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A semi-quantitative bioassay for the detection of fungitoxic tulipalins in aqueous solutions

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Extracts of tulip tissue contain the fungitoxic substance α-methylenebutyrolactone (Bergman et al., 1967; Tschesche et al., 1968) and extracts of leaves and pistils moreover contain β-hydroxy-α-methylenebutyrolactone (Tschesche et al., 1968). These compounds, called tulipalin A and B, respectively, and their precursors are thought to play a role in the resistance of the tulip against several fungi (Schönbeck, 1967; Bergman et al., 1967; Bergman and Beijersbergen, 1971; Schroeder, 1971).

A method based on ion exchange column chromatography (IECC) and UV spectrophotometry was developed for determination of the concentration of both tulipalins individually in extracts of tulip tissues (Beijersbergen, 1972).

It is possible that in some cases non-fungitoxic compounds with the same UV spectrum are eluted simultaneously with one or both tulipalins. Because the concentration of the tulipalins is too low to permit quantitative chemical analysis, a semi-quantitative bioassay was developed for routine checking.

In this method, the fractions (column: Dowex 50 W X 2 200/400 mesh, 18 × 1.8 cm; eluant: distilled water; fraction volume 3.0 ml) known to contain tulipalin A or B (fractions 25–36 or 17–24, respectively) were pooled and diluted until 800–1000 μg tulipalin/ml was present, as shown by UV measurements (Beijersbergen, 1972). Dilution ranges were made by pipetting 0, 0.5, 1, 1.5, ... 5 ml in petri dishes (⌀ 8.5 cm) and these volumes were brought up to 5 ml with distilled water. The same dilution range was made for pure tulipalin solutions in water containing 900 μg/ml.

The 5 ml aliquots were mixed with 10 ml PDA (40 g Oxoid Potato Dextrose Agar + 5 g Difco Noble Agar in 1000 ml water) at 55–60 °C. In this medium the tulipalins are stable (Beijersbergen and Lemmers, unpublished). The culture media were inoculated with 3 pieces (⌀ 3 mm) taken from the edge of a colony (⌀ 4–5 cm) of *Fusarium oxysporum* Schlecht *f. tulipae* Apt, growing on PDA, to demonstrate the presence of tulipalin A. In bioassays for tulipalin B *Phoma pomorum* Thüm. isolated from tulips was used, because *F. oxysporum* is very insensitive to this compound.

The growth inhibition (I) resulting from the presence of one of the tulipalins in the PDA is expressed as a percentage of the growth in the control on the basis of the formula:

$$I = (d_b - d_x)\frac{100}{d_b}\ \%$$

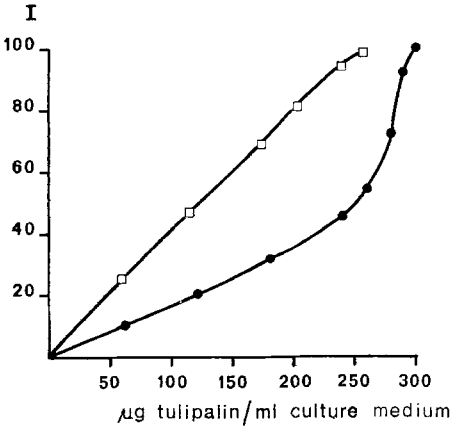

Fig. 1. Inhibitory effects of tulipalin A (●——●) and tulipalin B (□——□) on the growth of *Fusarium oxysporum* and *Phoma pomorum*, respectively.
I = growth inhibition as a percentage of the control; 100 = complete inhibition.

Fig. 1. Remmend effect van tulipaline A (●——●) *en tulipaline B* (□——□) *op de groei van respectievelijk Fusarium oxysporum en Phoma pomorum.*
I = groeiremming uitgedrukt in procenten van de controle; 100 = volledige groeiremming.

in which d_x is the diameter in mm (minus 3 mm) of the colony growing under the influence of the pooled IECC fractions or pure tulipalin solution and d_b the same in the control dish (Beijersbergen and Lemmers, 1972).

If p is the number of ml of the pooled IECC fractions present in the culture medium, the quantity Z (in µg) of the tulipalin in 1 ml culture medium is found from I_p and the parallel standard growth inhibition curve of the tulipalin (Fig. 1); reliable determination of Z was possible when $20 < I_p < 95$. The quantity Z_f (in µg) of the tulipalin present in 1 ml of the pooled fractions can be calculated from Z with the formula: $Z_f = 15/p \times Z$. The mean Z_f found within one dilution range of pooled fractions (p = 0.5, 1.0, 1.5, ... 5) gives the µg tulipalin /ml pooled fractions tested.

Table 1. Tulipalin B (in µg) in 1.0 ml pooled fractions 17–24 (see text) obtained by ion exchange column chromatography according to UV spectrophotometry and bioassay. For each experiment a fresh extract was made; the pooled fractions were diluted until 800–1000 µg tulipalin B/ml were present as shown by UV spectrophotometry.

Experiment No	UV spectrophotometry	Bioassay
1	910	885
2	1025	1090
3	860	1005
4	1040	930
5	780	765
6	860	780
7	800	900
8	790	720
average of 25 experiments	890	880

Tabel 1. Tulipaline B (in µg) in 1,0 ml van bijeengevoegde fracties 17–24 (zie tekst) verkregen na kolomchromatografie en bepaald met UV spectrofotometrie en biotesten. Voor elk experiment werd een vers extract vervaardigd; de bijeengevoegde fracties werden verdund tot 800–1000 µg tulipaline B/ml, bepaald met UV spectrofotometrie.

Since each Z_f value may differ from this mean value by 10–15%, the bioassay performed as described here must be considered only semi-quantitative.

Table 1 shows the data of a representative series of experiments (for each experiment a new extract of pistils was made and various cultivars were used) to determine the concentration of tulipalin B; similar results were obtained in a series to determine tulipalin A (average of 25 experiments: UV 840, bioassay 830).

Samenvatting

Een semi-quantitatieve biotest voor het aantonen van tulipalines in waterige oplossingen

Beschreven wordt een biotest om de onverzadigde fungitoxische lactonen tulipaline A en B semi-quantitatief te bepalen in fracties verkregen na kolomchromatografie van ruw tulpenextract. De methode wordt toegepast als controle op de bepaling van de concentratie van op de kolom gescheiden tulipalines met behulp van UV spectrofotometrie.

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