes the elongation of wheat coleoptiles when applied at the concentration of 50 µg/ml. The effect is similar to that of auxin which promotes growth by 250% which is 2.5 fold higher in comparison with the control. Obviously, high accumulation of lectins in the actively growing plant organs may be connected to the activity of growth stimulatory hormones like auxins.

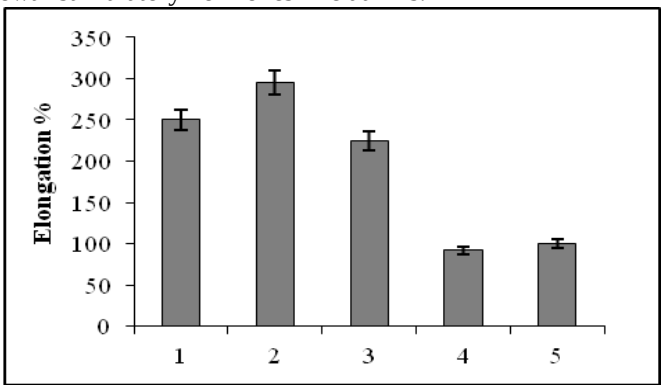

Fig.3 The growth stimulatory effect of galactose-specific mulberry lectin (MAL) on wheat coleoptiles 1) MAL (50 µg/ml); 2) endogenous auxin of mulberry leaves; 3) endogenous auxin of mulberry inflorescence; 4) MAL inhibited by 12.5mM D-galactose; 5) H_2O control. (ANOVA, n=20, $p<0.05$; Student's test).

Haemagglutination activity of MAL was inhibited by monosaccharide galactose at the concentration of 12.5 mM; interestingly, incubation with galactose negatively affected the elongation ability of MAL as well. Supposedly, sugar-binding moieties of MAL might be responsible for the ability to promote tissue elongation.

The results obtained demonstrate that endogenous lectins of mulberry and especially, the galactose-specific mulberry lectin (MAL) has presumably been involved of in the processes of plant growth and development. Apparently, this data may have importance concerning the realizing the physiological role of plant lectins and reveal the possible ways of growth processes in plants. The ability of endogenous carbohydrate-binding proteins to initiate growth and elongation of cells in physiologically active zones of plants may be the promising tool as the potential non-toxic growth-stimulating agents for agricultural application.

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