ISSN 1021-4437, Russian Journal of Plant Physiology, 2015, Vol. 62, No. 2, pp. 171–178. © Pleiades Publishing, Ltd., 2015.
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RESEARCH PAPERS

Character of Oligosaccharin OS-RG Participation in the IAA-Induced Formation of Adventitious Roots

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Received July 2, 2014

Abstract—The interaction between auxin and oligosaccharin OS-RG of natural origin (DP ~10) during the process of adventitious root formation was studied. Oligosaccharin isolated from pea seedlings increased by 20–25% the number of roots induced by IAA on both the segments of buckwheat (*Fagopyrum esculentum* Moench) hypocotyls and explants produced from the leaves of transgenic (*rolB-GUS*) tobacco *Nicotiana tabacum* L., cv Petit Havana. The highest effect was obtained after short-term treatment of explants with oligosaccharin before hormone adding. The optimal time of pretreatment depended on the used model system and was from 1–2 to 5–24 h for buckwheat hypocotyl segments and explants from tobacco leaves, respectively. Treatment with OS-RG after IAA did not affect the number of roots induced by the hormone. By using the explants from the leaves of transgenic tobacco plants harboring the reporter *GUS* gene under the control of the auxin-inducible promoter of the *rolB* gene permitted to reveal the explant response to the hormone at the early stages of root formation. The dynamics of GUS activity after IAA addition was characterized by the presence of two peaks. The histological analysis showed that the first peak coincided with the formation of 4-5-layer primordia, whereas the second peak – with the emergence of essentially developed roots. Pretreatment of explants from tobacco leaves with OS-RG activated IAA-induced GUS-activity and accelerated the response, i.e., the shift of the first peak to the beginning of culture without change in the position of the second peak. Thus, obtained data indicate that OS-RG action precedes that of the hormone at early stages of rhizogenesis. Possible mechanisms of interaction between IAA and oligosaccharin in the process of root formation are discussed.

Keywords: Nicotiana tabacum, Fagopyrum esculentum, oligosaccharins, IAA, transgenic plants, explants, rhizogenesis, GUS activity

DOI: 10.1134/S1021443715020120

INTRODUCTION

The interest for the studying the mechanisms of rhizogenesis follows not only from the importance of the root system in the maintenance of plant growth and nutrition, but also because the formation of lateral roots is a convenient model for studying the factors that determine a such characteristic feature of plant growth, as developmental plasticity [1]. In dependence on the availability of water and mineral nutrition, plants optimize the root architecture mainly due to the initiation and growth of lateral roots of different orders. At present, the stages of lateral root development are established including initial cell division, primordium development, the formation of active meristem, the appearance and growth of the root [2]; biochemical, physiological, and histological events directly related to this process are revealed [1–3].

Among the major constraints for understanding the mechanism of root formation is the lack of information about the inducers of rhizogenesis. The leading role in this process is assigned to auxin: the key role of its gradient is established [1, 4]; the components of its transport are identified [3]; the great number of IAA-inducible genes are characterized [5]; it is shown than the changes in the rate of IAA synthesis in mutants affect the amount of roots [4]. Nevertheless, the search for other endogenous factors involved in the root initiation process continues. Most effectors currently revealed function already after triggering rhizogenesis by the hormone [6, 7]. In addition, when auxin is considered as a key stimulus of the earliest events of lateral root initiation, including priming and getting by some cells the status of founder cells [1–3], most authors do not take into account the events taking place before the action of the hormone, and do not consider the mechanisms and signals that are involved in the selection of definite cells and the formation of their competence to auxin.

Starting from the 80s, the evidence appeared about oligosaccharins, fragments of the cell wall oligosaccharides manifesting biological activity [8]. A great number of oligosaccharins differing in their composi-

tion, structure, and physiological action have been identified [9]. Some of them participate in various morphogenetic reactions, which was demonstrated for some organisms [10–12]. However, the effects of oligosaccharins were mainly studied with compounds obtained by the chemical synthesis or after the hydrolysis of cell wall polymers. In our laboratory the methodology of biologically active oligosaccharin obtaining from plant homogenates without preliminary hydrolysis of cell wall polymers was developed [13], which confirms the in vivo occurrence of these regulatory molecules. One of isolated fractions of oligosaccharides (with the degree of polymerization of ~10) manifested a capability of stimulation of IAA-inducible rhizogenesis on the explants from buckwheat hypocotyls [14]. Although at present the signaling role of oligosaccharins is already commonly accepted [9, 15], little is known about the mechanisms of their actions in mediating IAA-induced processes in the plant.

For the investigation of the process of root formation, model systems are often used (segments of stems or leaves, thin-layers explants, etc.); in these cases, under certain conditions, a change in the developmental program of some cells occurs, which leads to the emergence of so-called adventitious roots [16]. The formation of adventitious roots in these model systems has some common features with the formation of lateral roots on the main root because they both develop post-emryonically from the cells, which must dedifferentiate to give rise to the new organ. The similarity was observed also at the stages of root development, which was confirmed in morphological observations [1, 17] and in the studies of some root-specific gene expression [18]. Therefore, the formation of adventitious roots produced on in vitro cultured plant tissues is a suitable system to study rhizogenesis under controlled conditions and under the influence of diverse factors.

Despite the significant progress made in the study of root formation [1–5, 19], yet there is no clear picture of how a multistage process of development of the root system is controlled during plant ontogeny and what mechanisms coordinate the complex responses to many external and internal signals. Further studies are required for decoding these mechanisms that will be more correctly describe the process of regulation of the earliest stages, as well as to understand the role of oligosaccharins as the regulatory molecules of the new class. Therefore, the objective of this work was to study the nature of the involvement of natural oligosaccharin stimulating rhizogenesis in the process of the IAA-induced formation of adventitious roots.

MATERIALS AND METHODS

Experiments were carried out on the segments of buckwheat (*Fagopyrum esculentum* Moench) hypocotyls and also explants from leaves and hypocotyls of transgenic plants of tobacco (*Nicotiana tabacum* L., cv Petit Havana) harboring the construct comprising the *rolB* promoter from *Agrobacterium rhizogenes* and the reporter *GUS* gene (encoding β-glucuronidase) from *Escherichia coli*. Plants were grown from seeds obtained in the Department of Biological Investigations "La Sapienza" University of Rome [11].

To obtain segments of buckwheat hypocotyls, the seeds were sterilized in 2% sodium hypochlorite for 10 min and then germinated for four days on agar-solidified half-strength MS medium [20]. Segments 1 cm in length were excised from the middle parts of hypocotyls, cut longitudinally into the two parts, which were placed by the cut surface down in Petri dishes with liquid half-strength MS medium and cultured in darkness at 25°C.

To obtain explants from tobacco leaves, plants were grown at 25°C and an irradiance of 20 W/m^2 with a 16-h photoperiod from the seeds preliminarily sterilized in 2% sodium hypochlorite for 8 min. Middle veins were removed from the leaves of top parts of seedlings at the age of 35–40 days, and segments 6 × 6 mm were excised. Explants were placed abaxial side down in Petri dishes on liquid (for determination of GUS activity) or agar-solidified (for root counting) half-strength MS medium. Explants were cultured in darkness at 25°C.

To obtain explants from tobacco hypocotyls, the seeds were germinated on hormone-free agar-solidified half-strength MS medium for 7 days in the temperature controlled cabinet at 25°C. Segments 6–8 mm in length were excised from the upper parts of seedlings, which were further cultured in the liquid half-strength MS medium in darkness at 25°C.

In all cases half-strength MS medium supplemented with 2% sucrose; 0.8% agar was added for it solidification. Plant growing and explant preparation and culture were performed aseptically.

For the assay of glucuronidase activity (GUS activity), 50 mg of explants from tobacco leaves or 5 segments from hypocotyls (~7 mm in length) were ground with a mortar and pestle in the extraction buffer (300 μL) containing 50 mM Na_2HPO_4, 5 mM DTT, 1 mM Na_2EDTA, 0.1% SDS, and 0.1% Triton X-100, pH 7.0. Extracts were centrifuged at 7000 *g* for 3 min. GUS activity was measured in the same buffer in the presence of 100 μM 4-methylumbelliferyl glucuronide. The reaction was started by the addition of 20 μL of the extract, and the amount of released 4-methylumbelliferone (4-MU) was measured during 30 min with intervals of 10 min, using the Perkin Elmer MPF-44B spectrofluorimeter (Perkin Elmer, United States) at the excitation at 455 nm and emission at 365 nm. Enzyme activity was expressed in 4-MU nmol/(mg fr wt min) (for explants from tobacco leaves) or 4-MU nmol/(sample min) (for tobacco hypocotyl segments).

The fraction of active oligosaccharide was isolated from pea (*Pisum sativum* L.) seedlings grown on tap

The number of roots formed on hypocotyl segments of buckwheat and explants from tobacco leaves, depending on the availability of IAA and oligosaccharin OS-RG in culture medium

Time of culture in the first medium, h	Number of roots per explant at different treatments (explant transfer from one to another medium)			
	I IAA → hormone-free	II hormone-free → IAA	III IAA → OS-RG	IV OS-RG → IAA
	a) *Segments of buckwheat hypocotyls*			
0.5	–	11.4 ± 0.9	3.4 ± 0.5	12.8 ± 1.9
1	4.3 ± 0.5	11.9 ± 1.5	3.5 ± 0.5	17.0 ± 2.7
2	3.5 ± 0.8	11.0 ± 1.6	3.3 ± 0.4	17.8 ± 3.3
5	3.0 ± 0.9	8.5 ± 1.6	3.4 ± 0.5	10.3 ± 1.1
24	12.1 ± 1.2	4.3 ± 0.8	12.0 ± 1.4	5.8 ± 1.2
48	12.0 ± 1.4	3.7 ± 0.9	12.0 ± 0.8	5.8 ± 0.8
	b) *Explants from tobacco leaves*			
1	1.3 ± 0.5	17.4 ± 1.3	1.2 ± 0.4	19.2 ± 2.3
2	2.0 ± 0.8	18.3 ± 2.9	1.1 ± 0.4	20.6 ± 2.3
5	1.5 ± 0.6	–	2.0 ± 0.8	22.3 ± 1.8
24	2.0 ± 0.7	19.0 ± 2.0	1.7 ± 0.5	22.0 ± 1.5
48	1.6 ± 0.5	16.9 ± 1.2	1.7 ± 0.5	18.8 ± 1.9
72	5.2 ± 0.8	9.3 ± 1.3	7.0 ± 0.9	–
96	–	8.1 ± 0.8	16.6 ± 1.3	8.9 ± 1.2
120	15.7 ± 1.1	7.0 ± 1.3	–	–
144	16.8 ± 1.8	–	18.7 ± 1.6	3.2 ± 0.8

Roman figures in the columns indicate the treatments corresponded to the scheme presented in Fig. 1. The time of culture in the first medium is indicated in the first column. After transfer to the second medium, explants were cultured in it until the end of the experiment. The number of roots on the segments of buckwheat hypocotyls was counted on the 5th day, and on the explants from tobacco leaves – on the 14th day, starting from the moment of explant placing on the first medium. The numbers of roots at constant culture of the segments of buckwheat hypocotyls on medium with IAA was 12.0 ± 0.9, with OS-RG + IAA – 14.4 ± 1.1, the corresponding values for explants from tobacco leaves were 14.0 ± 1.2 and 17.5 ± 1.3. IAA concentration was 3 μM and that of OS-RG – 5 μg/mL.

water at 25°C at an irradiance of 20 W/m^2 with a 12-h photoperiod. Seedlings were fixed at 100°C for 30 min, dried at 60°C until the constant weight, and powdered. Plant material was washed with organic solvents (chloroform : ethanol, 1 : 2, and 70% ethanol); then endogenous oligosaccharides were extracted with 50 mM KH_2PO_4 (pH 7.0). Further oligosaccharide purification was performed by successive chromatographic steps using different types of chromatography. The methodology of oligosaccharides isolation and purification based on the high hydrophilicity of neutral oligosaccharides and the lack of charges in them was developed in our laboratory. All conditions were chosen to provide intactness of the oligosaccharides during their extraction from plant tissues. The preliminary analysis of the obtained active fragment showed that it consisted mainly of arabinose and galactose [9]. More detail description of the method has been published earlier [13, 14]. The selected oligosaccharide fraction stimulating rhizogenesis was designated as oligosaccharin OS-RG.

The concentrations of IAA and oligosaccharin used for the highest effects were chosen in preliminary experiments. In all experiments with the buckwheat hypocotyl segments and explants from tobacco leaves, we used 3 μM IAA and 5 μg/mL of oligosaccharin. For segments of tobacco hypocotyls 0.5 μM IAA was used. The numbers of roots (not less than 2 mm in length) were counted visually on the 5th and 14th day of culture of buckwheat hypocotyl segments and explants from tobacco leaves, respectively. After longer culture, the identification of individual roots was difficult because of their higher lengths. The formation and development of primordia in the tissues of tobacco hypocotyl segments was assessed daily during 7 days on squashed preparations using a PZO light microscope (Poland) at a 125X magnification.

The following reagents were used: IAA, 4-umbelliferyl glucuronide, Na_2EDTA, SDS, Triton X-100, and growth regulators for explant culture were purchased from Sigma (United States). All other reagents of high quality were produced in Russia.

Experiments were performed in two–three replicates. No less than 10–15 explants were used for each measuring. Figures 2 and 3 and tables present means and standard deviations of one of typical experiments.

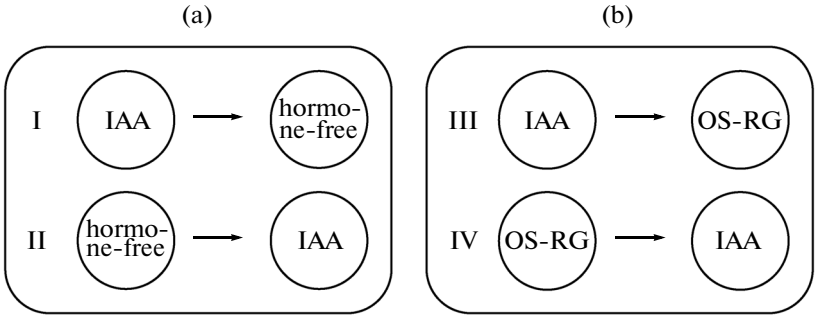

Fig. 1. Scheme of the experiment on the setting the periods of sensitivity to IAA and the oligosaccharin OS-RG.
IAA concentration is 3 μM; OS-RG concentration is 5 μg/mL.
I – transfer from medium with IAA to hormone-free medium;
II – transfer from hormone-free medium to medium with IAA;
III – transfer from medium with IAA to medium with OS-RG;
IV – transfer from medium with OS-RG to medium with IAA.

RESULTS

Determination of the Time of IAA Action Required for the Appearance of Adventitious Roots on Explants

Segments of buckwheat hypocotyls. First visible roots on the explant surface (~2 mm in length) appeared in the third day of culture. On the 5th–6th day of culture on hormone-free medium, only 3.3 ± 0.3 roots were formed per explant, whereas on medium with IAA (3 μM) 12.0 ± 0.9 roots per explant were formed.

To determine the duration of culture in the presence of the hormone required for the effective induction of adventitious roots, explants were first placed on the medium with IAA and then after culture during different periods (from 30 min to 48 h) they were transferred to hormone-free medium, where they were kept until root counting (Fig. 1a, treatment I). Or explants were first cultured on hormone-free medium using the same time points, and then they were transferred to the medium with IAA until the end of culture (Fig. 1a, treatment II). The number of roots per explant as assessed on the fifth day decreased when initial incubation with IAA was less than for 24 h (table (a), column I). When explants were transferred from hormone-free medium to the medium with IAA, it was found that the delay in the IAA application within 2 h did not exert any negative effect (table (a), column II): explants produced the same number of roots as in the case of their culture with IAA during the whole tested period. However, the number of roots reduced approximately by 30 and 65% at the culture on hormone-free medium for 5 and 24 h, respectively. When the hormone was added after 48 h after the start of culture, its effect was absent and the number of roots was similar to that on the hormone-free medium, during the whole tested period (3.3 ± 0.3).

Explants from tobacco leaves. First roots on explants from tobacco leaves appeared on the 6th–7th day of culture and on the 14th day their number reached 14.0 ± 1.2 roots per explant when 3 μM IAA was constantly present in medium, whereas roots were not developed on hormone-free medium.

For the determination of the optimal time of culture with the hormone, the same scheme was used as in the case of buckwheat explants (Fig. 1a) but the time points were in a wider range (from 1 to 144 h). Incubation with the hormone for 72 h was not sufficient for the appearance of the same number of roots as at the longer exposure to IAA (table (b), column I). When explants were transferred from hormone-free medium to the medium with IAA, culture without the hormone during two days induced even the higher number of roots than at explant incubation with the hormone during the whole period of culture. However, when the hormone was supplied only on the third day or later, the number of roots was substantially reduced (table (b), column II).

Duration of adventitious root formation differed sharply in two tested model systems; however, for both cases, it is possible to reveal close stages reflecting explant sensitivity to the hormone. The first stage when the absence of the hormone in medium did not affect the number of roots; it lasted for 2 to 48 h after the start of culture for buckwheat and tobacco explants, respectively. Thus, this period can be designated as an IAA-insensitive stage. The next stage can be designated as IAA-dependent one (2–24 h for the first model and 48–144 h for the second one), because the absence of exogenous IAA during this period led to the reduction of the number of roots, and finally the following period (starting from 24 h for buckwheat and 144 h for tobacco explants) was the IAA-independent period when the presence of IAA in medium was not necessary.

Effect of OS-RG Oligosaccharin on the Formation of Adventitious Roots

The oligosaccharin OS-RG (5 μg/mL) added to the medium together with IAA for the whole period of culture, induced the additional increase in the number of roots in both model systems as compared with the effect of IAA alone: from 12.0 ± 0.9 to 14.4 ± 1.1 in buckwheat hypocotyl segments and from 14.0 ± 1. 2 to 17.5 ± 1.3 in explants from tobacco leaves, i.e., in both cases stimulation was by ~20–25%.

To study short-term action of oligosaccharin and to compare the periods of its activity with the above described periods of sensitivity to auxin, we performed experiments with the same time intervals; however, instead of hormone-free medium the medium containing 5 μg/mL of OS-RG was used (Fig. 1b). When explants were pre-incubated in the medium with the oligosaccharin with their further transfer to the medium with IAA (columns IV in both parts of the table), the number of roots was higher than at the incubation with the hormone alone or with a treatment when IAA and OS-RG were added simultaneously and were present during the whole period of culture. The period of pre-incubation with the oligosaccharin when the highest effect was observed was 1–2 h for segments of buckwheat hypocotyls and from 5 to 24 h for explants from tobacco leaves, which corresponds to the time interval designated by us as IAA-insensitive, because the efficiency of the hormone presence in medium during this period was not high. When explants were pre-incubated with IAA and then transferred to the medium with oligosaccharin (columns III in both parts of the table), the number of roots was small and the overall pattern was almost consistent with a treatment with explant transfer on hormone-free medium (columns I in both parts of the table).

Effects of Oligosaccharin OS-RG and IAA on GUS Activity

The use of explants from the leaves of transgenic tobacco plants containing *GUS* gene under the control of the *rolB* promoter gives a possibility to assess early IAA-induced events by GUS activity still before the appearance of first visible roots because auxin, by triggering the expression of *GUS*-gene, stimulates the formation of the enzyme hydrolyzing exogenous substrate (4-methylumbelliferyl glucuronide) with the formation of fluorescent product. Enzyme activity assessed from the intensity of fluorescence is actually the detector of auxin action. Like in the case of root development, changes in GUS activity in tobacco explants were observed only in the presence of IAA.

The change in GUS activity during explant culture was characterized by the two peaks (Fig. 2). When IAA was present in the medium during the whole period of culture, the first maximum was observed on the third day, and the second – on the fifth day. A short-term

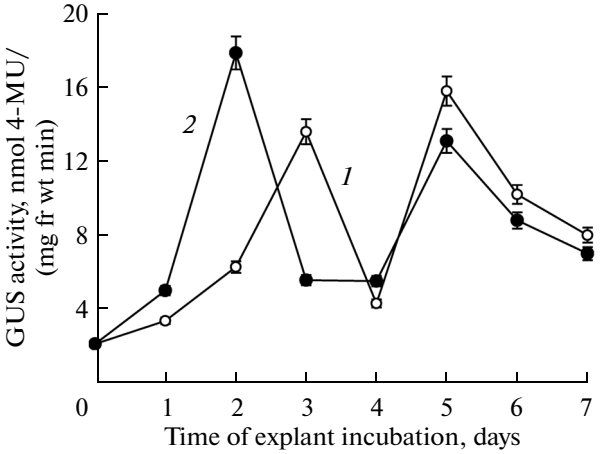

Fig. 2. Effects of oligosaccharin OS-RG and IAA on GUS activity in explants from transgenic (*rolB-GUS*) tobacco leaves.

(*1*) Incubation on medium with 3 μM IAA during the whole period of culture (open symbols);

(*2*) Explant pre-incubation on medium with 5 μg/mL of OS-RG (1 h) with subsequent transfer to the medium with 3 μM IAA (filled symbols).

(for 1 h) explant pre-incubation in the medium with 5 μg/mL of the oligosaccharin OS-RG with subsequent transfer to the medium with IAA increased the number of roots. In this case, not only GUS activity was enhanced, but also the response was accelerated, as indicated a shift of the first peak on the graph to the left, i.e., to the start of culture. The position of the second peak was not changed (Fig. 2).

To compare GUS activity with morphological changes occurring in tissues after treatment with IAA, we used segments from tobacco hypocotyls. At the treatment with 0.5 μM IAA (optimal for this model system), GUS activity had a well expressed peak on the third day of culture and a shoulder on the 4th–5th day (Fig. 3a). An increase in GUS activity coincided with the formation and development of various cell structures, from simply dividing groups of cells in the initial period to multilayer cellular structures (Fig. 3b). Since we were not dealing with a synchronous cell culture, different figures representing different stages of root development can be in the field of view simultaneously; however, at each time point one of them was predominant (Fig. 3c). Thus, within the first day after the start of explant culture, only dense cell aggregations were seen; on the second day a great number of structures in the form of meristematic tubercle appeared; by the third day of culture, when the first peak of GUS activity was observed, mainly 4–5-layered primordia of semi-spherical shape were seen in the explant tissue (Fig. 3). The shoulder on the curve of GUS activity (corresponding to the second peak of activity in explants from tobacco leaves in Fig. 2) coincided with the appearance of already almost developed roots.

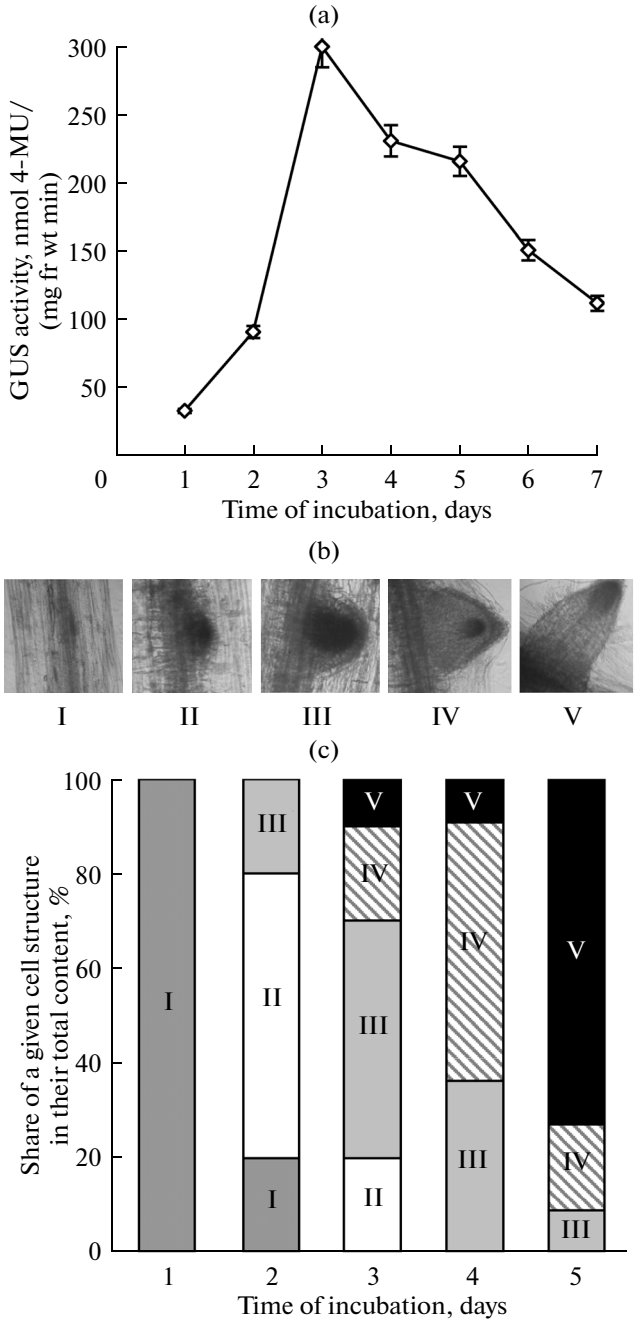

Fig. 3. Stages of root formation and GUS activity in the segments of transgenic (*rolB-GUS*) tobacco hypocotyls.
(a) Dynamics of GUS activity; (b) types of cellular structures in explant tissues during root development (125X magnification); (c) shares of different cellular structures appearing during definite periods of explant culture expressed in percents of their total number. Roman figures correspond to Fig. 3b. IAA concentration is 0.5 μM.

DISCUSSION

In the process of adventitious root development on explants from buckwheat hypocotyls and tobacco leaves, there are definite time intervals differing in the sensitivity to auxin (table). It has been shown earlier on other plant material [21, 22] that auxin is not necessary during all phases of root development. A comparison of such periods differing in the sensitivity to auxin with morphological changes occurring during the development of lateral and adventitious roots allowed a suggestion about the two stages of this process: root initiation requiring the presence of auxin and root appearance when auxin is not needed [22, 23]. Most researchers, even working with model systems, ignore the existence of a short period before the stage of increased explant demand to the hormone, which we called as insensitive to auxin. Duration of this period in our experiments depended on the plant material used and was 1–2 h or 2 days for the segments of buckwheat hypocotyls and tobacco leaves, respectively (table (a, b)). The occurrence of this period of insensitivity to the hormone in the beginning of explant culture is hardly related to the amount of endogenous IAA in our samples because such period was observed in both model systems. While the segments of buckwheat hypocotyls can develop some small amount of roots (3–4 per explant) even without the addition of the exogenous IAA due to its endogenous content, the roots never appeared on explants from tobacco leaves on hormone-free medium, although it is known that the content of IAA in leaf explants is rather high [16]. The existence of the period of insensitivity to auxin was noted in others although very numerically small numbers of studies of adventitious root formation [21, 24]. Thus, the highest number of roots was produced by the apple stem pieces when auxin was added not in the beginning but at 24–96 h after the start of culture [24]. It is supposed that during such lag-period dedifferentiation occurs when the cells become competent to respond to growth regulators, auxin in particular. Obviously, just this time interval is a key one for the determination of further cell fate, although molecular and physiological mechanisms and signals underlying these processes and determining the cell fate are not well clear until now. We demonstrated that the efficiency of the oligosaccharin isolated by us was higher in both segments of buckwheat hypocotyls and explants from tobacco leaves when it was applied just in this period before IAA. This means that this effector participates in the early stages of root formation before the point when the process would be triggered by the hormone, i.e., evidently still before cell asymmetric division, which is considered as the first visible event induced by auxin during rhizogenesis [1, 2].

Experiments with explants from the leaves of transgenic tobacco allowed revealing the cell responses on the stages before visible root appearance. According to Altmura et al. [25], who studied the expression of *rolB-GUS* gene in transgenic tobacco plants, the *rolB* promoter was activated in the initial cells competent to the formation of new organs and the phases of increased and decreased GUS activity reflect different states of these cells. It is believed than the enhancement of *rolB-GUS* gene

expression occurs in the primordia of lateral organs, starting from the appearance of initial cells, which is reflected in enzyme activation. When the pattern of the further organ is ready, the expression of the *rolB-GUS* gene ceases and GUS activity reduces. Initial cells are essentially absent at this stage. Subsequently, the developing organ starts to initiate its own initials, the expression of *rolB-GUS* is resumed, and GUS activity increases again [26]. This can explain the rise and fall of GUS activity observed by us at explant incubation on medium with 3 μM IAA (Fig. 2), when the first peak of GUS activity with the maximum at the third day of culture reflects the initiation and early stages of rhizogenesis, whereas the second peak is related to the appearance of already almost completely developed adventitious roots, as it was demonstrated in histological examinations of tobacco hypocotyls (Fig. 3). Since the position and amplitude of the second peak at oligosaccharin action are not changed, it confirms once more that its effect is limited to the early stages of root development, and, in all probability, is associated with an increase in the number of initial cells, because treatment with the oligosaccharin caused a steady increase in the number of roots.

If the relationship between changes in enzyme activity and the stages of root development is clear, the question of what mechanisms underlie this process and the interaction between the oligosaccharin and IAA remains open. Auxin plays a key role in the regulation of *rolB* promoter activity in tobacco plants and, according to the opinion of some researchers [11, 25], positive or negative effect of treatment with auxin in different tissues can reflect a difference in the cell sensitivity to the hormone. Thus, a shift of the enzyme activity peak under the influence of the short-term treatment with OS-RG, which we observed in our experiments (Fig. 2), can be associated with auxin redistribution and the formation of its gradient, which, in the opinion of some researchers, triggers the first asymmetric division and subsequent events related to the root development [1, 2] or the interaction of two effectors occurs at the level of auxin perception and oligosaccharin sensibilized auxin receptors. May be this is the reason why oligosaccharin was quite ineffective at its addition after the hormone in comparison with short-term pretreatment.

The data obtained indicate that endogenous oligosaccharin OS-RG isolated from pea seedlings is active during the early stages of root formation and is involved in the signaling pathway of rhizogenesis regulation triggered and controlled by auxin. Its effect was manifested in different model systems. Oligosaccharin-induced stimulation of rhizogenesis was observed on explants prepared from tissues of dicotyledonous plants belonging to different orders: Solanales (tobacco) and Caryophyllales (buckwheat). Experiments were performed on explants of different types: leaf explants of tobacco and segments of buckwheat hypocotyls, and this indicates a similarity in the responses to OS-RG of different initial cells, because adventitious roots are formed on leaf explants from the cells located near the veins, similar to procambial ones [16], whereas adventitious roots developing on hypocotyls arise probably from the pericycle cells [17]. This indicates a universal nature of oligosaccharin participation in the cell processes occurring during the development of adventitious roots.

ACKNOWLEDGMENTS

The authors are grateful to colleagues from the Department of Biological Research, University of Rome "La Sapienza" (Italy) and personally to Zabotina O.A. (now a professor at Iowa State University, USA) for providing seeds of transgenic tobacco plants.

The work was partially supported by the Russian Foundation for Basic Research, project no. 14-04-01591.

REFERENCES

1. Dastidar, M.G., Jouannet, V., and Maizel, A., Root branching: mechanisms, robustness and plasticity, *Dev. Biol.*, 2012, vol. 1, pp. 329–343.
2. Peret, B., Larrieu, A., and Bennett, M.J., Lateral root emergence: a difficult birth, *J. Exp. Bot.*, 2009, vol. 60, pp. 3637–3643.
3. Lavenus, J., Goh, T., Roberts, I., Guyomarch, H., Lucas, M., de Smet, I., Fukaki, H., Beeckman, T., Bennett, M., and Laplaze, L., Lateral root development in *Arabidopsis*: fifty shades of auxin, *Trends Plant Sci.*, 2013, vol. 18, pp. 450–457.
4. Moriwaki, T., Miyazawa, Y., Kobayashi, A., Uchida, M., Watanabe, C., Fujii, N., and Takahashi, H., Hormonal regulation of lateral root development in *Arabidopsis* modulated by *MIZ1* and requirement of GNOM activity for *MIZ1* function, *Plant Physiol.*, 2011, vol. 157, pp. 1209–1220.
5. Parizot, B., de Rybel, B., and Beecman, T., VisuaLRTC: a new view on lateral root initiation by combining specific transcriptome data sets, *Plant Physiol.*, 2010, vol. 153, pp. 34–40.
6. Li, S.W. and Xue, L., The interaction between H_2O_2 and NO, Ca^{2+}, cGMP, and MAPKs during adventitious rooting in mung bean seedlings, *In Vitro Cell Dev. Biol. – Plant,* 2010, vol. 46, pp. 142–148.
7. Takahashi, F., Sato-Nara, K., Kobayashi, K., Suzuki, M., and Suzuki, H., Sugar-induced adventitious roots in *Arabidopsis* seedlings, *J. Plant Res.*, 2003, vol. 116, pp. 83–91.
8. Albersheim, P., Darvil, A., McNeil, M., Valent, B., Sharp, J., Nothnagel, E., Davis, K., Yamazaki, N., Gollin, D., York, W., Dudman, W., Darvill, J., and Dell, A., Oligosaccharines, naturally occurring carbohydrates with biological regulatory functions, *Structure and Function of Plant Genomes*, Ciferri, O. and Dure, L., Eds., New York: Plenum Press, 1983, pp. 293–311.
9. Zabotina, O., Zabotin, A., Biologically active oligosaccharide functions in plant cell: updates and prospects,

Oligosaccharides: Sources, Properties and Applications, Gordon, N.S., Ed., New York: Nova Science Publ., Inc., 2010, pp. 1–34.

10. Tran Than Van K., Toubart, P., and Cousson, A., Manipulation of the morphogenetic pathways of tobacco explants by oligosaccharins, *Nature*, 1985, vol. 314, pp. 615–617.
11. Bellincampi, D., Cardarelli, M., Zaghi, D., Serino, G., Salvi, G., Gatz, C., Cervone, F., Altamura, M., Costantino, P., and de Lorenzo, G., Oligogalacturonides prevent rhizogenesis in *rolB* transformed tobacco explants by inhibiting auxin-induced expression of the *rolB* gene, *Plant Cell*, 1996, vol. 8, pp. 477–487.
12. Kollarova, K., Henselova, M., and Liskova, D., Effect of auxins and plant oligosaccharides on root formation and elongation growth of mung bean hypocotyls, *Plant Growth Regul.*, 2006, vol. 46, pp. 1–9.
13. Zabotina, O.A., Gur'yanov, O.P., Malikov, R.G., Ayupova, D.A., Beldman, G., Voragen, A.D., and Lozovaya, V.V., The isolation and biological activity of oligosaccharins from the pea shoots, *Russ. J. Plant Physiol.*, 1995, vol. 42, pp. 366–371.
14. Zabotina, O.A., Gur'yanov, O.P., Ayupova, D.A., Lozovaya, V.V., Beldman, G., and Voragen, A.D., Isolation and analysis of soluble oligosaccharides stimulating root formation from pea shoots, *Biochemistry* (Moscow), 1997, vol. 62, pp. 845–849.
15. Seifert, G.J. and Blaukopf, C., Irritable walls: the plant extracellular matrix and signaling, *Plant Physiol.*, 2010, vol. 153, pp. 467–478.
16. Ling, A.P.K., Kok, K.M., Hussein, S., and Ong, S.L., Effects of plant growth regulators on adventitious roots induction from different explants of *Orthosiphon stamineus, Am.-Eur. J. Sust. Agric.*, 2009, vol. 3, pp. 493–501.
17. Falasca, G. and Altamura, M.M., Histological analysis of adventitious rooting in *Arabidopsis thaliana* (L.) Heynh seedlings, *Plant Biosyst.*, 2003, vol. 137, pp. 265–274.
18. Lucas, M., Swarup, R., Paponov, I., Swarup, K., Casimiro, I., Lake, D., Peret, B., Zappala, S., Mairhofer, S., Whitworth, M., Wang, J., Ljung, K., Marchant, A., Sandberg, G., Holdsworth, M., Palme, K., Pridmore, T., Mooney, S., and Bennett, M., SHORT-ROOT regulates primary, lateral, and adventitious root development in *Arabidopsis, Plant Physiol.*, 2011, vol. 155, pp. 384–398.
19. De Smet, I., White, P., Bengough, A., Dupuy, L., Parizot, B., Casimiro, I., Heidstra, R., Laskowski, M., Lepetit, M., Hochholdinger, F., Draye, X., Zhang, H., Broadley, M., Peret, B., Hammond, J., Fukaki, H., Mooney, S., Lynch, J., Nacry, P., Schurr, U., Laplaze, L., Benfey, P., Beeckman, T., and Bennett, M., Analyzing lateral root development: how to move forward, *Plant Cell*, 2012, vol. 24, pp. 15–20.
20. Murashige, T. and Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue culture, *Physiol. Plant.*, 1962, vol. 15, pp. 473–497.
21. Pelosi, A., Lee, M., Chandler, S., and Hamill, J., Hormonal control of root primordial differentiation and root formation in cultured explants of *Eucalyptus globules* seedlings, *Aust. J. Plant Physiol.*, 1995, vol. 22, pp. 409–415.
22. De Klerk, G.J., Hormone requirements during the successive phases of rooting of *Malus* microcuttings, *Current Issues in Plant Cellular and Molecular Biology*, Terzi, M., Cella, R., Falavigna, A., Eds., Dordrecht: Kluwer, 1995, pp. 111–116.
23. Williams, M.E., Nusbaum, H.C., and Sussex, I.M., Formation of lateral root meristems is a two-stage process, *Development*, 1995, vol. 121, pp. 3303–3310.
24. De Klerk, G.J., Keppel, M., Brugge, J., and Meekes, H., Timing of the phases in adventitious root formation in apple microcuttings, *J. Exp. Bot.*, 1995, vol. 46, pp. 965–972.
25. Altamura, M., The role of hormones on morphogenesis of thin layer explants from normal and transgenic tobacco plants, *Physiol. Plant.*, 1992, vol. 84, pp. 555–560.
26. Altamura, M., Archilletti, T., Capone, I., and Constantino, P., Histological analysis of the expression of *Agrobacterium rhizogenes rolB-GUS* gene fusions in transgenic tobacco, *New Phytol.*, 1991, vol. 118, pp. 69–78.

Translated by N. Klyachko