CHLOROGENIC ACID - AN INDEPENDENT MORPHOGENESIS REGULATOR OR A COFACTOR

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Abstract

The involvement of intermediates of the Cinnamic acid-lignin pathway on flower bud differentiation, rooting and callus development was studied. The endogenous level of CHA in olive (Olea europaea L.) leaves of fruit bearing trees was 3-4 times higher than the non bearing ones. Application of CHA decreased the amount of differentiating buds when injected prior to flower bud induction and had no effect when applied thereafter. The increase in the CHA level in leaves of fruiting trees was accompanied by an increase in total protein of those leaves. A quantitative change was found in proteins of 14, 32 and 60 Kd. The protein changes were specific according to cultivars. CHA could replace the auxin requirement for olive callus growth in vitro. A similar but weaker response was found with cinnamic acid. Quercus, Syringa, Praxincus and Malus callus growth was promoted by some of the phenolic acids either with or without auxin. On the other hand the rooting ability of in vitro grown lilac shoots was enhanced by CHA and CAA when applied alone and even more so in the presence of auxin. Thus both an independent effect of the Cinnamate-lignin pathway acids and an interaction with auxin on growth and differentiation was found.

INTRODUCTION

Chlorogenic acid and other related phenolic acids were described as metabolic cofactors in auxin related metabolism and morphogenesis (Feucht and Johel 1977, Lavee et al. 1986, Bauer et al. 1983). It has been shown that phenolic acids were involved mainly in controlling the activity of IAA-oxidase (Pilet 1964, Feucht 1991). Both enhancement and inhibition of IAA mediated processes were reported (Stonio et al. 1979). This antagonistic response of plant tissue to the phenolic acids and particularly those of the cinnamic acid-lignin pathway raises the questions of the nature of their involvement in IAA-induced reactions such as tissue growth, morphogenesis, etc. The response of different plants and in vitro grown tissues to phenolic acid is not uniform. In some cases a clear promotion of auxin mediated processes such as tissue growth were reported (Lavee et al., 1986) while in others the phenolic acids caused inhibition (Jordan et al. 1980). Thus the suggestion that the level of hydroxilation of the phenolic acids is responsible for its activity could not be accepted as is. Furthermore, in some in vitro systems such as olive callus, specific phenolic acids were shown to control growth independently compensating for the lack of auxin in the system (Lavee and Avidan, 1982). A similar response was shown in other tissues though only in the presence of some auxin as well. In various other tissues the same phenolic acid i.e. chlorogenic acid, caused a complete inhibition of tissue growth. It was also shown lately that different phenolic acids
of the cinnamic acid-lignin pathway might have a similar response in
different tissues and vice versa. Thus the question rises to what
extend these phenolic acids can act as independent metabolic
regulators or are involved mainly as cofactors to auxin mediated
metabolism. In the present study we tried to determine the dependence
or independence of the intermediary acids of the cinnamic acid-lignin
pathway on auxin in controlling growth, rooting and flower bud
differentiation in different plant systems.

Materials and methods

In vitro callus cultures were prepared from Olea europaea,
Quercus robur fastigiata, Fraxinus excelsior, Syringa vulgaris and
Malus domestica using the LBD media in the presence of an auxin and a
cytokinin. The subcultures were transferred to media containing
different amounts of phenolic acids and auxins. Similarly in vitro
grown shoots of lilac rootstock A2 (Pierik et al. 1992) were planted
on the same media without cytokinins and the rooting ability
determined.

The content of chlorogenic acid (CHA) was determined in leaves of
fruit bearing and non bearing olive trees using both TLC-GC and HPLC
systems. The protein content and its nature in those leaves was also
determined. The effect of CHA and caffeic acid (CAA) on flower bud
differentiation was determined by injecting the acids into the xylem
stream of cv. Manzanillo olives in the early and late winter recording
their effect on flowering in the following spring.

Results and discussions

In previous studies (Lavee et al., 1986) we have shown that the
leaves of fruit bearing olive trees contain a 3-4 times higher level
of CHA than the leaves of non bearing ones (Fig. 1). This was further
followed over a number of consecutive years. When the fruitlets were
removed after fruit set the level of chlorogenic acid during the
following season remained low. A pressure injection of CHA into the
xylem sap of the olive trees during the early winter caused a 50-60%
reduction in bud differentiation. Injection during the late winter,
after the secondary bud induction took place, had no effect on
the amount of developing inflorescences (Lavee et al. 1986). CHA was
the only acid of the cinnamate-lignin pathway which had this effect on
olive bud differentiation while the others had no noticeable influence
on either shoot growth or bud differentiation. Thus, it could be
concluded that the CHA content in the leaves is involved in
controlling flower bud initiation. It was also deductible that the
developing embryos are responsible for the secretion of a signal
translocated to the leaves where a metabolic change takes place
leading to the synthesis of CHA. This CHA, on a feedback basis, will
inhibit either the primary induction for differentiation during summer
or the secondary one during winter or both (Lavee, 1989). Presently
we could demonstrate metabolic change in the leaves of "on" and "off"
years also by the total amount of protein in the leaves and by the
relative quantitative changes of specific proteins. The total amount
of protein was higher in the leaves of four olive cultivars tested
during the "on" year than in the "off" year, though unsignificant in
leaves of cv. Koronaiki (Table 1).
Table 1: The protein content of mature olive leaves of four cultivars during "on" and "off" years. Leaves were sampled in late summer during fruit development. Results are expressed as µg protein per 1g fr. weight. Different letters represent significance of P=0.05.

<table>
<thead>
<tr>
<th>Year</th>
<th>Manzanillo</th>
<th>Barnea</th>
<th>Uovo de Piccione</th>
<th>Koronaiki</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;off&quot;</td>
<td>295 a</td>
<td>310 a</td>
<td>370 a</td>
<td>475 b c</td>
</tr>
<tr>
<td>&quot;on&quot;</td>
<td>510 b</td>
<td>405 c</td>
<td>510 b</td>
<td>530 a b</td>
</tr>
</tbody>
</table>

The difference found in specific proteins in leaves of "on" and "off" trees were only quantitative and specific for each cultivar (Fig. 2). The main proteins involved were at 14, 32 and 60 kd in accordance with the different cultivars.

It should be noted that while the changes in the relative quantities of the proteins involved were specific for each cultivar tested the changes in the amount of CHA and total protein between fruiting and non-fruiting trees were found in all cultivars tested.

In order to test the specific effect of the phenolic acids of the cinnamate-lingpin pathways on olive tissues, callus cultures were planted on media containing the various acids in the presence and absence of IAA. Both cinnamic acid (CIA) and CHA induced olive callus growth without the presence of auxin in the media. To a lesser extent ferulic acid (FEA) also enhanced callus growth. The addition of IAA had a similar effect as increasing the concentration of the active phenolic acid. Thus supraoptimal inhibitions of growth were noted (Fig. 3). On the other hand CAA had no noticeable promoting effect on the olive callus growth and p-coumaric acid (COA) only a minimal one. No inhibiting effect at the tested range of concentrations was noted either. Thus it could be concluded that CHA has an independent auxin-like effect on in vitro olive callus growth. The results also point to a specific pathway leading to the CHA in the tissue probably via cinnamoyl quinic acid. It seems that the anticipated pathway with CAA as an intermediate does not function in this case.

As the olive has a high content of endogenous phenolic acid, oak, another species with a high phenolic acid content was chosen to test the effect of the acids of the lignin pathway on in vitro callus growth. Callus from a seedling of Quercus robur (Jr) grown in vitro for 6 subcultures was planted on LBD media in the presence of a Riboside-ctokinin (IPA) and fed with either auxin or the different tested phenolic acids. Good callus growth was achieved in this case only with NAA however, CAA, FEA and slightly also CHA, induced callus growth (Fig. 4). On the other hand when the tissue was grown on a more suitable non Riboside cytokinin (2IP) an independent growth effect due to CHA could be noted. This effect reamined detectable also when auxin was added to the media as well (Table 2). In this system however, increasing the concentrations of the phenolic acids to 10^{-7}M caused an inhibition of growth particularly when no or a low concentration of auxin or the more suitable cytokinin for Oak, BA, which also induces some independent growth were added to the media. Thus, although the growth induction of the oak callus by CHA was somewhat weaker than that of NAA, it did seem independent and additive to the growth effect of the latter.
Table 2: The reciprocal effect of CAA or CHA and NAA on in vitro 
growth of oak callus (media LBD, cytokinin = 2iP, results - mg/fw).

<table>
<thead>
<tr>
<th>10^{-4}M</th>
<th>0</th>
<th>0.1</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>329 ± 41</td>
<td>547 ± 52</td>
<td>898 ± 78</td>
</tr>
<tr>
<td>CAA</td>
<td>394 ± 36</td>
<td>594 ± 61</td>
<td>989 ± 92</td>
</tr>
<tr>
<td>CHA</td>
<td>517 ± 57</td>
<td>716 ± 81</td>
<td>1079 ± 89</td>
</tr>
</tbody>
</table>

In the presence of a high concentration of NAA (3x10^{-5}M) except 
for the CIA all other acids tested enhanced the oak callus growth over 
that of NAA alone (Fig. 5). The effect of CHA was also in this system 
the strongest but only slightly above that of CAA. Thus, it could be 
concluded that also in the oak CHA was a potent callus growth 
promoter. However, the pathway leading to CHA probably differs from 
that in the olive and passes via the CAA junction which leads also to 
the FEA showing in this tissue also growth induction activity. A 
possible reverse metabolism from FEA to tryptophan has been already 
previously suggested (Lavee 1989).

The effectiveness of CHA on callus growth was, hence demonstrated 
in two different species which have a common high endogenous phenolic 
acid content. We further tested the effect of the cinnamate-lignin 
pathway intermediates on the growth of callus of Syringa vulgaris 
which belongs to the same family - Oleaceae - as the olive. Neither of 
the phenolic acids tested had any promoting effect on the in vitro 
growth of Lilac callus and when applied in the presence of NAA clearly 
inhibited callus growth at the higher concentration (Table 3).

Table 3: The effects of NAA, CHA and CAA on the growth of lilac callus 
in vitro. (Planted June 4th, Recorded July 2, 1992 as Fwt in mg).

<table>
<thead>
<tr>
<th>NAA (mg/1)</th>
<th>CHA (M)</th>
<th>CAA (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^{-4}</td>
<td>10^{-3}</td>
</tr>
<tr>
<td>0</td>
<td>189</td>
<td>111</td>
</tr>
<tr>
<td>0.1</td>
<td>681</td>
<td>519</td>
</tr>
<tr>
<td>1.0</td>
<td>833</td>
<td>711</td>
</tr>
</tbody>
</table>

In the presence of 1 mg/l NAA in the media the phenolic acids 
tested, CIA, COA, CAA, FEA and CHA at 5x10^{-4}M had no effect on callus 
growth of two different lilac cultivars except for a lighter color of 
the tissue grown on CIA. In one experiment, when the IPA cytokinin 
was used, CIA induced callus growth. Recently we found a similar 
phenomena with callus of two apple cultivars when the tissue used 
originated from cultures grown on media containing both NAA and CHA. 
Apple tissue from mother cultures grown on NAA alone did not respond 
to CIA.
The response of in vitro grown callus of Fraxinus (also from the Oleaceae family) to phenolic acids of the cinnamate pathway was somewhat different from that of other species studied. None of the phenolic acids could independently induce callus growth, however, in the presence of NAA some clearly affected the growth of ash callus. When grown on COA and FEA callus growth was enhanced while on CHA, CAA and CIA it was inhibited (Fig. 6). Thus it seems that in this case the phenolic acids acted as cofactors to the auxin in a metabolic pathway different from that in tissues of other woody plants tested.

A strong dual effect of CHA and CAA was found on the rooting ability of lilac. In vitro grown single node segments of lilac rooted up to 30% when NAA was added to the medium and up to 40% when CHA or CAA was added. However, when both NAA and CHA were applied the rooting of cuttings increased to 100% (Fig. 7). Similar results with some quantitative differences were obtained with three different clones.

Thus it could be concluded that specific phenolic acids and particularly the chlorogenic acid might have in some systems an independent growth inducing and morphogenetic effect while in others, it effects the developmental potential by interaction with auxin. It also became apparent that the active phenolic acid on one hand and the metabolic pathway leading to it on the other, are somewhat different and apparently specific in the various tissues of different plants.

References


Fig. 1: A schematic description of the level of chlorogenic acid in the leaves of olive trees during "on" and "off" years and after fruit removal.

Fig. 2: Protein distribution in leaves of "on" and "off" olive trees from four cultivars. K=Koronaiki; U=Uivo de piccione; B=Barnea; M=Manzanillo.
Fig. 3: In vitro growth of cv. Manzanillo olive callus on media containing tryptophan (TRY) and 4 phenolic acids of the cinnamate-lignin pathway in the presence (+) and absence (-) of IAA.

Fig. 4: In vitro oak callus growth, [Quercus robur seedling strain (J_5)], on LBD medium with riboside cytokinin (IPA) and either NAA or phenolic acids of cinnamate-lignin pathway.

Fig. 5: The effect of phenolic acids of the cinnamate-lignin pathway on in vitro callus growth of oak strain J_5 in the presence of a high concentration of 3x10^{-5} M NAA. (LBD medium with 1 mg/l BA.)
Fig. 6: The effect of phenolic acids of the cinnamate-lignin pathway in the presence of NAA on Fraxinus callus growth in vitro. (LBD medium with 1 mg/l IPA).

Fig. 7: The effect of CAA and CHA acids on rooting of cuttings from in vitro grown lilac rootstock A2 shoots in the presence and absence NAA in the LBD medium.