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Root Formation in Pea Stem Sections and its Inhibition by Kinetin, Ethionine and Chloramphenicol

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Abstract. Root formation in the etiolated pea stem sections and inhibition of this process is described in the present paper. Sodium fluoride, iodoacetic acid, norvaline, phenyl-serine, 5-bromuracil and 2-thiouracil did not inhibit the root formation completely. Complete inhibition, however, was observed after treatment of pea stem sections by kinetin, ethionine and chloramphenicol (5×10^{-5} M, 1×10^{-2} M, and 1×10^{-2} M 16 hours after sectioning). The concentration of kinetin which produced complete inhibition of root formation simultaneously stimulated the growth of the lateral buds.

Root formation under the conditions described below can be divided into two stages. The first stage 64 hours from the beginning of the experiment, the second stage 64 hours later. Further, the first stage includes the formation of the meristematic cells in the pericycle areas. In the second stage are included the growth of roots and differentiation of root-tissues. Roots were formed, first of all, in the short vertical region of the sections near to the basal buds. Secondary xylem formation was also observed during the cultivation of the sections. This process was stimulated by kinetin.

Root formation in the pea stem sections of plants is a condition of organ regeneration, including the process of differentiation of cells and tissues. Such stem sections during the process of differentiation may be useful for the studies of relationship between structural differentiation and metabolism.

A suitable experimental design for such work has to satisfy at least the following demands:

1. Cultivation of experimental material in sufficient scale under standard conditions.
2. Control of the root formation by specific means with the possibility to completely inhibit this process.
3. Diagnosis of the beginning of root formation at the anatomical level in the very early stages.
4. A suitable ratio between differentiated and non-differentiated cells and eventually close localization of the process of differentiation.

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It is necessary to know for the further experimental work the root development at various intervals of time under standard conditions of cultivation.

The object of this study was (1) to find out the chemicals inhibiting completely the root formation, (2) to study the development of roots and its inhibition at the anatomical level as dependent on time.

The root formation in the second node region of the pea stem sections was studied by SOROKIN et al. (1962). Extensive root formation which they observed was completely inhibited by kinetin. Their result is in full agreement with the works by SKOOG and MILLER (1957), HUMPHRIES (1960) and KOBLITZ (1961). Many other chemicals are also able to inhibit the root formation. Some of them were used in the present experiments, such as ethionine, chloramphenicol, 2-thiouracil, 5-bromuracil etc. The inhibition effect of higher ethionine concentrations on root formation was described by LOWTER and BOLL (1960). Inhibition of root formation by chloramphenicol has not been reported so far, but has thought to be responsible for the inhibition of protein biosynthesis (WEBSTER 1957, JACOBY and SUTCLIFFE 1962). It was expected by the present author that the root formation is limited after the treatment of pea stem sections by chloramphenicol. The inhibition of root formation by 2-thiouracil was reported in *Tradescantia* (HÖHN 1954, 1955), sunflower and pea (MELICHAR 1964) stem sections. WOODSTOCK and BROWN (1963) found that 2-thiouracil stimulates the root elongation and inhibits cell division. The effect of 5-bromuracil is, however, more complicated. This uracil derivate in low concentrations promotes the growth of plants (ŠORMOVÁ 1961), cell division in the root tips of onion and root formation in the pea and sunflower stem sections (MELICHAR 1964). Higher concentrations of 5-bromuracil have opposite effects on the growth processes (FUJII 1963).

The development of roots under specific experimental conditions as dependent on time has not been reported, although valuable information in this field are presented in the work of SOROKIN et al. (1962).

Material and Methods

The seeds of pea (*Pisum sativum* L., cv. "Lincoln") were partially sterilised in 70% ethanol solution for 3 min., washed and soaked in distilled water for twelve hours. Swelled seeds were sowed on the cellulose wool saturated by half concentrated Knop's solution containing 1 p.p.m. $CuSO_4$ as antiseptic. The seeds were covered by clean 1 cm thick sand. Peas were grown in a 26° C dark room. Stem sections were cut from eight days old plants by serving the parts below and above the first and second nodes (Fig. 1). The basal sides of sections with buds were submerged into 2% sucrose solution in phosphate buffer (pH 5·6, 0·3 M) along with the chemical substances which were thought to effect root formation. After sixteen-hours of incubation period in the dark at 26° C, the sections were rinsed by distilled water and cultivated in the vertical position by the basal side on the cellulose wool saturated by distilled water containing 1 p.p.m. $CuSO_4$ as antiseptic. All operations involving sections were carried out in a weak green light. After ten days of cultivation in the dark at 26° C, the number of roots per one section was calculated. Each experiment was repeated trice with forty sections in each group.

The development of root formation and its inhibition by kinetin, ethionine, chloramphenicol and other chemicals were observed in additional experiments. The internodial stem sections (without apical and basal buds) sectioned from the first internode were also used in this work. The cultivation of pea and pea stem sections, as well as the application of chemical substances, were the same as described above. The basal parts of sections were removed for fixation (in the FAA solution) at the subsequent intervals from the beginning of the experiment, i.e. 0, 16, 40, 64, 88, 136, 184 and 232 hours. These intervals were chosen with respect to the possibility of distinguishing the first anatomical indications of differentiation, the period of differentiation of cells and the period of growth of the newly-formed roots. The fixed material was dried in tert.-butyl alcohol and transferred into paraffin. Both transverse and longitudal sections cca. 7 μ thick, were stained by alciane violet and by Ehrlich's hematoxiline according to the method described by OPATRNÁ at al. (1964). Photographs were made on 35 mm camera film Agfa-Isopan FF. The sections from the most extensive root formation region (from 0 to 2 mm below the basal bud) treated with various chemicals were recorded.

Results and Discussion

Inhibition of root formation. Kinetin in concentration 5×10^{-5} M, ethionine and chloramphenicol in concentration 1×10^{-2} M completely inhibit the root formation in pea stem sections (Table 1). Other chemical substances were also tested. Some of them partially inhibited the root formation. The treatment of sections by solutions of the following substances was found to be significant (5% confidence limit): sodium fluoride

Table 1
Inhibition of the root formation by kinetin, ethionine and chloramphenicol

Concentration M	Kinetin		Ethionine		Chloramphenicol	
	Number of roots per one section $\bar{x} \pm s_{\bar{x}}$	Inhibition %	Number of roots per one section $\bar{x} \pm s_{\bar{x}}$	Inhibition %	Number of roots per one section $\bar{x} \pm s_{\bar{x}}$	Inhibition %
0	3·23 ± 0·22	—	3·30 ± 0·12	—	3·60 ± 0·05	—
1×10^{-8}	1·76 ± 0·18	45·5	—	—	—	—
1×10^{-7}	1·02 ± 0·16	68·4	—	—	—	—
1×10^{-6}	0·76 ± 0·05	76·5	—	—	—	—
5×10^{-5}	0·00	100·0	—	—	—	—
1×10^{-4}	—	—	1·62 ± 0·15	50·9	—	—
1×10^{-3}	—	—	0·32 ± 0·11	90·3	1·50 ± 0·11	58·3
1×10^{-2}	—	—	0·00	100·0	0·00	100·0

1×10^{-5} M: 56·6%, iodoacetic acid 1×10^{-4} M: 65·6%, norvaline $1 \times \times 10^{-2}$ M: 50·8%, thienylalanine 1×10^{-2} M: 87·3%, phenyl-serine $1 \times \times 10^{-2}$ M: 94·9%, 5-bromuracil 1×10^{-2} M: 72·9%, 2-thiouracil 1×10^{-2} M: 64·8%. The inhibition is expressed in percentage.

Kinetin, ethionine end chloramphenicol, which produced the complete inhibition of root formation, were used for inhibition effects in such experiments where the root development at various intervals of time were recorded. Since kinetin also stimulates the growth of the lateral buds, as reported by WICKSON and THIMANN (1958, 1960), this effect has already been checked by the present author (1965).

Development of root formation. The root formation in the pea stem sections after the treatment of sucrose solution is shown in microphotographs (Figs. 2—6). The vascular tissue arrangement is similar to that described by SOROKIN et al. (1962) in the second node region of the pea stem sections.

The first indications of the root formation were observed 40 hours after the beginning of the experiment (after cutting). The foci of 2 to 8 cells were usually observed in each section in the pericycle region (Fig. 3). These cells contained big nucleus and their cytoplasma stained more by Ehrlich's hematoxyline. The foci were first formed in the pericycle region among the vascular bundles. Cell division and increase in the number of cell in the foci were observed during the next 24 hours of the experiment (Fig. 4). The foci formation was achieved at 64 hours and the roots were formed by their subsequent development.

Clear indications of root growth were observed 88 hours after the beginning of the experiment. The organised root meristems were formed at that time, procambium differentiation started, new roots increased in volume and roots penetrated through the cortex (Fig. 5). The development of the roots continue by differentiation of procambium and by the gradual decomposition of the stem sections (Fig. 6). The activation of cambium and formation of the secondary xylem also occurred at that time.

Development of roots in the pea stem sections was similar to that described by SOROKIN et al. (1962) who treated their sections by IAA. These authors did not record the root formation at various time-intervals.

Treatment of the stem sections by kinetin (5×10^{-5} M) prevented foci formation in the pericycle. The cambium was activated, resulting in the formation of the secondary xylem which formed several ring layers of cells. Secondary xylem was found to be very thick after 232 hours from the beginning of the experiment, as shown in Fig. 7. SOROKIN et. al. (1962) observed similar inhibition of the root formation and activation of the cambium by kinetin in pea stem sections. The thickening of stem sections after treatment with kinetin, as observed by KATSUMI (1962) is, perhaps, due to the increase in the secondary xylem formation which has been frequently recorded in the present experiments. It is interesting to note that kinetin affects the inhibition of root formation as well as growth-stimulation of the adjacent bud, as reported by the present author (1965). The cause of this complicated phenomenon is due to kinetin, the interactions of kinin and auxin in various proportions in respect to the morphological differentiation of the plant tissue as explained by SKOOG and MILLER (1957). Another explanation, as given by SOROKIN and THIMANN (1964), is due to the effect of kinetin on xylem differentiation, resulting in the complete contact of the bud and the vascular bundles of the stem.

Chloramphenicol (1×10^{-2} M), like kinetin, prevented the formation of

the foci of meristematic cells in the pericycle region. Activity of meristems was not stopped completely. Progressive interfascicular cambium and thin layer secondary xylem formation was observed after 88 hours from the beginning of the experiment (Fig. 8). Secondary xylem formation is much less extensive with chloramphenicol treatment, than with kinetin treatment. It is quite possible that the effect of chloramphenicol decreases gradually after the treatment of sections.

Ethionine (1×10^{-2} M) also affected the root formation like chloramphenicol. Light formation of secondary xylem was observed 88 hours from the beginning of the experiment in the chloramphenicol-treated sections (Fig. 9).

The extensive cell division was also found in the basal side of the internodial stem sections (Fig. 10). It is difficult to distinguish whether this process is the beginning of the root or calus formation. Differentiation of such cells ceases at this stage. Occurrence of cell division in section representing the basal area indicates the formation of calus.

Pea stem sections seem to be convenient material for use in the studies of the root formation. The experimental arrangement, as described above, facilitates the control of the root formation. The root formation might be prevented by the treatment of the stem sections by kinetin, ethionine and chloramphenicol. The present experimental design is also convenient for the study of secondary xylem formation and the growth of buds after treatment with kinetin.

The root formation passes two stages. The first stage, which is completed after 64 hours from the beginning of the experiment, includes the differentiation of cells in the pericycle region resulting in the formation of foci of big cells. The second stage includes the root growth and root tissue differentiation.

Further experiments in relation to the metabolism of the pea stem sections during root formation and its inhibition are being carried out and will be reported soon.

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Byla sledována tvorba kořenů v etiolovaných úsecích hrachových stonků a její inhibice. Fluorid sodný, jodoctová kyselina, norvalin, fenyl-serin, 5-bromuracil a 2-tiouracil neinhibovaly úplně tvorbu kořenů. Úplná inhibice tvorby kořenů byla pozorována po aplikaci kinetinu, etioninu a chloramfenikolu (5×10^{-5} M, 1×10^{-2} M, 1×10^{-2} M 16 hod. po odřezání úseků). Koncentrace kinetinu působící úplnou inhibici tvorby kořenů stimulovala růst postranních pupenů.

Tvorbu kořenů za uvedených podmínek lze rozdělit na dvě stadia. První — 64 hod. od začátku pokusu, druhé — 64 hod. později. V prvním stadiu se tvoří meristematické buňky v pericyklu. Ve druhém stadiu rostou kořeny a probíhá differenciace kořenového pletiva. Nejprve se tvoří kořeny v krátkém vertikálním pásmu úseku poblíž basálního pupene. Při pěstování úseků byla rovněž pozorována tvorba druhotného xylému. Tento proces byl stimulován kinetinem.

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В работе исследовалось образование корней и его ингибирование в этиолированных отрезках стебля гороха. Фтористый натрий, йодуксусная кислота, норвалин, фенилсерин, 5-бромурацил и 2-тиоурацил не ингибировали полностью образование корней. Полное подавление корнеобразования при применении кинетина, этионина и хлорамфеникола (5×10^{-5} м, 1×10^{-2} м, 1×10^{-2} м, 16 часов после взятия отрезков). Концентрация кинетина, полностью подавляющая корнеобразование, стимулировала рост боковых почек.

Корнеобразование в данных условиях можно разделить на две стадии. Первая — 64 часа от начала опыта, вторая — следующие 64 часа. В первой стадии образуются меристематические клетки в перицикле. Во второй стадии растут корни и происходит дифференциация корневых тканей. Сперва образуются корни в короткой вертикальной зоне отрезка вблизи базальной почки. При выращивании отрезков также наблюдалось образование вторичной ксилемы. Этот процесс стимулировался кинетином.