## Post-Infection Changes in Sugar and Organic Acid Contents of Chilli (*Capsicum annuum* L.) and Mango (*Mangifera indica* L.) Leaves

The infection of chilli (*Capsicum annuum* L.) and mango (*Mangifera indica* L.) leaves by *Curvularia ovoidea* (HIROE and WATANABE[1]) Muntanola and *Curvularia lunata* (Wakker) BOEDIJN[2] var. aeria (BATISTA, LIMA and VASCONCELOS[3]) M.B. Ellis, respectively caused considerable changes in sugar and organic acid contents. Studies by SRIVASTAVA and TANDON[4] have revealed that fungal invasions cause marked changes in the chemical composition of different parts of plants. But so far there has been no proper study of the changes induced by the above organisms. A detailed study was therefore undertaken.

*Materials and methods.* Leaves of the same age were inoculated with the respective pathogens. 1 g each of healthy and diseased oven-dried and finely-powdered leaves was taken. Extracts were prepared with 25 ml of 80% ethanol. They were filtered and evaporated to dryness. The residues left were dissolved in 1 ml of 20% ethanol and were centrifuged at 2000 rpm for 30 min. The clear supernatant liquids were decanted and used for chromatographic analysis.

The circular paper chromatographic technique employed by RANJAN et al.[5] was followed for detection of sugars. The chromatograms were sectored pieces of Whatman filter paper No. 1 (27 cm in diameter). They were run in *n*-butanol–acetic acid–water (4:1:5, v/v) and were subsequently sprayed with aniline-diphenyl amine-*ortho*phosphoric acid (5 vol. of 4% aniline, 5 vol. of 4% diphenyl amine and 1 vol. of *ortho*phosphoric acid), as recommended by BUCHAN and SAVAGE[6]. They were then heated in an electric oven at 110 °C for 90 sec.

LUGG and OVERALL'S[7] technique of one-dimensional paper chromatography was employed for detection of organic acids. The running solvent was *n*-butanol–formic acid–water (10:2:5, v/v) and spray reagent was 0.04% bromophenol blue in 90% alcohol (w/v). The Rf values of these sugars and organic acids were compared with those of known ones.

*Results and discussion.* The results are recorded in the Table. It is evident from the Table that 3 sugars, viz. sucrose, glucose and fructose, and 2 organic acids, namely malic acid and citric acid, were present in the healthy chilli and mango leaves. Besides these, healthy leaves of mango contained oxalic acid also. It was observed that the 3 sugars present in the healthy leaves were not present in the diseased leaves of the 2 hosts. The amount of organic acids was also markedly reduced under such conditions. The total absence of the sugars and a considerable decrease in the organic acids in infected tissues may be attributed to their utilization by the pathogens.

*Zusammenfassung.* Die Infektion der Blätter von *Capsicum annuum* und *Mangifera indica* mit den Blattpilzen *Curvularia ovoidea* beziehungsweise *lunata* hat zur Folge, dass die freien Zucker Saccharose, Glukose und Fruktose verschwinden und dass der Gehalt an organischen Säuren (Apfelsäure, Zitronensäure, Oxalsäure) vermindert wird.

Sugar and organic acid contents of healthy and diseased leaves of chilli and mango

| Sugar and organic acid | Chilli leaves | | Mango leaves | |
|---|---|---|---|---|
| | Healthy | Diseased | Healthy | Diseased |
| Sucrose | $2^+$ | — | + | — |
| Glucose | + | — | + | — |
| Fructose | + | — | + | — |
| Malic acid | $2^+$ | + | $2^+$ | + |
| Citric acid | $2^+$ | + | $2^+$ | + |
| Oxalic acid | — | — | $2^+$ | + |

The signs +, $2^+$ indicate comparative concentrations; — indicates absence.

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## Interaction between Morphactin, AMO-1618 and Different Gibberellins in Potato Sprout Growth

Although it has been shown that gibberellic acid ($GA_3$) can negate the inhibition of internodal elongation caused by morphactins[1–4] and in pea stem elongation test morphactins have been claimed to act as competitive gibberellin-antagonist[4] but the interaction between morphactins and other gibberellins remains to be seen. Therefore, it was considered of interest to study the interaction between morphactin (methyl-2-chlora-9-hydroxyfluorene-(9)-carboxylate) and different gibberellins and to compare these interactions with those between AMO-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylate methyl chloride) and these gibberellins. The results obtained show that while AMO-induced inhibition is reversed by all the gibberellins used that induced by morphactin is negated only by $GA_3$.

Twenty 'eyes' (1 × 0.5 cm) excised from freshly harvested 'OT-NO' potato tubers were floated for 24 h in each test solution separately in petri-dishes and thereafter these were planted in sand and kept under continuous light (2000 lux intensity). Observations were recorded on the date of commencement of sprouting. The length of sprouts was measured 14 days after treatment. The re-

sults, together with the concentration of test solutions, are summarized in the Table.

Effect of 1 and 10 mg/l of $GA_3$, 4, 7, 13, morphactin, and AMO-1618 alone and in combination is given. It was observed that sprouting starts almost simultaneously in control and treated 'eyes'. Morphactin inhibited sprout elongation, the inhibitory effect increasing with concentration. AMO, on the other hand, stimulated sprout growth at low concentration but was inhibitory at higher concentration. Gibberellins stimulated elongation of sprout growth, the stimulatory effect being more in higher concentration. The 4 gibberellins tested showed differential response in an order of $GA_7 > GA_3 > GA_4 > GA_{13}$. Water-treated 'eyes' showed minimum growth. The order of activity of different GAs observed here is different to that obtained by other workers in other biological tests[5-8].

Considering the interaction between different gibberellins and morphactin or AMO, the results showed that inhibition caused by morphactin is reversed only by $GA_3$ whereas AMO-induced inhibition was negated by all the gibberellins, although the length of sprouts when treated with different gibberellins in combination with AMO was less as compared to those treated with respective gibberellins alone.

The results indicate that morphactin in contrast to AMO – a gibberellin antagonist[9], is not the competitive inhibitor of action of all the gibberellins tested and is specific only to $GA_3$ in its mutual antagonist effect, because if it was not so that application of other gibberellins should have caused reversal of morphactin-induced inhibition as they did when used in combination with AMO-1618[10].

Length of sprouts, produced on excised dormant potato 'eyes' treated with different concentrations of gibberellins, morphactin and AMO-1618 alone and in combination, observed 14 days after the treatment

| Treatment | Concentration (mg/l) | Mean length of sprout (cm ± S.E.) |
|---|---|---|
| Control | – | 0.32 ± 0.07 |
| | 1.0 | 0.99 ± 0.36 |
| $GA_3$ | 10.0 | 3.47 ± 0.94 |
| | 1.0 | 0.61 ± 0.20 |
| $GA_4$ | 10.0 | 1.66 ± 0.48 |
| | 1.0 | 3.01 ± 0.90 |
| $GA_7$ | 10.0 | 4.10 ± 0.80 |
| | 1.0 | 0.74 ± 0.30 |
| $GA_{13}$ | 10.0 | 0.67 ± 0.40 |
| | 1.0 | 0.29 ± 0.07 |
| Morphactin | 10.0 | 0.26 ± 0.10 |
| | 1.0 | 0.69 ± 0.10 |
| AMO-1618 | 10.0 | 0.30 ± 0.12 |
| $GA_3$ + Morphactin | 10.0 each | 2.54 ± 0.82 |
| $GA_4$ + Morphactin | 10.0 each | 0.34 ± 0.21 |
| $GA_7$ + Morphactin | 10.0 each | 0.10 ± 0.02 |
| $GA_{13}$ + Morphactin | 10.0 each | 0.28 ± 0.11 |
| $GA_3$ + AMO-1618 | 10.0 each | 2.50 ± 0.69 |
| $GA_4$ + AMO-1618 | 10.0 each | 1.37 ± 0.43 |
| $GA_7$ + AMO-1618 | 10.0 each | 2.09 ± 0.68 |
| $GA_{13}$ + AMO-1618 | 10.0 each | 1.01 ± 0.23 |

*Zusammenfassung.* Die Hemmwirkung, verursacht durch AMO-1618, auf das Auswachsen isolierter Kartoffelknospen lässt sich durch 4 getestete Gibberelline ($GA_7$, $GA_3$, $GA_4$ und $GA_{13}$) aufheben. Die Hemmung durch Morphactin wird jedoch nur von $GA_3$ aufgehoben. Morphactin scheint kein kompetitiver Inhibitor der getesteten Gibberelline zu sein.

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## Bacterial Mutants of Hydrogenomonas Lacking Poly-β-Hydroxybutyric Acid

Poly-β-hydroxybutyric acid (PHBA) is a storage product of many aerobic and phototrophic bacteria. The accumulation of this lipid occurs preferentially when energy and carbon are available in excess and when growth is limited by the absence of utilizable compounds of nitrogen, sulphur or phosphorus. The lack of oxygen also results in the storage of PHBA. In *Hydrogenomonas* intracellular PHBA-deposition can amount to 65% of the cellular dry weight when the cells are incubated in a growth medium lacking a nitrogen source[1].

Mutants of *Hydrogenomonas H16* which are not able to store PHBA can be recognized and isolated by the following procedure: the cell suspension is distributed on nutrient agar plates (100–200 cells per plate) and incubated at 30 °C for 2 days. The colonies are transferred by replica plating to agar plates containing a medium low in nitrogen (0.005% ammonium chloride) and rich in carbon source (0.5% fructose). After 4 or 5 days incubation the colonies grown are treated with Sudanblack B: the agar plates are flooded with 10 ml of a 0.2% solution of Sudanblack B in 96% ethanol. After 20 min this solution is replaced by 10 ml of 96% ethanol, and after 1 min this is poured off. While the colonies of the PHBA-rich cells retain the dye and remain dark blue coloured, the colonies of the PHBA-poor or PHBA-free cells are completely decolorized during the differentiation process and appear