Reduced Indole-3-acetic Acid Decomposition Causes Enhanced Growth of Plums Affected by the Fungus *Taphrina pruni*

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Z. Naturforsch. **52c,** 504–507 (1997); received December 1996/April 16, 1997

Indole-3-acetic Acid, Growth, *Taphrina pruni*, Catechins, Caffeoyl-D-quinic Acids

Plums attacked by the fungus *Taphrina pruni* produce great amounts of catechins and caffeoyl-D-quinic acids. This reduces – as we show in this paper – the degradation of indole-3-acetic acid. The increased indole-3-acetic acid concentration causes enhanced growth of infected plums in comparison to not infected ones.

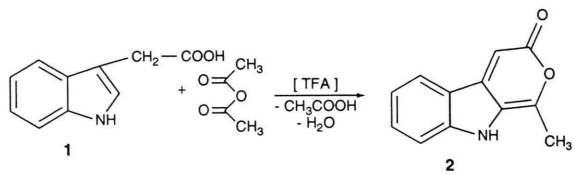
Introduction

Indole-3-acetic acid (IAA) is ubiquitous in higher plants, many algae and fungi (Gruen, 1959; Augier, 1978). It plays an important role in many physiological processes in plants, such as cell elongation and cell division (Pope, 1993; Shinkle and Briggs, 1984; T. Yang *et al.*, 1993).

Several reports demonstrate increased IAA-concentration as a result of parasitic attack or interaction with symbionts. This causes increased plant growth (Pegg, 1976). Plums infected by the fungus *Taphrina pruni* show particular striking growth symptoms: Affected fruits not only have increased growth and develop no cernel but also desiccate prematurely and fall off the tree. We demonstrate in this paper that enhanced IAA-concentration (Hirata, 1978) and growth is due to reduced IAA-decomposition. This reduced IAA-decomposition is caused by increased amounts of caffeoyl-D-quinic acid esters and catechins.

Results and Discussion

The IAA-amount of plants can be determined by the rapid and sensitive indolo-α-pyrone-method (Stoessl and Venis, 1970): The method is based on the specific reaction of IAA with acetic anhydride to an indolo-pyrone:



1 2

Fig. 1. Reaction of IAA (**1**) and acetic anhydride to a pyrone (**2**).

Due to their fluorescence these pyrones (excitation 440 nm; emission 490 nm) are detectable up to 1 ng. IAA-conjugates produce no pyrone (Stoessl and Venis, 1970).

Addition of the antioxidant 2,6-di-*tert*-butyl-*p*-cresol (BHT) increased the stability of IAA in solution and thus reproducability of the measurement (Iino *et al.*, 1980). Absolute IAA-quantities are determined using a calibration curve with known IAA-amounts.

Figure 2 shows that the IAA-concentration rises in infected plums until 45th day after blossom. Then it decreased rapidly. Summing up the IAA-concentration of infected plums accumulated up to 63 ng, to values three times higher as observed in healthy plums.

Even with the sensitive indolo-α-pyrone-method only trace amounts of IAA (in 1 g fungal mycel : a relative fluorescence of 3 was found corresponding to 5 ng IAA / g fungal mycel) were determined in *Taphrina pruni* (Hirata, 1978).

The subsequent experiments prove that reduced catabolism caused the different IAA-concentrations: Auxin-inactivation **1** proceeds by two different catabolic pathways: Either the side chain of

Abbreviations: IAA, indole-3-acetic acid; TFA, trifluoro acetic acid.

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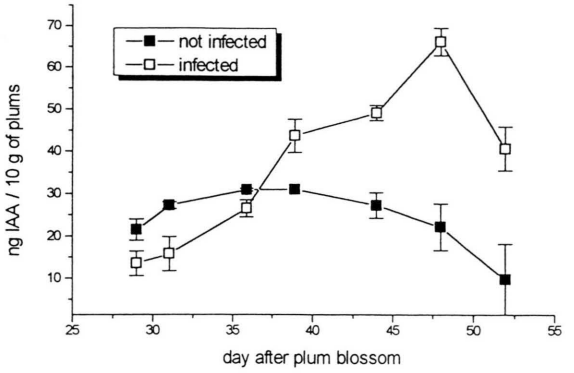
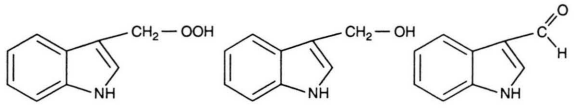


Fig. 2. Time-dependent IAA-concentration of non-infected and infected plums. (Data are mean values of 4 measurements).

the molecule may be oxidatively decarboxylated or the indole group itself may be oxidized.

The oxidative decarboxylation of the IAA (**1**) is catalyzed by IAA-oxidases. Many of them have peroxidase activity (Osswald *et al.*, 1988; Waldrum *et al.*, 1981; Gelinas *et al.*, 1973; Wiese, 1986). This IAA-decomposition probably proceeds via an indole-3-methyl-hydroperoxide (**3**) intermediate (Nakajima and Yamazaki, 1979; Kobayashi *et al.*, 1984) to indole-3-methanol (**4**) and indole-3-carbaldehyde (**5**) (Tsurumi and Wada, 1985). With appropriate electron donors present, e.g. phenols, indole-3-methanol is the only product (Sembdner *et al.*, 1980), while without electron donors in position 2 oxidized indoles are generated (Tsurumi and Wada, 1985; Reinecke and Bandurski, 1983; Grambow and Lagenbeck-Schwich, 1983).



3 4 5

Fig. 3. Compounds produced by oxidative decarboxylation of indole-3-acetic acid (**1**).

S. Krylov *et al.* (1993) describe a „threshold effect" of caffeic acid towards IAA-decomposition: Peroxidase-catalysed IAA-decomposition was instantly stopped if the caffeic acid concentration exceeded a certain threshold concentration. The structural analogy of chlorogenic acid (a caffeic acid ester) to caffeic acid suggests that there are similar effects for chlorogenic acid.

We therefore determined the formation of IAA oxidation products (mainly indole-3-methanol) (Volpert *et al.*, 1995) by measuring the absorbance difference between 242 and 296 nm (wavelengths with the same absorbance for IAA) compared to a reference of known IAA-concentration. This absorbance difference after the reaction showed the extent of IAA-decomposition and the extent of generated oxidation products, respectively.

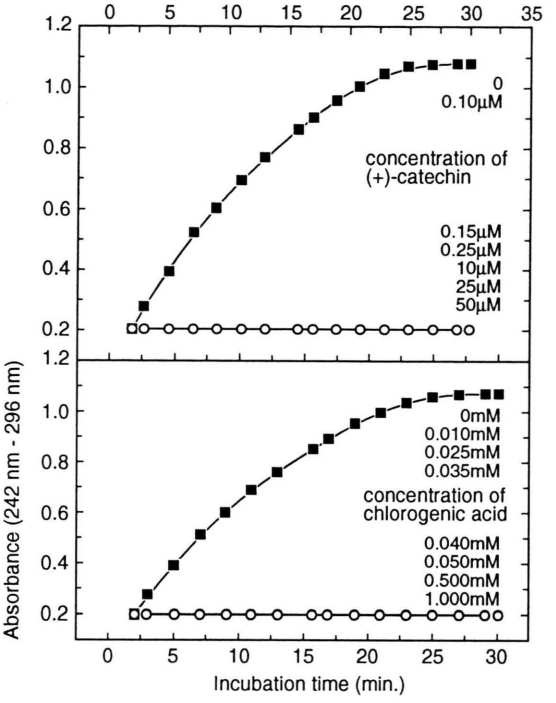


Fig. 4. Effect of different concentrations of chlorogenic acid and (+)-catechin on the kinetics of peroxidase catalysed IAA-oxidation.

The threshold concentrations for chlorogenic acid and (+)-catechin were 0.035–0.040 mM and 0.10–0.15 µM. Therefore (+)-catechin is 20–100 fold more effecient as IAA-antioxidant than any compound cited in the literature (Krylov *et al.*, 1993; Volpert *et al.*, 1995). Even in low concentrations catechins have a high potential as antioxidants towards IAA-decomposition.

The investigations described above implicate that increased concentrations of caffeoyl-D-quinic acids and catechins lead to an improved protection of IAA towards oxidative decomposition, thus causing accumulation of IAA and enhanced growth.

Experimental

Fluorescence spectroscopy

Fluorescence measurements were performed with a Kontron SFM 25 spectrometer.

Indole-3-acetic acid, trifluoroacetic acid, chlorogenic acid, (+)-catechin and horseradish peroxidase (EC 1.11.1.17) were obtained from Fluka Chemie AG, Neu-Ulm.

Plant material

Origin

50 g of infected and non-infected plums were harvested near Bayreuth at intervals of about four days from the same tree.

Extraction

20 g of plums were frozen in liquid nitrogen, homogenized and extracted four times with 500 ml MeOH/H_2O (9:1, v/v) or acetone/H_2O (8:2, v/v) at 20 °C.

Taphrina pruni

Taphrina pruni (CBS Number 358.35) was obtained from Zentraal Bureau voor Schimmel Kultures (P. O. Ox 273 Oosterstraad 1, NL 3740 A6 Baarn).

Culture conditions

Taphrina pruni was grown on malt extract/agar 2%. A suspension of 11.6 g malt extract, 8.7 g agar and 580 ml H_2O was adjusted to pH 7 with diluted KOH and boiled for one hour. The hot solution was poured into plastic petri dishes.

Indolo-α-pyrone fluorescence method

Indole-3-acetic acid was determined according to the method of Wiese (Wiese, 1986).

Acknowledgements

We thank Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie for financial support of this work.

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