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Gravitropic Response of Adventitious Roots Cultivated in Light and Darkness on Sucrose-Free Medium

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Summary

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Elongation of adventitious roots of *Dracaena fragrans* was investigated under photoautotrophic conditions. Root elongation decreased and stoped when cultures were transferred to darkness. Upon return to light roots renewed growth after a 5 day lag period. During the first two days of intensive new growth roots were agravitropic elongating in random directions. Investigation showed that transient absence of geotropic response was connected with disappearance of starch grains in root tip which occurred due to sucrose starvation of cultures in continuous darkness.

Introduction

According to IVERSEN & al. 1968 there are numerous reports which indicate a close correlation between the occurrence of movable amyloplasts and geotropic sensitivity. Observations of this type are basis of the traditional starch-statolith hypothesis which gives a simple answer for the mechanism of the first step in a process through which plants perceive presence and direction of gravity.

Amyloplast rearrangement is not the only early event in the induction of gravitropic response, According to PICKARD 1985 it includes also: transient release of ethylene, lateral gradients of voltage, IAA, Ca^{2+} accelerated transport, deposition of callose and increased occurrence of small voltage transients.

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Several approaches have been used to test the validity of the starch-statolith hypothesis. One of them is to use mutants with altered starch content as in Arabidopsis (KISS & al. 1989, 1996). Here roots of wild type were more sensitive to gravity then roots of a starch-less mutant Similar results were obtained with hypocotyls of Arabidopsis mutants (KISS & al. 1997).

Interesting approach was presented by IVERSEN 1969 who devised a system in which cress roots were made starch-free by treatment with gibberellic acid and kinetin for 29 hours at 35°C in darkness.

Material and Methods

All cultures were maintained on MS (1962) type medium in a growth room adjusted to 25 ± 2 °C , photoperiod 16/8 hours light to darkness with cool-white fluorescent lamps providing 33-46.5 $\mu mol.m^{-2}s^{-1}$. Details for the establishment of shoot cultures were as previously published (VINTERHALTER & VINTERHALTER 1997). Dracaena shoot cultures were maintained on medium with 0.44 µM BA and 5.37µM NAA. Single 30-40mm long shoots were used as treatment explants. Treatments were performed in Φ 18 x 180 mm test tubes with stationary liquid medium and stainless steel supports for explants. Experiments were repeated at least three times with 24-32 replicates per treatment. Starch grains were observed in fresh hand sectioned material stained with IIK reagent

Results and Discussion

Adventitious roots of *D. fragrans* on media with MS inorganic salts +/- sucrose grow straight down through the medium manifesting a strong gravitropic reaction. In presence of exogenous sucrose roots elongate both in light and darkness. On sucrose-free medium roots can elongate only in light.

If cultures are transferred from light to continuous darkness then the rate or root elongation declines until it stops after 4-7 days (Fig. 1) When such cultures consisting of shoots with dormant roots are transferred back to light there is a 5 day lag period after which roots commence to elongate at fast rate.

The new growth often starts with a sharp turn - change in the direction of growth. The angle at which tip elongates seems to be random indicating loose of gravitropic orientation. This period of agravitropic growth is short lasting approx. 2 days during which gravitropic response gets restored. Agravitropic period ends with a turn which reorients the root tip downwards.

Having in mind the statolith theory we investigated distribution of starch grains in shoot explants and later adventitious roots. Starch grains were scarce even in light. In shoots they appeared only in the endodermis (starch sheet) and in roots they were present only in the root tip, under the root cap.

After cultures spent two weeks in darkness starch grains completely disappeared from the root tip remaining only in starch sheet of the shoot. Starch grains in the rot tip were also absent through the 5 day lag period in cultures transferred from darkness back to light.

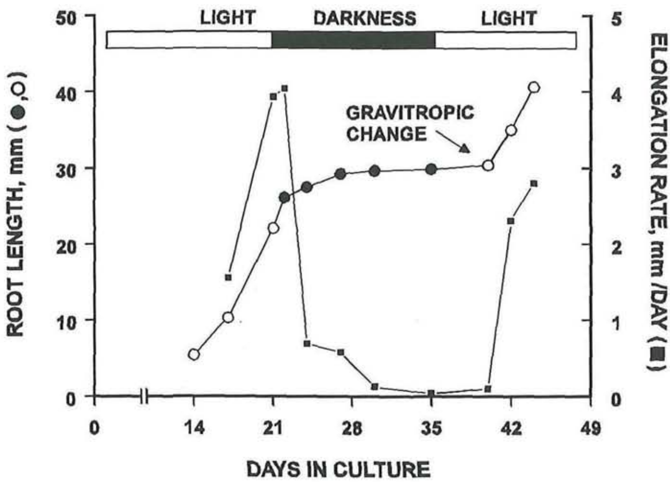

Fig. 1. Gravitropic change after 2 weeks of sucrose starvation in darkness.

They were also absent at the beginning of the period of agravitropic growth. Grains which reappeared during the restoration period were present in all roots which attained gravitropic response.

Our results seem to be in accordance with the findings of IVERSEN 1969 that starch depleted roots are unable to respond to gravity but retain fast rate of elongation. Advantage of our system is that the change in gravitropic response was induced by change in light regime and not by supplementation of exogenous substances.

Conclusion made through this investigation was that transient lose of gravitropic orientation in adventitious roots of *D. fragrans* in vitro cultures was caused by resorption of starch grains during sucrose starvation in darkness. This results indicate that in vitro cultures can be used as a useful model system to study perception of gravity under variable metabolic regimes.

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