

Activities of enzymes connected with IAA oxidation in cucumbers of various sex types

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Abstract

Activity of peroxidase, catalase, and IAA-oxidase in flower buds, stems and stem callus cultures derived from four pure genetic lines of *Cucumis sativus* L., i.e. male, monoecious, female and hermaphrodite was determined. The activities of all enzymes in flower buds and callus cultures are considerably higher in the female lines than in male ones. IAA-oxidase activity is the highest in female line, independently of the type of plant material under examination. The activities of the remaining two enzymes depend on the type of tissue or organ under examination.

INTRODUCTION

Previous investigations on sex determination in cucumber were performed with plants *in vivo* (Kubicki 1965, 1966; Kubicki and Borkowski 1965, Galun 1959; Astmon and Galun 1962, Galun et al. 1965) and with isolated floral buds cultured *in vitro* (Galun, Jung and Lang 1962, 1963; Mitchell and Wittwer 1962). These results do not explain, however, the formation of definite sex features. The mechanism responsible for these transformations is also unknown.

Considering the unquestionable role of auxins in sex determination we examined, the activities of enzymes connected with IAA oxidation as related to the sex type of cucumbers. As it results from our previous research (Maciejewska-Potapczykowa et al. 1970) great differences occur in IAA-se activity between female and monoecious cucumber plants. Only very few data concerning the mechanism of the action of enzymes oxidizing IAA are available.

In view of the considerable genetic differences in the material under examination and its metabolism depending on the sex type (Maciejewska-Potapczykowa et al. 1972) we examined the activities of peroxidase, catalase and IAA-oxidase in callus tissues cultured continuously *in vitro* derived from fragments of plants belonging to four sex types of *Cucumis sativus* L.

Since the metabolism of plant tissues can undergo transformation in the course of *in vitro* culture, we also performed investigations on analogous plant fragments, that is stem parts situated below the buds and floral buds, in order to prove a relationship between the activity of the enzymes.

MATERIAL AND METHODS

The same plants were used as in previous investigations (Maciejewska-Potapczykowa et al. 1972). Sub-bud stem fragments, callus tissues isolated previously from subapical stem fragments, and flower buds were analysed. Male flower buds were taken only from monoecious plants, whereas in the case of other sex lines the buds were of the same sex as the line. The buds chosen for isolation were old enough to pass an early ontogenetic phase of bisexuality. At the moment of isolation the final sex type was clearly fixed.

The activity of catalase was determined by the method of Bonnichsen et al. (1947), that of peroxidase according to Reifer and Grabianowska (1952) and the activity of IAA-oxidase by the method of Stutz (1957). Approximately 200 mg samples were prepared from several plants (stem fragments and floral buds). The samples of callus tissues (passage 6-9) weighing about 250 mg each contained parts of three different tissue colonies of the same series. The activities of catalase and peroxidase are expressed in units per 100 mg of fresh weight, the activity of IAA-oxidase in μl of O_2 per hour per 100 mg of fresh weight. Each result represents the mean value of four determinations.

RESULTS

Callus tissues. The results of experiments on the activities of catalase, peroxidase and IAA-se are summarised in Table 1. The highest activities of these enzymes were found in callus tissues of the female line, the lowest in those of the monoecious line. Maximal differences occurred in the case of IAA-oxidase. The activity

Table 1

Enzyme activities in callus tissues of various genetic lines of cucumber

Lines	Mo	An	H	G
Peroxidase	131.4 69.4%	188.4 100%	256.2 136%	294.7 156.3%
Catalase	30000 78.9%	38000 100%	50000 131.4%	62500 164.0%
IAA-oxidase	833.5 43.0%	1936.2 100%	2480.7 128.1%	2906.7 150.1%

Lines: Mo — monoecious; An — male; H — hermaphrodite; G — female.

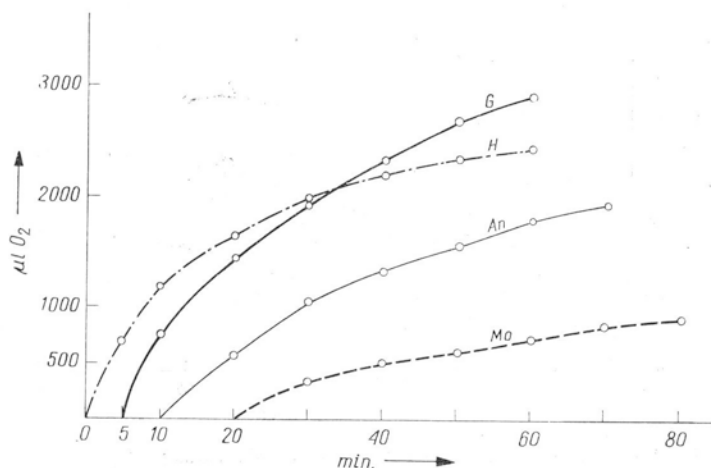


Diagram 1. IAA-se activity in callus cultures derived from stem fragments of four pure genetic lines of *Cucumis sativus* L.

of this enzyme in the tissues of the female line, expressed in $\mu\text{l O}_2$ per hour was 2906,7 whereas in those of the monoecious line scarcely 833.5, that is approximately three times lower. Along with an increase on this enzyme activity in the tissues of various genetic lines, the duration of the lag-phase period decreased, proving the presence of IAA-se inhibitors (Diagram 1). The longest lag-phase period was found in the case of monoecious line tissues, the shortest in female line. IAA-se activity in the tissues of hermaphrodite line was highest during initial 20 minutes (lack of lag-phase), then dropped below the value attained by the female line tissue. The values obtained for the male line were higher by 50% than those for monoecious line, however, the values established for the hermaphrodite line were lower by 20% than those for the female line.

Table 2

Enzyme activities in apical stem fragments of various genetic lines of cucumber

Lines	Mo	An	H	G
Peroxidase	11.2 122.0%	9.2 100%	6.0 65.3%	9.2 100%
Catalase	3280 195.0%	1680 100%	2520 150.0%	1120 66.6%
IAA-oxidase	297.8 106.3%	280.1 100%	214.2 76.4%	370.4 130.3%

Explanations as in table 1.

Stem fragments situated below floral buds. The results of analogous experiments made with these fragments are summarised in Table 2. The respective

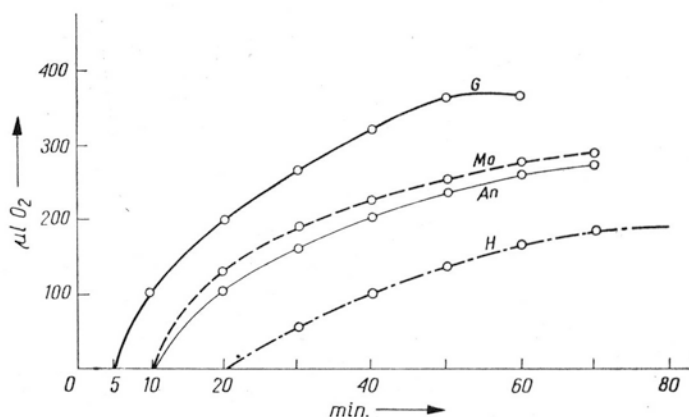


Diagram 2. IAA-se activity in apical stem fragments of various genetic lines of cucumber

activities of the enzymes under examination deviate from the values obtained for callus tissues. Highest activities of peroxidase and catalase were found in the stem fragments of the monoecious line, lowest in the hermaphrodite (peroxidase) and female (catalase) lines. IAA-se activity however, similarly as in callus tissues, was the highest in the stems of the female line — 370.4; in the male line — 280 $\mu\text{l O}_2/\text{hour}$.

The lag-phase periods in male and female lines were identical as in callus tissues (Diagram 2) despite the fact that absolute values are almost tenfold lower than in the case of calluses.

Table 3

Enzyme activities in floral buds of various genetic lines of cucumber

Lines	Mo	An	H	G
Peroxidase	19.2	14.4	16.8	25.2
	133.0%	100%	116.6%	175.0%
Catalase	2320	1400	2080	2720
	165.7%	100%	148.5%	194.2%
IAA-oxidase	190.1	231.2	241.6	533.6
	82.1%	100%	104.4%	230.8%

Explanations as in table 1.

Floral buds. The activities of enzymes oxidizing IAA in the buds of four cucumber sex types are shown in Table 3. Highest activity of peroxidase and catalase was found in the female line, lowest in the male line. It is characteristic that catalase activity is very high as compared with the activity of the remaining enzymes. The highest IAA-se activity expressed in $\mu\text{l O}_2/\text{hour}$, was found in the female line — 533.6, the lowest in the monoecious and male lines — 190.1 and 230.2 respectively. As seen in diagram 3 IAA-se activity in the buds of the female line is twice as high as that of the remaining lines and in this only case the lag-phase

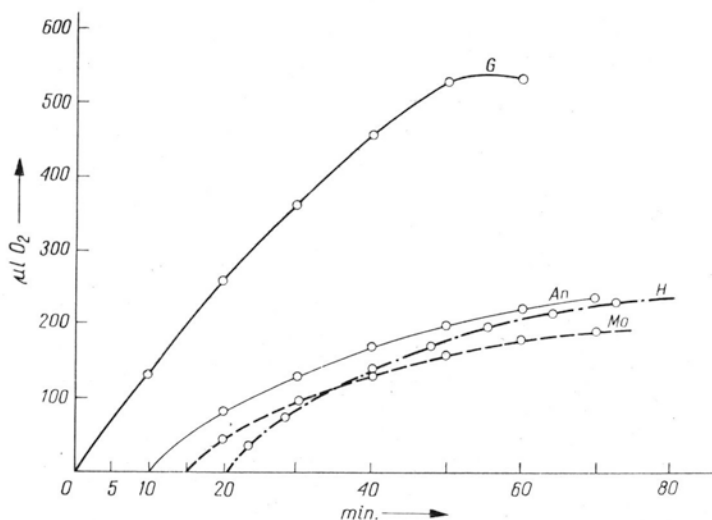


Diagram 3. IAA-se activity in floral buds of various genetic lines of cucumber plants

does not occur. The activity of this enzyme in the buds of the remaining lines is similar, lag-phase periods, however are within the limits 10 minutes in the male line to 20 minutes in the hermaphrodite line.

DISCUSSION

Among scarce papers dealing with the biochemical aspects of sex determination Sidorskiĭ's (1965) investigation should be noted. This investigation showed considerable differences in the content of some carbohydrates and in the redox potential occurring in leaves as related to the sex type of the developing flowers. The formation of female flowers is connected with a higher redox potential than that of male ones. This difference disappears after the end of flowering.

The results of our studies indicate certain relationships between the activities of catalase, peroxidase and IAA-se in some tissues and organs of four *Cucumis sativus* sex types. On the basis of the data shown in tables 1 and 3 it appears, that the activities of the enzymes under examination in floral buds and callus tissues are the highest in the female line. A comparison of the absolute values of respective enzymes in various tissues (floral buds, apical stem fragments and corresponding callus tissues) of cucumber leads to the conclusion that the highest activities of the enzymes under examination occurs in callus tissues, the lowest in stems. Low enzyme activities in the stems as compared with that in flower buds reflect a natural physiological activity of these organs. The tenfold higher activities found in calluses are connected with their meristematic character. Differentiated stem cells cultured in vitro pass to a stage of intensive multiplication and of great metabolic activity.

It is characteristic that the results obtained for flower buds and calluses of female and male lines show a permanent tendency, that is: the activities of all enzymes under examination in the female line are high and in the male line low. In mixed lines (as regards the sex type) IAA-se activity is higher in the hermaphrodite line as compared with the monoecious one. The values obtained for the remaining enzymes change, however, according to the kind of tissue or organ. It should be emphasized that IAA-se activity and lag-phase periods in the hermaphrodite line are identical for both: flower buds and stems; in calluses the activity of this enzyme is exactly tenfold higher (Tables 1, 2, 3, Figs. 1, 2). This exceptional IAA-se stability confirms the results of Galun et al. (1963) who found that bisexual flower buds in cucumber hermaphrodite plants, not possessing any mechanism regulating the formation of stamens or pistils (the lack of A-gene), are more resistant to the factors modifying their development.

The results of our previous investigations (Maciejewska-Potapczykowa et al. 1970) seem to confirm the assumption that the activity of enzymes connected with IAA destruction corresponds to a certain degree to the level of endogenous auxin: high enzyme activity meaning low auxin content and vice versa. Under such an assumption the results of this study seem to be paradoxical. The data of Galun et al. (1963) indicate that auxin takes part in the mechanism of ovary formation but only at lower concentrations of this hormone (0.1 mg/l), its higher concentrations did not stimulate ovary development. If we assume, that the enzyme induction is the result of IAA appearance and the factor counteracting an excess of its optimal level, a high activity of IAA-se in flowers and some tissues of female line (as compared with monoecious and male ones) seems to be explained.

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*Aktywność enzymów utleniających IAA u roślin czterech różnych linii genetycznych
Cucumis sativus L.*

Streszczenie

Porównywano aktywność peroksydazy, katalazy i IAA-zy w pąkach kwiatowych, szczytowych fragmentach łodyg i odpowiadających im tkankach kalusowych roślin czterech, czystych linii genetycznych *Cucumis sativus* L., a mianowicie: jednopienniej (Mo-298), męskiej (An-314), hermafrodytycznej (H-713-5), żeńskiej (G-MSU-713-5). Stwierdzono, że:

1. Aktywności wszystkich badanych enzymów w pąkach kwiatowych i tkankach kalusowych są najwyższe dla linii żeńskiej.

2. Wyniki otrzymane dla pąków kwiatowych i kalusów linii żeńskiej i męskiej wyrażają pewną stałą tendencję, tzn. że aktywności wszystkich badanych enzymów są wysokie u linii żeńskiej i niskie u linii męskiej. U linii mieszanych pod względem płci zależność ta dotyczy tylko IAA-zy, której aktywność jest wyższa u linii hermafrodytycznej w porównaniu z jednopienną. Wartości uzyskane dla pozostałych enzymów zmieniają się zależnie od rodzaju badanej tkanki czy organu.

3. Aktywność IAA-azy u linii hermafrodytycznej wykazuje dużą stabilność: bezwzględne wartości oraz okresy lag są identyczne zarówno dla pąków kwiatowych, jak i łodyg, a dla kalusów dokładnie $10\times$ wyższe.

4. Aktywność IAA-azy jest najwyższa zawsze u linii żeńskiej niezależnie od rodzaju badanej tkanki czy organu.

5. Ogólnie najwyższe wartości dla badanych enzymów otrzymano w tkankach kalusowych, a najniższe w łodygach.

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