Nuclei of Plants as a Sink for Flavanols

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Onion cepa (L.) and *Tsuga canadensis* (L.) Carr. were investigated histochemically on the association of flavanols to nuclei. The young roots of *Onion cepa* are totally devoid of flavanol structures. Therefore, the excised roots tips were directly incubated into different solutions of flavanols. After 3 h of incubation a flavanol binding on the nuclei was recognizable, as seen by a yellowish-brown tanning reaction. Still to ensure the presence of flavanols on the nuclei, subsequent staining with the p-dimethylaminocinnamaldehyde reagent (DMACA) resulted in an intense blue colouration. *Tsuga canadensis* has significant amounts of vacuolar flavanol deposits in all parts of the tree as indicated by the DMACA reagent. It is obvious that also the nuclei were associated strongly with flavanols which can be demonstrated particularly elegant in the cells of the seed wings by histochemical methods. However, the mode of flavanol release from the original deposits is not yet clear.

Introduction

The role of flavanols in constitutive and induced resistance of tree species, particularly with respect to barrier formation, was investigated extensively since the last two decades (Feucht and Treutter, 1999). These investigations were mainly based on the use of the DMACA reagent yielding a blue colouration exclusively for flavanols at a maximum of 640 nm when measured spectrophotometrically (Treutter, 1989). During the course of these studies some conifer species showed frequently nuclei which reacted with the DMACA reagent. Indeed, isolated globular proteins such as bovine serum albumin and nucleic acids allow complexation with oligomeric flavanols (Hagerman and Butler, 1981; Haslam *et al.*, 1989). There is until now no information concerning an association of flavanols to nuclei. Among numerous tissues investigated the seed wings of *Tsuga canadensis* proved to be an unique system to deal more thoroughly the unexpected phenomenon.

Materials and Methods

Young rootlets *Allium cepa* (L.) were obtained from germinating seeds in petri dishes. Their root tips, about 6–7 mm in length, were placed in a buffered solution (pH 5.8) of the macroelements of Murashige and Skoog (1962) containing monomeric and oligomeric flavanols extracted with water from grape seeds (10 mg dry powder in 0.2 ml aqua dest) or catechin at 1 mM. The incubation time should be at least 3 h.

Seed wings of *Tsuga canadensis* (L.) Carr. were collected from 2 trees each about 20 years old. Staining of the nuclei can be performed with the intact seed wings immediately after sampling. The staining procedure with DMACA (1% of *p*-dimethylaminocinnamaldehyde dissolved in 1-butanol with 1.5 N sulfuric acid) was finished after 10 min. To obtain a bright blue colour for photographic documentation the excess of reagent was removed with water. Imbibition in flavanol solutions was performed as described for *Allium*.

The flavanols of the seed wings were determined colorimetrically and by TLC (Feucht and Khan 1973). Oligomeric flavanols were additionally detected with 1-butanol-HCl after boiling 40 min (Swain and Hillis, 1959).

The histological investigations were performed over a period of 5 years. About 80 seed wings per year were sampled at different dates from May to December. Per seed wing about 2 500 blue stained nuclei can easily be recognized.

Results

Allium cepa

After 3 h of incubation into a flavanol solution (catechin or grape seed extract) the nuclei of the root tissues turned a brownish-yellow colour which was more intense than the remainder of the cells (Fig. 1). This reaction was retarded for some hours in the thick-walled epidermis. On the con-

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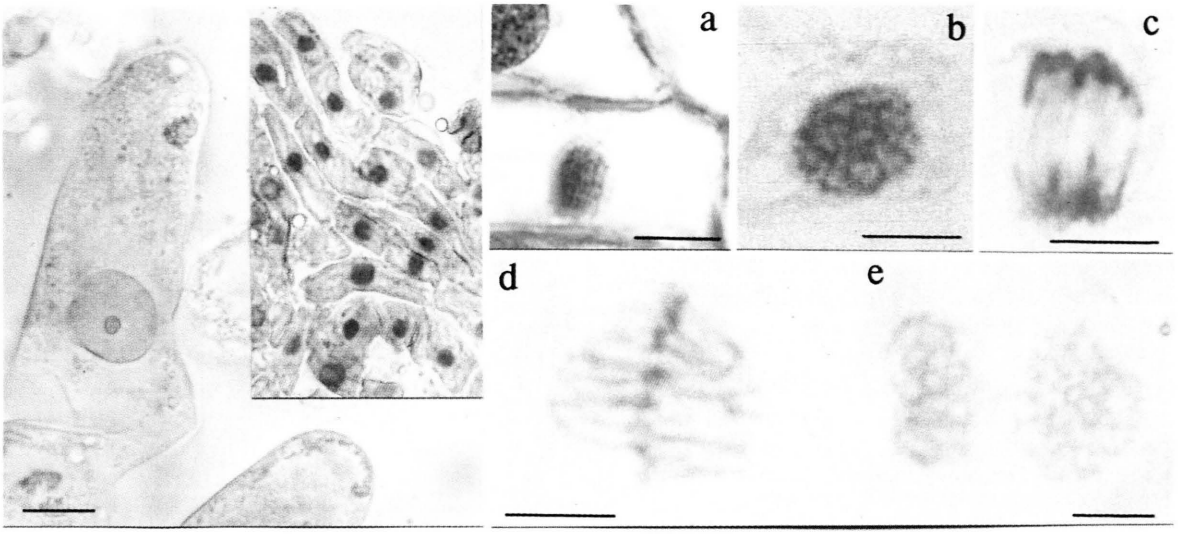

Fig. 1 (left). Root hair of *Allium cepa* showing a brown nucleus after incubation in flavanols. (Inset, browned nuclei turn blue when treated with DMACA). Bar = 5 μm.
Fig. 2 (right). Nuclear structures of *Tsuga canadensis*. a. Seed wings incubated in flavanols show a blue DMACA staining reaction of the cell walls. b. Interphase (total cell visible as a faint shaddow). c and d. Mid–anaphase and early–anaphase occupying nearly 75% of the total cell (very faint shaddow) e. telophase with both daughter cells. Bars = 5 μm.

trary, the solutions entered rather rapidly into the smooth hairs emerging from the root tips. The nuclei changed to a brownish colour at about 1 h of incubation. The colouration could be intensified in all tissues by prolonging the incubation period up to 12 h. Some plasmic compounds, probably proteins, were additionally shown to turn brownish with added oligomeric flavanols from grape seeds. All controls were constantly pale.

The flavanol-treated browned tissues can additionally be stained with DMACA to further prove the presence of flavanols (inset, Fig. 1). A dense blue staining of the nuclei was evident. Diffuse cytoplasm staining a pale blue was observed on occasions.

Tsuga canadensis

All parts of the *Tsuga* tree, either needles, shoots or roots, revealed consistently the blue staining nuclei. Most suited for the studies were the seed wings.

Adding external solutions of flavanols to the seed wings, as in the case of *Allium*, resulted already after 5 s in a blue haze on the cell walls which turned to a prominent blue after 10 min (with DMACA reagent, Fig. 2 a). Controls remained pale. The nuclei themselves did not attain a visibly more prominent blue colour as usual.

The formation of the young seed wings started in early-May and finished in late-June. Senescence (browning) symptoms appeared not until mid-winter. Thereafter, the necrotic nuclei failed to stain with DMACA. During the early growth phase in May most wing cells were found to be in an active cycling state. The "blue" nuclei with different chromosomal configurations occupied between 50 to 80% of the cellular volume (Fig. 2 b,c and d). Even small amounts of vacuolar flavanols were not observed in these cells.

A major portion of the seed wing flavanols (nuclei) was extractable by aqueous ethanol (50:50, v/v). The main flavanolic compound was the monomeric catechin. Extractable flavanols accounted for 8.5 mg (g DW^{-1}). The non-extractable flavanols consisted of oligomeric proanthocyanidins. The nuclei turned reddish, as evidenced by boiling the extracted seed wings with n-butanol/HCl (Swain and Hillis, 1959).

Discussion

Roots and bulbs of *Allium cepa* invariably do not synthesize flavanols. Upon incubating intact tissues such as root tips in flavanol solutions, the nuclei turn to a brownish colour. Thus, these nuclei subject to normal physiological conditions act directly as a sink for flavanols. Using the DMACA reagent as a further indication for flavanols the nuclei gave a blue colouration.

Woody plants such as *Tsuga canadensis* favour the shikimate pathway and the formation of flavanols (Ribereau-Gayon, 1961). This tree species is capable to store large amounts of vacuolar flavanols in all parts of the tree and the nuclei were constantly found to bind those flavanols. However, in the case of *Tsuga* it cannot be excluded that application of the DMACA reagent containing 1.5 N sulfuric acid could have facilitated leakiness of the tonoplast and artificial diffusion of vacuolar flavanols into the cytosolic site. The lipoprotein structures of bilayered tonoplasts are highly variable in modifying their channel function and some release or passive diffusion of flavanols through ultrastructurally modified pores cannot be negated with security. There is a need for more basic research in this respect.

If the above view of vacuolar leaching caused by the DMACA reagent is really true, then a flooding bulk of flavanols has to be targeted to both nuclei and cell walls. However, the rather small cells in which mitosis uniquely occurs were devoid of vacuolar flavanols, at least when investigated by light microscopy. To obtain the intense blue staining of the large nuclei, a rich source of extranuclear flavanols would be necessary. All these observations argue against an uncontrolled leaching.

Seeds and seed wings guarantee the survival of conifers and therefore need an effective overall protection. Apart from environmental stress (Stapleton and Walbot, 1994), scavenging by flavanols of intracellular toxic compounds such oxygen free radicals is also of vital importance for the nuclei (Britt, 1996; Davies, 1995). In keeping with this survival concept, Sarma and Sharma (1999) found a mutual protection to occur by complex formation between anthocyanin and DNA.

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