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Biochemical Changes in Lettuce Plants Infected with Lettuce Mosaic Virus

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Abstract. Lettuce leaves from 0-30 days after inoculation with lettuce mosaic virus (LMV) were examined and compared with healthy leaves for chlorophyll (total, a and b), nitrate, nitrite contents, polyphenol oxidase, and peroxidase enzyme activities. Samples were collected at 5-day intervals. Total chlorophyll, chlorophyll a and chlorophyll b decreased in infected lettuce cv. Eskandrany, with a pronounced effect in about 30 days, when symptoms appeared conspicuous. There was practically no difference in NO_3 content except at 15 days after inoculation where it was significantly higher in infected plants as compared to its counterpart healthy plants. An increase in most of the free amino acid content was observed in 5,15 and 30 days after inoculation.

Polyphenol oxidase activity was considerably higher in leaves 10 days after inoculation, whereas peroxidases showed similar effect in 15 days samples. While the activity of cell wall-ionically bound PO did not change at 20 days post-inoculation, the activity of cell wall-covalently bound PO was doubled by infection with an increase of 7.26% in the total PO activity.

Introduction

Lettuce mosaic virus (LMV) has been shown to be one of the most destructive viruses to lettuce production [1]. The first symptom to appear on lettuce is vein clearing of the young expanding leaves followed by the development of mosaic symptoms which may, sometimes, be associated with veinal necrosis. In general, diseased plants appear stunted with rosetted and loose heads of poor quality [2].

Most of the work that has so far been published on this virus host combination has concerned with isolation and identification of the virus [3], virus seed transmissibility and factors affecting it [4], its cytopathology and host resistance [5].

Very little is known about the biochemical alterations due to LMV infection. The present work was, therefore, undertaken, to understand the changes in

chlorophyll content, polyphenol oxidase and peroxidase activities following the infection with LMV.

Materials and Methods

A virus isolate previously identified as lettuce mosaic virus [6] was used throughout this study. Inoculum preparation and inoculation procedure adopted by the same authors were followed.

Total chlorophyll was extracted according to the method of Goodwin [7, p. 583]. Concentration of a,b and total chlorophyll was calculated as described by Ross [8].

Nitrite (NO_2) and nitrate (NO_3) were determined according to the method of Chapman and Pratt [9] and Bremner [10, pp. 1179-1237]. Nitrite and nitrate nitrogen concentration was determined in μg/ml using a calibration curve relating NO_2-N (0.2 to 1.0 μg/ml) or NO_3-N (0-50 μg/ml) concentration to absorbance at 530 and 420 nm respectively.

Tissues to be analysed were washed with distilled water and dried at 80°C until no change in weight was noticeable. Portions of dry material each of 0.5 g was extracted with 70% hot ethyl alcohol (1 g/50 ml) followed by centrifugation at 2500 $\times$g for 5 min. The dry residue was, then, dissolved in 2.5 ml of 0.2 M citrate buffer, pH 7.5, and filtered through glass wool. Filtrates were kept at -20°C until assayed by Beckman 116 GL amino acid analyzer.

Peroxidase (PO) and polyphenoloxidase (PPO) enzymes were extracted from frozen leaf tissue using the method described by Wagih and Coutts [11]. Cell wall peroxidase enzyme was extracted according to the method of Birecka and Miller [12]. PO activity was assayed as outlined by Wagih and Coutts [11], whereas PPO activity was measured by following the oxidation of catechol at 495 nm [13]. Absolute PO and PPO activity values were expressed as $\triangle$ A/min./g F.wt. All assays were performed in triplicate.

Results and Discussion

Biochemical changes in lettuce plants infected with LMV chlorophyll content

Changes in chlorophyll content as the disease progressed were remarkable as evident from Fig. 1. This effect became detectable 15 days post-inoculation when symptoms were in their early development and was most noticeable 30 days after inoculation when symptoms were conspicuous. The total chlorophyll in LMV-infected Eskandrany leaves as compared to healthy ones decreased by 7.33, 28.38 and 42.93% at 10, 15 and 30 days after inoculation. The corresponding reduction in

chlorophyll a content was 5.13, 26.5 and 34.21%, respectively. These were associated with reduction in chlorophyll b amounting to 0.00, 32.25 and 54.86%, respectively. These findings, agree with those of Fegla *et al.* [14] who found that the reduction in total chlorophyll content of lettuce leaves was greater when infection with LMV occurred at an early stage of plant development. Decrease in chlorophyll content was also demonstrated with other virus diseases and this reduction was attributed to the higher chlorophyllase activity in the virus infected tissue [15].

Nitrite and nitrate concentrations

The nitrate (NO_3) concentration in diseased and healthy Eskandrany leaves were 2.0, 3.3, 4.6 and 1,9, 6.4, 4.4 μg/ml at 0, 15 and 30 days after inoculation. There was practically no difference in NO_3 concentration between healthy and diseased plants at 0 and 30 days. However, NO_3 decreased drastically, 15 days after inoculation. This change coincided with the onset of symptom development. An increase in nitrate reductase at this stage is suspected of causing this reduction by converting nitrates into ammonia. This possibility is suported by the finding that nitrate nitrogen leads to resistance while ammonical nitrogen leads to susceptibility [16]. However, the linear increase in NO_3^- concentration observed in infected tissue is difficult to explain unless concomitant increase in ammonical nitrogen transformed from other sources is speculated. Nitrite was not detected in diseased or healthy tissues.

Free amino acids

Seventeen free amino acids: Alanine, Argenine, Aspartic Cysteine, Glutamic, Glycine, Histidin, L-Leucine, Leucine, Lysine Methionine, Phenylalanine, Proline, Serine, Threonine, Tyrosine, Valin as well as Amonia were detected in healthy and infected leave.

As compared to healthy control, a noticeable increase in all amino acids was noticed 5 and 15 days after inoculation. A similar increase in the concentration of most amino acids except aspartic acid, methionine, tyrosine and histidine was detected 30 days post-inoculation. In contrast, a fall in the concentration of all amino acids except proline, leucine, tyrosine and lysine was observed 10 days after inoculation (Table 1). These results agree to some extent with those of Fegla and Sheir [17] who reported a decrease in the concentration of most amino acids during the early stages of virus infection and a general tendancy towards increase when the disease became well established. Bozarth and Diener [18] found that the total amino acid concentration was greater in tobacco leaves infected with potato virus Y or potato virus X and Y than the healthy ones. But the detectable change in the concentration of a specific amino acid (s) or amide (s) in any virus infected plant seems to depend on the host virus combination and time of infection.

Table 1. Effect of lettuce mosaic virus infection on the free amino acids of the susceptible lettuce cultivar, Eskandrany.

Amino acid \ Days after inoculation.	Concentration of amino acids (mg/g dry weight)*											
	Healthy						Infected					
	0	5	10	15	30	$\bar{x}$	0	5	10	15	30	$\bar{x}$
Alanine	31	51	44	9	59	39	11	90	21	24	78	45
Amonia	13	18	23	8	20	16	7	28	3	15	30	17
Argenine	2	40	24	2	22	18	2	72	8	4	23	22
Aspartic	16	30	30	8	69	31	8	82	19	18	61	38
Glutamic acid	47	70	39	18	115	58	17	146	27	26	110	65
Glycine	3	5	5	3	7	5	2	8	4	5	10	6
Histidine	5	11	8	3	15	8	2	35	3	1	14	11
L.leucine	7	14	13	8	17	12	6	35	15	9	32	19
Leucine	15	26	27	9	41	23	3	92	23	20	53	38
Lysine	1	2	2	1	3	2	1	7	3	3	4	4
Methionine	18	33	36	18	30	27	21	50	35	26	18	30
Phenylalanine	5	10	11	2	13	8	1	33	8	5	21	14
Proline	11	57	62	24	77	46	8	66	70	34	133	62
Serine	10	36	26	9	29	22	5	62	13	11	44	27
Threonine	6	17	15	4	9	10	2	29	8	6	16	12
Tyrosine	18	28	29	14	20	22	16	45	35	22	15	27
Valine	5	14	10	3	23	11	1	40	9	8	42	20
Total	211	461	405	143	568	21	112	919	307	234	703	27

* Concentrations shown are 100 times the actual values.

Peroxidase activity

Peroxidase (PO) activity (1.5×10^{-4}, $\triangle$A/min/g.F.wt) did not significantly change until the tenth day. At 15 days after inoculation, a significant increase (3.4×10^{-4}) was detected in virus-infected tissue as compared to mock inoculated plants (1.5×10^{-4}). This high activity returned to normal, 30 days following inoculation. When the activity of soluble, cell wall-ionically bound and cell wall-covalently bound peroxidase was estimated in Eskandrany, 20 days after inoculation, the activity of peroxidases did not significantly change between uninoculated and inoculated plants with very close to total activity amounting to 3.72×10^{-4} and 3.99×10^{-4}, respectively. Specifically, the activity of cell wall-ionically bound PO (1.4×10^{-4}) did not change at this time whereas that of cell wall-covalently bound PO (0.7×10^{-4}) was doubled.

In other host-virus combinations, enhanced PO activity has frequently been correlated with symptom severity [19,20] but this does not seem to be the case in LMV-

infected lettuce. This is concluded from the fact that PO activity decreased slightly after 15 days post-inoculation and became not significantly different from the naturally increased activity in healthy tissue at 30 days post-inoculation.

Polyphenol oxidase

No change in the activity (1.5×10^{-4} , $\triangle$A/min/g.F.wt) of polyphenol oxidase (PPO) was detected at the fifth day, but the activity reached its highest level (8.4×10^{-4}), 10 days-post-inoculation. No significant difference occurred between healthy and infected plants. PPO activity returned to normal levels from the 15 th day onwards.

As regards PPO, the results obtained in this study showed a pattern of activity in infected tissue similar to that in healthy tissue. The pronounced relative increase in PPO activity in infected tissue at 10 days post inoculation seems to be a response to inoculation rather than infection and/or symptom development because of the similar increase observed in healthy tissue at the same time after inoculation.

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التغيرات البيوكيميائية في نباتات الخس المصابة بفيروس موزايك الخس

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ملخص البحث. تمت في هذه الدراسة مقارنة عينات من أوراق الخس مأخوذة على فترات من صفر ـ ٣٠ يوم بعد التلقيح بفيروس موزايك الخس بعينات من الأوراق السليمة فيما يختص بمحتواها من الكلوروفيل (الكلي، كلوروفيل أ، كلوروفيل ب) ومحتواها من النترات والنيتريت وكذا نشاط إنزيم البولي فينول أوكسيديز وإنزيم البيروكسيديز وقد جمعت العينات على فترات طولها خمسة أيام. واتضح من الدراسة أن الكلوروفيل الكلي وكلوروفيل أ وكلوروفيل ب انخفض محتواها في أوراق الصنف إسكندراني المصابة بالفيروس وكان التأثير أكثر وضوحًا عند ٣٠ يوم من التلقيح عندما كانت الأعراض ظاهرة بوضوح. هذا ولم يكن هناك فرق من الناحية العملية بين الأوراق المصابة والأوراق السليمة في محتواها من النترات فيما عدا عند ١٥ يوم من التلقيح حيث كان محتوى النترات أكبر في الأوراق المصابة عنه في الأوراق السليمة. وقد ظهرت زيادة في محتوى الأوراق المصابة من الحموض الأمينية الحرة عند ٥، ١٥ و٣٠ يوم بعد التلقيح.

وقد كان نشاط إنزيم البولي فينول أوكسيديز أعلى في الأوراق المصابة عند ١٠ أيام بعد التلقيح في حين تساوى نشاط إنزيم البيروكسيديز في العينات المأخوذة ١٥ يوم بعد التلقيح. وبينما لم يتغير نشاط إنزيم البيروكسيديز المرتبط أيونيًا بالجدار الخلوي عند ٢٠ يوم بعد التلقيح فإن نشاط إنزيم البيروكسيديز المرتبط تعاونيًا بالجدار الخلوي قد تضاعفت نتيجة الإصابة بزيادة بلغت ٧,٢٦٪ في النشاط الكلي لإنزيم البيروكسيديز.

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