Amyloplast distribution in hairy roots induced by infection with *Agrobacterium rhizogenes*

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Abstract To elucidate the rapid and plagiotropic growth of hairy root induced by *A. rhizogenes*, a root apex was investigated with respect to its amyloplast deposition, activity of α -amylase and glucose content. The amyloplasts distributed in the hairy roots were fewer than those of the adventitious root. Since auxin availability is enhanced in hairy roots, it could affect the statolith degradation by elevating α -amylase activity so that the energy requirement for rapid growth could be fulfilled as represented of glucose content. Consequently, it is suggested the overall decrease of starch grains could result in the lack of gravi-response in hairy roots.

Key words: Amyloplasts, Carrot, Gravitropism, Hairy root, Statolith.

Introduction

Hairy roots induced by infection with *A. rhizogenes* have some unusual morphological and physiological symptoms. Those include adventitious root formation with a high growth rate and plagiotropic growth of roots (Tepfer 1984). Such an alteration is caused by a natural genetic engineering event, namely the transfer of a specific region of DNA from Ri plasmid in *A. rhizogenes* into a plant cell (White et al. 1985). The expression of the transferred genes results in the development of hairy roots (Webb et al. 1990).

Since the hairy roots rarely respond to the stimulus of gravity, this characteristic provides a good opportunity to study the cause of plagiotropic phenomenon in the root system. For this purpose, it is necessary to examine the root apex where the primary process of gravitropic response occurs.

On the other hand, some phytohormones such

as gibberellic acid and cytokinin can cause destarching of amyloplasts due to enhancement of amylase activity in the cells located at the root apex (Busch and Sivers, 1990). It was also reported that auxin is related to the activation of amylase in peas (Hirasawa and Yamamoto 1991). Therefore, it is possible that some phytohormonal regulation is involved in statolith deposition.

In this study, the biochemical and histochemical aspects of hairy roots were examined in order to elucidate their loss of gravitropic response. Particular interest focused on whether hairy roots have additional effects on the structure of statoliths. Therefore, we observed a root tip to characterize the distribution of statolith using a light microscope and analyzed the amylase activity and glucose content in both a hairy root and an adventitious root.

Materials and Methods

The tap root of a carrot (*Daucus carota* L.) was transversely sliced and inoculated with *A. rhizogenes* R1000 by smearing. The carrot explants were kept on 1% solidified agar without nutrient or growth regulator. After 1 week, hairy roots developed mainly on the cambial ring. The hairy roots after 3 weeks incubation and untransformed

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Figure 1. Distribution of PAS positive material (starch) in root apex of non-infected adventitious root (A) and hairy root (B). (RC : root cap)

*Arrows indicate Amyloplasts.

adventitious roots were double fixed with 2% glutaraldehyde and 1% OsO_4 in 0.1 M phosphate buffer (pH 7.0), dehydrated with alcohol and embedded in epon 812. Semi-thin sections were stained with Periodic acid Schiff reagent (PAS) and then slightly counterstained with 0.1% toluidine blue.

α -amylase activity was measured according to Bernfeld (1955) and represented as mannitol quantity. Free glucose was determined as described by Buysse and Merckx(1993).

Results and Discussion

Hairy roots induced by infection with *A. rhizogenes* formed on the inoculated surface of the carrot disk a week after infection. The hairy roots mainly formed on cambial ring and grew plagiotrophically. In contrast, non-inoculated carrot explants have never formed adventitious roots on the cutting region in this experimental condition. The hairy roots were incubated to enlarge them to a size comparable to adventitious roots. As an untransformed counterpart, adventitious roots on the tap root were used as the untransformed control showing gravitropic response.

According to histological observation, the main differences were revealed in root morphology and amyloplast distribution. The tip of the hairy roots were slightly swollen while adventitious

roots were tapered. The expanded root cap of hairy roots had significantly less amyloplasts and numbers of tiny amyloplasts appeared along the vascular region toward the distal portion of the root (Fig. 1B). In contrast, abundant amyloplasts were located in the root cap of normal adventitious roots (Fig. 1A).

Amyloplast has long been suggested to function as statolith in gravity perception and its presence is important in the initial process of the perception (Volkmann and Sievers 1979, Bjorkman 1988). In this experiment, the statolith emerged to be significantly reduced in the root cap of hairy root.

On the other hand, α -amylase activity was almost doubled in hairy roots compared to that of normal root and glucose content was also much higher in hairy roots (Fig. 2). The hairy roots seem to have a much higher energy requirement since the hormonal balance in hairy roots is changed by the expression of Ri T-DNA which is integrated into plant genome (white et al. 1985). Actually, the hairy roots grew very rapidly. The metabolic pathways in transformed roots might have been shifted due to the elevated sensitivity to auxin through either increased concentration or increased sensitivity (Schaerer and Pilet 1993). Sutter and Luza (1993) reported that the anatomical development of hairy roots resembled untransformed roots which were treated with exogenous auxin. It was concluded that auxin was involved in the response to *A. rhizogenes*.

Figure 2. Amylose activity, represented as maltose content, and glucose content in non-infected adventitious root and hairy root.

*Data are represented as means of triplicate of 30 roots per replicate.

It is well known that gibberellin is important in regulating the production of α -amylase. This starch degrading enzyme could also be included by endogenous or exogenous auxin (Hirasawa and Yamamoto 1991). Therefore, the elevated auxin sensitivity in hairy roots could affect α -amylase activity so that starch is degraded to fulfill the energy requirement for rapid growth and it could also explain the increase of glucose content in hairy roots.

It seems reasonable that the overall decrease of starch grains in the root cap could cause the lack of graviresponse in hairy roots because amyloplast zonation is evidently important for gravisensitivity in root. Most likely, the diminished geotropism of hairy roots is correlated with the presence of less statolith.

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