Stimulating effect of distilled water

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Summary: It is an early observation that plants in poor soil are developing roots quicker and more abundantly than on rich one. There is a similar correlation between the nutrient status of medium and adventitious root formation.
In order to throw more light on the background of this strange phenomenon we started a systematic experimental program in which the biological effects of distilled water as model factor was investigated.
The experiments proved that the root formation of Pinto bean *(Phaseolus vulgaris* L.*)* cuttings with 3 cm long hypocotyls was promoted by distilled water.
The phenomenon above accompanied with slower decline and faster recovery of total and also water-soluble protein content, more intensive efflux of amino acids, greater amount of tryptophane and increased uptake of water compared to those in control hypocotyls. From other data obtained we may suspect that some additional active substance unknown for us also contributes to the stimulation of root initiation in distilled water.

Key words: bean, roof formation, distilled water

Introduction

The stimulating effect of distilled water was observed in a few experiments. It manifested itself in the growth (Crescimanno, 1954) and initiation of root *(Zatykó,* 1962). Since there was no real good explanation for the phenomenon, an intensive research program was launched using the explants of Pinto bean seedlings.

Material and method

Pregerminated seeds were sown in a mixture of peat, perlit and sand. Seedlings were grown at 20–22 °C under continuous light of 2500 lux provided by low pressure mercury-vapour lamps. Seedlings were used on the 6th day. We prepared cuttings with 3 cm long hypocotyls and after removing the cotyledons they were placed in test tubes filled with distilled water and Pfeffer mineral solution respectively. Evaluation took place on the seventh day, when the number of adventitious roots developed on the hypocotyl was counted. Determination of total protein was accomplished by the Kjeldahl method. The water-soluble proteins were measured according to Lowry and co-workers (1951). For detecting indol-acetic acid and indol-derivatives essentially *Fletcher & Zalik* (1963) were followed.

Results and discussion

Cuttings in distilled water developed significantly more roots on their hypocotyls than those in Pfeffer solution *(Table 1).*

Table 1 Effect of distilled water on the root formation of Pinto bean cuttings

Liquid	Number of roots/hypocotyls %
Pfeffer solution	100 a
Tap water	125 b
Distilled water 1x	144 c
2x	163 d
3x	155 d

All figures followed by the same letter are not significantly different at the 5% level.

The number of roots increased with the second distillation but the third one did not cause any further improvement. The first visible signs of adventitious root formation appeared on the third day in distilled water and fourth day in mineral solution *(Fig. 1).*

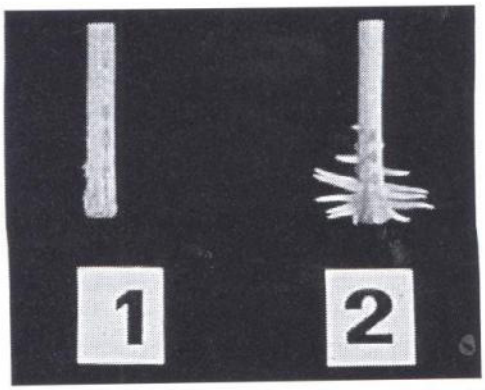

Figure 1 Adventitious root formation on the hypocotyls of Pinto bean explants held in Pfeffer solution (1) and distilled water (2) for 4 days.

The curves representing the daily change of the protein content in hypocotyls of Pinto bean explants express faster decrease of both total and water-soluble proteins in Pfeffer solution than in distilled water *(Fig. 2, 3)*.

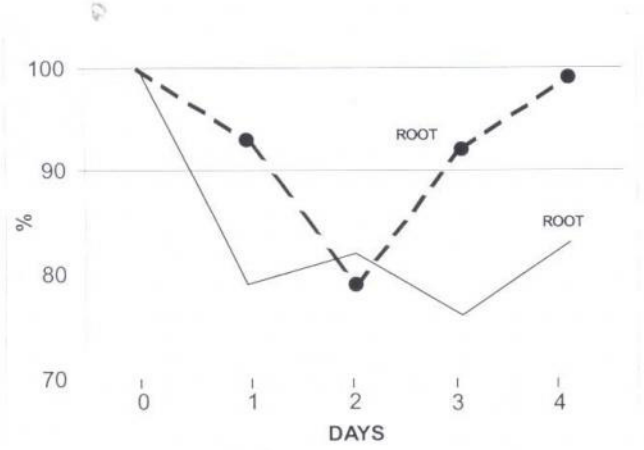

Figure 2 Daily change of the total protein content in the hypocotyls of Pinto bean explants placed in Pfeffer solution (–) and distilled water (—) respectively. Data were expressed in the percentage of zero-time samples and calculated on dry matter basis. Data marked by the same symbol are not significantly different at the 5% level.

At the same time the original level of proteins was regained faster in the hypocotyls held in distilled water. It seems that the turnover of protein synthesis is more rapid in that later solution.

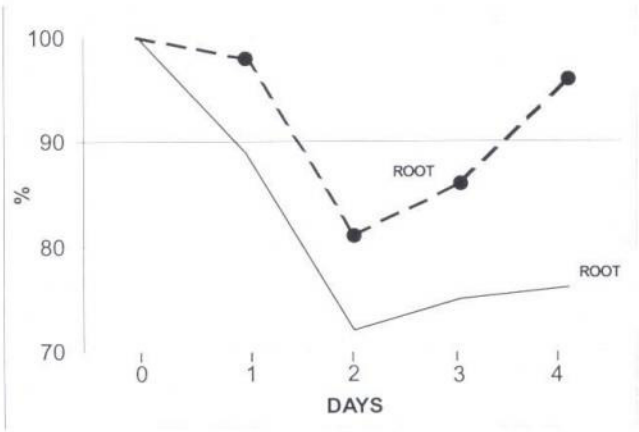

Figure 3 Daily change of the water-soluble protein content in the hypocotyls of Pinto bean explants placed in Pfeffer solution (–) and distilled water (—) respectively. Data were expressed in the percentage of zero-time samples and calculated on dry matter basis.

The increase of protein content coincides with the appearance of adventitious roots. That increase is rather the consequence than the cause of the latter. Already *Chibnall* (1964) suggested that roots must supply some factor, which is necessary for the maintenance of protein synthesis. *Parthier* (1964) also demonstrated that the initial decline in capacity for protein synthesis in detached leaves of *Nicotiana rustica* was followed by recovery when roots appeared on petiole.

Although the faster recovery of protein level in distilled water treated samples can be explained by the earlier root formation, we do not know why distilled water retards the decomposition or enhances the synthesis of proteins at the beginning of experiment.

The characteristic pattern of protein metabolism in the samples held in distilled water was accompanied with an intensive efflux of amino acids. At the same time there was no chromatographically detectable amount of amino acids in the Pfeffer solution.

Due to osmotic pressure, water uptake of the explants in distilled water must be more intensive than in Pfeffer solution. Data concerning the dry matter percent of the hypocotyls support this idea representing lower values in distilled water *(Fig. 4)*.

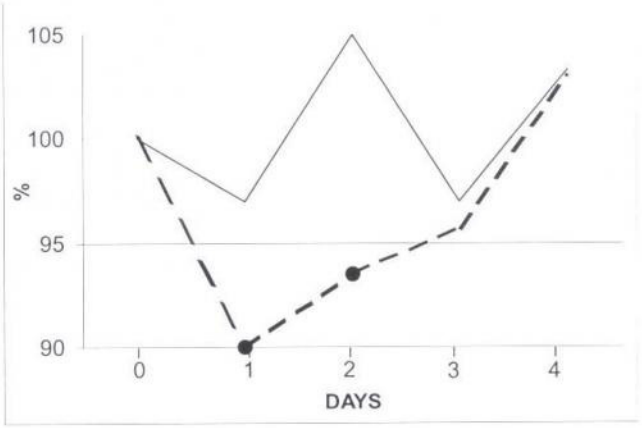

Figure 4 Daily change of the dry matter content in the hypocotyls of Pinto bean explants placed in Pfeffer solution (—) and distilled water (- - -) respectively. Data were expressed in the percentage of zero-time samples.

Among indole compounds only tryptophane could be found and characterized. Its concentration proved rather high (20–60 µg/g fresh weight). The hypocotyls in distilled water contained more free tryptophane than those submerged in mineral solution. Since zero-time controls contained always the greatest quantity of that substance, this superiority only means that the decline of free tryptophane level has been more moderate in distilled water than in mineral solution *(Fig. 5)*.

One may think that higher amount of tryptophane can be an explanation for the enhanced root formation in distilled water. Therefore we added the natural tryptophane from the hypocotyls treated differently to distilled water and tested their efficiency with Pinto bean explants.

All figures followed by the same letter are not significantly different at the 5% level.

Chromatographically purified tryptophane from hypocotyls of bean explants held in distilled water for 2 days increased the number of roots significantly *(Table 2)*, while tryptophane from other sources, extracted from mineral solution, treated and zero-time hypocotyls did not exert any

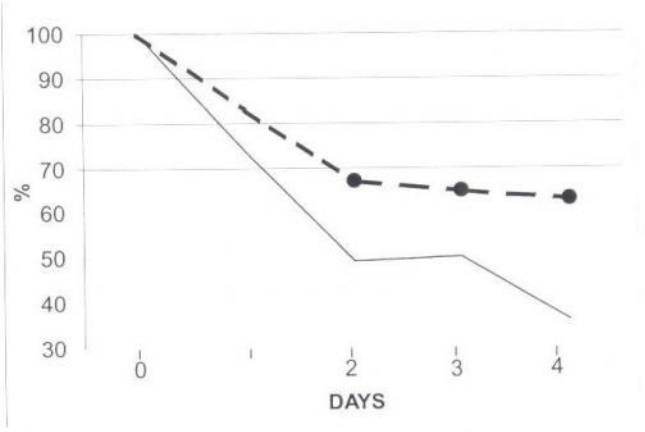

Figure 5 Daily change of the tryptophane content in the hypocotyls of Pinto bean explants placed in Pfeffer solution (—) and distilled water (- - -) respectively. Data were expressed in the percentage of zero-time samples and calculated on dry matter basis.

significant stimulating effect. Since zero-time hypocotyls contained always the greatest quantity of free tryptophane, we may suspect that in spite of purifications the tryptophane samples of hypocotyls treated by distilled water were contaminated with some active substance unknown for us, which contributes to the enhanced root formation too.

Table 2 The effect of natural tryptophane on the root formation of Pinto bean cuttings

Source of tryptophane	Number of roots/hypocotyls %
Control	100.0 a
Hypocotyl at the beginning of experiment	111.8 a
Hypocotyl in Pfeffer solution for 2 days	99.7 a
Hypocotyl in distilled water for 2 days	126.6 b

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