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Isoperoxidase and isopolyphenol oxidase spectra in male and female tissues of *Actinidia deliciosa in vitro*

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Abstract

The isoperoxidase and isopolyphenol oxidase patterns were studied in undifferentiated calli and regenerated shoots and roots of two cultivars of *Actinidia deliciosa*: one female cv. Hayward and one male cv. Matua. One characteristic, very stable isoperoxidase band ($0.85 < Rf < 0.90$) was found only in the male callus, shoots and roots cultured on media supplemented with zeatin (ZEA) or indole-3-butyric acid (IBA). In contrary, one isopolyphenol oxidase band ($0.35 < Rf < 0.40$) was typical for male callus and shoots cultured on the medium enriched with ZEA and another one ($0.85 < Rf < 0.90$) for male shoots and roots cultured on the medium with IBA. These specific bands had never been found in female cultivar.

Introduction

It is well known that plant tissues *in vitro* have to undergo a complex adaptation to overcome several stress factors during isolation and culture. Some isoenzymes, especially peroxidases and polyphenol oxidases are very sensitive to wounding, exogenous hormones and variable environmental factors (light, gravity, *etc.*).

Peroxidases often increase as a response to stress and one of their principle roles is the cellular protection from oxidative reactions. Moreover, they have very great catalytic versatility which enables them to work as multifaceted enzymes (Siegel 1993). Polyphenol oxidase has no clearly established function. Its primary function is modulation of photosystem I, reduction of molecular oxygen (pseudocyclic photophosphorylation). Moreover, polyphenol oxidase also catalyses the oxidation of phenolic compounds, especially *o*-diphenols. Reaction products can serve as

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Abbreviations: BAP - 6-benzylaminopurine; 2,4-D - 2,4-dichlorophenoxyacetic acid; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; KIN - kinetin; MS - Murashige-Skoog medium; Rf - electrophoretic mobility; ZEA - zeatin.

hydrogen carriers and enable to oxidise various substrates in biological systems (Sherman *et al.* 1991).

Recently, the peroxidases have proved to play important role in determining of some physiological and morphogenic processes *in vitro*. Some specific peroxidases appear during floral initiation in shoot apices (Nakanishi and Fujii 1992), others can serve as a predictive marker of rooting performance in micropropagated shoots (Gaspar *et al.* 1992) or as a marker of somatic embryogenesis in embryogenic and non-embryogenic callus lines (Zhou *et al.* 1992).

Some authors also claimed that peroxidases can be used as markers of sexual differentiation (Champault *et al.* 1981, Hirsch and Fortune 1984). Hirsch and Fortune (1984) reported that tissue cultures of dioecious plant seem to be an excellent tool for investigations of the hormonal regulation of sexual differentiation. According to these authors, sexual genes are not efficient only at the moment of the differentiation of sexual organs (flower buds), but also in undifferentiated callus tissue devoid of any sexual morphogenesis. Markova *et al.* (1992) have found higher peroxidase levels in the stamens of tomato male-sterile lines than in fertile ones and also the presence of specific isoperoxidases in male-sterile lines only.

The isoperoxidase and isopolyphenol oxidase patterns were studied in this work to compare two cultivars of *Actinidia deliciosa* cv. Hayward and cv. Matua representing different sexes. Isoenzyme spectra of undifferentiated calli and during morphogenic processes *in vitro* (shooting and rooting) were also studied.

Materials and methods

Plant material and tissue cultures: Lateral buds (from shoot tip vines) of two kiwifruit cultivars representing two sexes: female cv. Hayward and male cv. Matua were used as explants. Stem segments with lateral buds were harvested and sterilized in Chloramin B (800 mg dm^{-3}). After five rinses with sterile distilled water explants were isolated and placed on the shoot-induction MS medium (Murashige and Skoog, 1962) complemented with 5 mg dm^{-3} BAP or 1 mg dm^{-3} ZEA, 500 mg dm^{-3} myoinositol, 20 g dm^{-3} saccharose and 7 g dm^{-3} agar (*Bacto-Difco*), pH was adjusted to 5.5 with 1M NaOH before autoclaving at 120 °C for 20 min. Regenerated shoots were harvested and subcultured every 4 weeks on MS medium with ZEA (1 mg dm^{-3}). Single shoots 25 - 30 mm in length were transferred for rooting on half-strength MS medium containing IBA (0.5 mg dm^{-3}) with or without agar. Calli, which had arisen on shoot bases were grown on the MS medium with ZEA (1 mg dm^{-3}). Callus tissues, shoots and roots of male and female plants were analysed for isoenzyme composition.

Biochemical techniques: Samples for detection of isoperoxidases and isopolyphenol oxidases were taken from shoots and calli grown on shoot-induction medium enriched with 1 mg dm^{-3} ZEA and shoots and roots grown on rooting medium with 0.5 mg dm^{-3} IBA of both genotypes representing different sexes.

Crude enzyme extracts were prepared by grinding plant material in 150 mM TRIS-citrate buffer containing 6 mM ascorbic acid, 6 mM cystein hypochlorite and 0.5 M saccharose (pH 8.3). The homogenates were centrifuged (12 000 *g*, 5 min, 0 - 4 °C) and supernatants were used for enzyme analysis. Isoenzyme patterns were determined using vertical disc electrophoresis on 7 % polyacrylamide gel (pH 8.9) according to Ornstein (1964) and Davis (1964). Electrophoresis was carried out at 3 mA per tube and 40 mm^3 of enzyme extracts (corresponding to 150 - 200 μg proteins) were loaded on each tube. Isoperoxidases were visualised with 0.2 % quaiacol or 0.1 % benzidine in 0.1 M acetate buffer (pH 5.5) containing 0.1 % H_2O_2 and polyphenol oxidases with 0.1 % *p*-phenylenediamine in 0.1 M acetate buffer (pH 5.6). After staining, the distances of bands from the origin were calculated as electrophoretic mobility (Rf).

Results and discussion

Isoenzyme composition of peroxidase visualised with benzidine: Five isoperoxidase bands from male callus and four from female were obtained on the media MS supplemented with ZEA (Fig. 1). Peroxidase isoenzyme occurring in the band

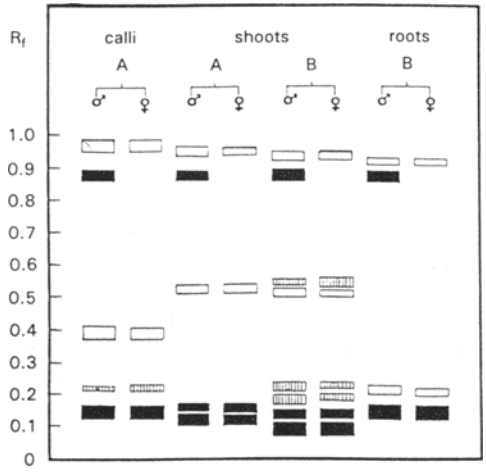

Fig. 1. Isoperoxidase pattern of calli, shoots and roots visualised with benzidine. Plant material was obtained from male plant or female plant calli, shoots and roots grown on MS medium with 1 mg dm^{-3} ZEA (*A*) or 0.5 mg dm^{-3} IBA (*B*). Band intensity: high (*closed columns*), faint (*stripped columns*), very faint (*open columns*).

0.85 < Rf < 0.90 was typical for male callus only. Male shoots possessed five isoperoxidase bands, while the female only four on the same media.

When shoots were cultured on the MS medium with IBA the increase of peroxidase bands was detected. In this case, male shoots contained eight isoperoxidase bands, while female seven only (Fig. 1). Male shoot material possesses

the characteristic isoperoxidase band $0.85 < Rf < 0.90$ on both nutritional media. The same characteristic band was found also in the male root material. The number of isoperoxidase bands in roots decreased to four in male and three in female material (Fig. 1).

Isoenzyme composition of peroxidase visualised with quaiacol: Callus originating from male plants possessed four isoperoxidase bands, while female only three on the medium supplemented with ZEA. Male and female shoots contained the same number of isoenzymes on the same medium, however their mobilities were different (Fig. 2).

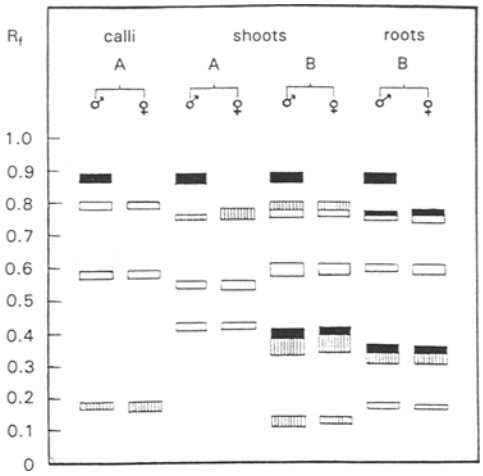

Fig. 2. Isoperoxidase pattern of calli, shoots and roots visualised with quaiacol. Details are the same as given in the legend to Fig. 1.

Shoots growing on the root-induction medium with IBA were characterized with greater number of isoperoxidases than those on the shoot-induction medium enriched with ZEA. Gaspar *et al.* (1992) reported that specific peroxidase activity increase before initiation and growth of root primordia in micropropagated shoots can serve as a predictive marker of rooting performance. Male shoots had seven bands, the female only six on the medium with IBA (Fig. 2). Roots originating from such shoots on the same media possessed also the same number of isoperoxidases (Fig. 2) in the male and female material (seven and six, respectively). The specific isoperoxidase band $0.85 < Rf < 0.90$ was present only in male calli, shoots and roots grown on the media with ZEA or IBA. It was never found in the female material.

Isoenzyme composition of polyphenol oxidase visualised with *p*-phenylenediamine: Male callus contained four isopolyphenol oxidase bands, and the female three on the medium with ZEA (Fig. 3). Male shoots possessed six isopolyphenol oxidase bands, while the female only five on the same medium.

The number of isopolyphenol oxidases in shoots increased on the medium with IBA. Male shoots had seven isopolyphenol oxidase bands, while female six. Male roots originated from shoots on this medium contained five isopolyphenol oxidase bands and female four (Fig. 3).

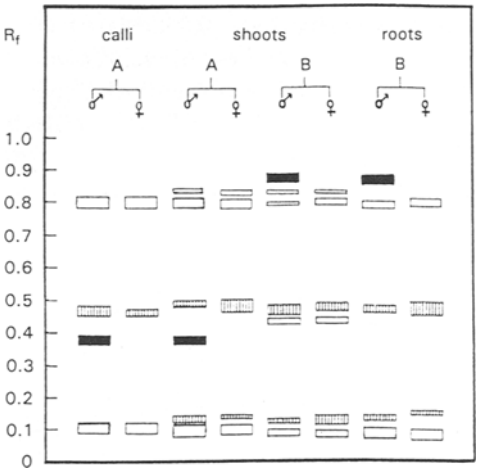

Fig. 3. Polyphenol oxidase pattern of calli, shoots and roots visualised with *p*-phenylenediamine. Details are the same as given in the legend to Fig. 1.

Specific characteristic isopolyphenol oxidase band $0.35 < Rf < 0.40$ was found in the male calli and shoots cultured on the medium enriched with ZEA. Moreover, other characteristic isopolyphenol oxidase band $0.85 < Rf < 0.90$ was found in the male shoots and roots cultured on the medium supplemented with IBA. These specific bands had never been present in the female calli, shoots and roots.

In male shoots and roots cultured on the medium with IBA was found one characteristic isoperoxidase and one isopolyphenol oxidase band with the same electrophoretic mobility ($0.85 < Rf < 0.90$), however, with different substrate specificity. Similar results were obtained by Jaeger-Wunderer (1980), who identified peroxidases, polyphenol oxidases and IAA oxidases as polyfunctional in *Riella helicophylla*. Badiani *et al.* (1990) reported the existence of polyfunctional peroxidase isoenzymes and found phenoloxidase-acting isoperoxidases in winter wheat. Our results suggest similar polyfunctionality of isoperoxidases which requires further study.

Marino and Bertazza (1990) claimed that cytokinin/auxin balance is higher for male genotype of *Actinidia deliciosa* than for female one, what may be of importance in sex expression. Endogenous hormone balance is supposed to be very important for sex expression of some dioecious angiosperms and also in kiwifruit (Marino and Bertazza 1990). Moreover, application of exogenous hormones can lead to sex modification in staminate cv. Matua and pistillate cv. Hayward of *Actinidia deliciosa* var. *deliciosa*. Exogenously applied gibberellin caused feminization in male kiwifruit clone (Marchetti *et al.* 1992).

In our work there were found specific peroxidase and polyphenol oxidase isoenzyme patterns different in male and female cultivars. One characteristic, very stable anodic isoperoxidase band ($0.85 < Rf < 0.90$) was found only in male cv. Matua grown on media supplemented with ZEA or IBA. One specific cathodic isopolyphenol oxidase band ($0.35 < Rf < 0.40$) was present only in male calli and shoots on the medium with ZEA, and another ($0.85 < Rf < 0.90$) anodic one, only in male shoots and roots on medium with IBA. Hirsch *et al.* (1977) reported that peroxidase activity remains higher in male kiwifruit flower buds than in female ones. Specific anodic isoperoxidases were also detected in the male flower buds (Hirsch and Bligny-Fortune 1979). On the contrary, Hirsch and Fortune (1984) found specific isoperoxidase fractions only in female long-term cultivated calli and suspensions, but they were grown in the media containing completely different hormones (2,4-D, KIN, IAA) compared with our experiments, and plant genotypes were not mentioned in this work. It seems that various isoenzyme spectra can be caused by different plant genotypes, but more probably they are due to different plant sexes in correlation to hormone balance.

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