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# COMPARISON OF INDUCED PEROXIDASE ACTIVITY IN COTTON PLANTS RESISTANT AND SUSCEPTIBLE TO *VERTICILLIUM DAHLIAE*

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## Summary

Peroxidases participate in many reactions and regulate multiple processes in the plant. Among these, chitin-binding peroxidases play a key role. We have investigated peroxidase activity in seven Upland cotton cultivars, and we found that a two-fold increase occurred in the resistant cotton variety AN-BAYAUT – 2 after inoculation with *Verticillium dahliae*. The peroxidase activity in the resistant variety was due to biosynthesis of chitin binding peroxidase isozymes. The susceptible cotton line C-4727 also showed an increase in peroxidase activity, but most of these peroxidase isozymes did not bind to chitin.

## Aims of the investigation

Study of cotton peroxidases and the role of their isoforms in protective function against phytopathogens attack.

## Objects

Two cotton varieties - AN-Bayaut-2 - resistant and C-4727 – susceptible to pathogens action.
Biochemical methods can be used for early diagnostics of some cotton diseases and for determination cotton sorts under influence of *Verticillium dahliae*.

## Results

We have investigated peroxidase activity in seven cotton cultivars, revealed, that most resistant cultivar is AN-Bayaut-2 among them, and there is less sort C-4727, which is less susceptible for infection (Table 1). We have shown that peroxidase activity increases in leaves of AN-Bayut-2 in 2,2 times on the first day after infection (Fig 1.b). At C-4727 the increase in activity in 2 times was observed on the 5th day only (Fig 1.g). Chitin-specific peroxidases isoforms have been isolated from leaves of the same cotton cultivars on column with chitin. Increase in enzyme activity in the fraction binded with chitin is revealed at sort AN-Bayut-2. It agrees with the electrophoresis data. The peroxidase activity increasing on the first day at AN-Bayaut-2 occurs the account isoform, binded with chitin (Fig 1.d), at C-4727 - for the account isoform, which is not contacted with chitin as it was shown by electrophoresis. Isoelectric focusing has revealed the increase in activity acidic isoform with pI 4.7 and 3.5, the last one is chitin-specific isoform (Fig 2). It was confirmed by dates of capillary electrophoresis (Fig 3). Peroxidase activity in rootlet at 7-day seedling of AN-Bayaut-2 cultivar is higher on 50-65% than control after influence *V.dahliae* and this data is correlated with change of isoenzyme's content demonstrated by electrophoresis (Fig.4 a, b, d, e) . The peroxidase spectrum of rootlets consists of acidic isoforms with pI 3.7 and 4.5 (data not shown). Chitin-specific peroxidase isoforms are isolated from the control seedling of the researched cotton cultivars (Table 1). An isoform with Rf-0,27 is binded with chitin (Fig 4.c,f).

## Conclusion

Under the investigation of enzyme activity changes in leaves of cotton cultivars AN-Bayaut- 2 and C-4727, it is possible to draw a conclusion, that AN-Bayaut-2 shows quick response to pathogen *V.dahliae* penetration. However activity of enzyme is reduced up to control value on the fifth day after infection. The increase of peroxidase activity at C-4727 is insignificant on the first day, but on the fifth day activity grows considerably, the plant continues to render counteraction to pathogen. So anion peroxidase's isoforms can serve as a biochemical marker for selection of cotton cultivars resistant against fungi infection.

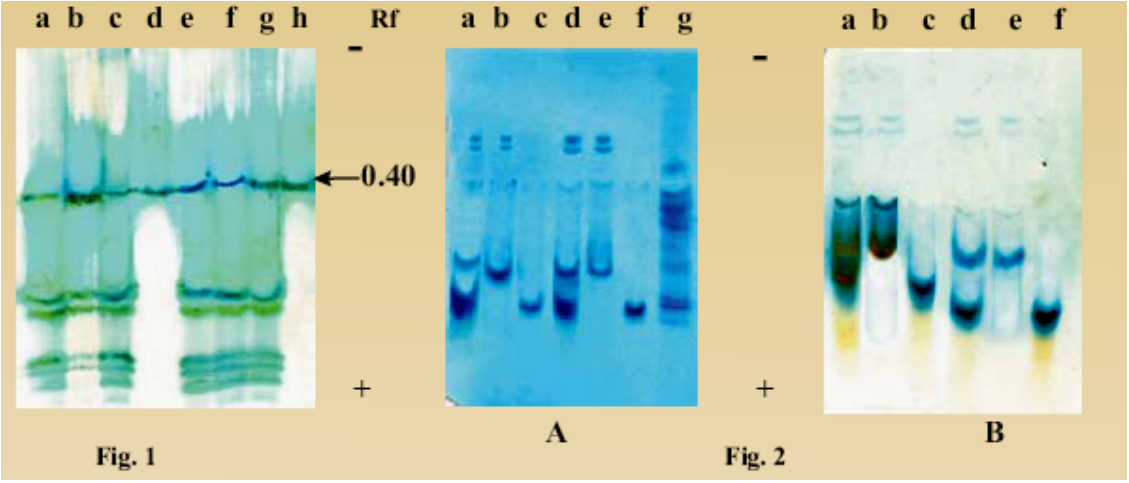


Fig.1 Electrophoretic analysis on 10 % PAGE of peroxidase from leaves of cotton species An-Bayaut-2 and C-4727 infected by *Verticillium dahliae:* a - Control plant An-Bayaut-2 mock inoculated with sterile water; b - Experiment, 1 day after inoculation *Verticillium dahliae;* c - Experiment, 5 day after inoculation *Verticillium dahliae;* d- Fraction bound by chitin; e- Control, leaves of cotton species C-4727; f- Experiment, 1 day after inoculation *Verticillium dahliae*; g- Experiment, 5 day after inoculation *Verticillium dahliae*; h - Fraction bound with chitin

Fig. 2 Isoelectric focusing on PAGE slabs containing ampholine in diapozone pH 3.5 - 10.0 of peroxidase from leaves of cotton species An-Bayaut-2 (A) and C-4727 (B) after infected by *Verticillium dahliae:*
A. Control: a - starting fraction; b - fraction unconnected with chitin; c - fraction connected with chitin; Experiment: d - starting fraction; e - fraction unconnected with chitin; f - fraction connected with chitin; g - IEF Mix 3.6 - 6.6 pH.
B. Control: a - starting fraction; b - fraction unconnected with chitin; c - fraction connected with chitin. Experiment: d - starting fraction; e - fraction unconnected with chitin; f - fraction connected with chitin.

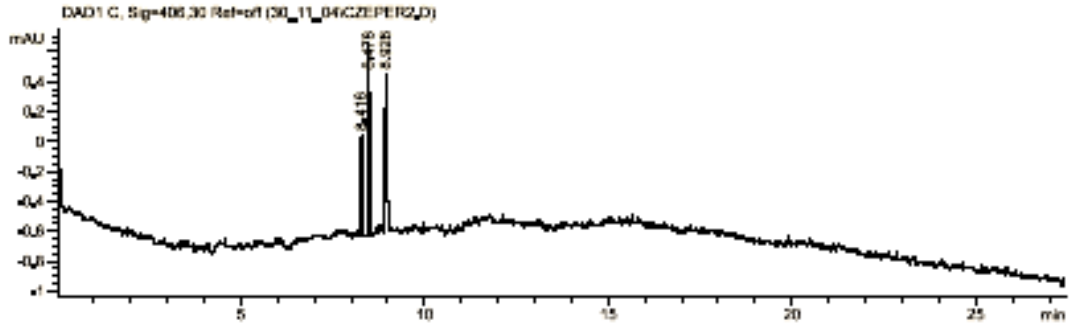


Fig 3: Capillary electrophoresis of enzyme extract from An-Bayaut-2 leaves treated with *Verticillium dahliae*
Run buffer: Borate 20 mM, pH 9,3 ; Capillary: effective length 56 cm internal diameter 50mkm; Injecting: 200 mbar; Temperature: 25 $^{0}$C ; Voltage: 30 kV; Polarity: positive; Concentration of a sample: 0,1 mg/ml

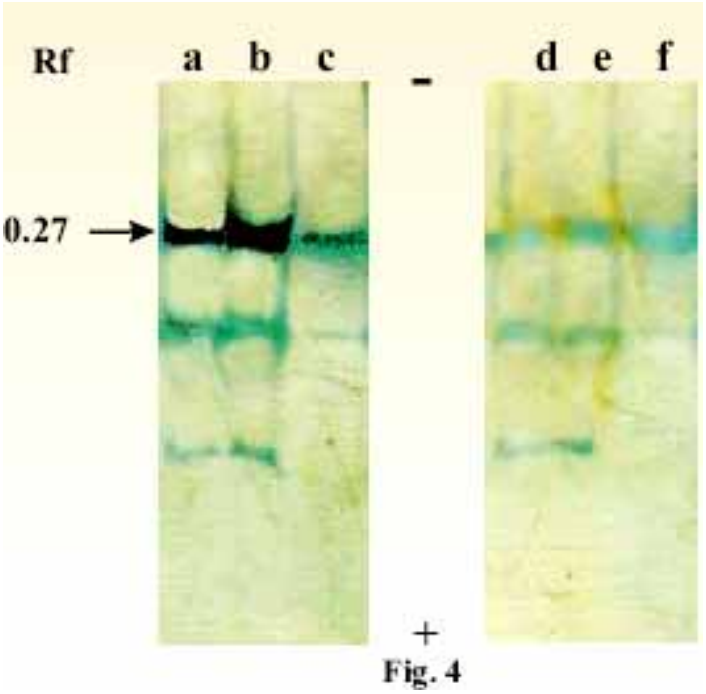


Fig. 4 Electrophoretic analysis on 10 % PAGE of peroxidase from rootlets of seedling by seven days of species An-Bayaut-2 (a, b, c) and C-4727 (d, e, f) of cotton after infected by *Verticillium dahliae:* a, d - Control; b, e - experiment; c, f - fraction bound by chitin from control plants.

Table 1

**Table 1. Peroxidase activity (unit/mg) of fractions binding and non-binding to chitin in control plants from rootlets of seedling of species of cotton**

| Species of cotton | Crud Extract | Chitin Binding | Chitin non-binding |
|---|---|---|---|
| An-Bayaut-2 | 12.10 | 17.20 | 1.98 |
| C-4727 | 9.00 | 1.96 | 8.60 |