CHLOROGENIC ACID - AN INDEPENDENT MORPHGENESIS 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, Fraxincus 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. 1989). 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 (Stonier 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 exelsior, 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. Similarily in vitro grown shoots of lilac rootstock A_3 (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 presure 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 flower 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 noticable 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 deductable 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.

Year	Manzanillo	Barnea	Uovo de Piccione	Koronaiki
"off"	295 a	310 a	370 a	475 b c
"on"	510 b	405 c	510 b	530 a b

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-lingin 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 acid 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 (J_5) grown in vitro for 6 subcultures was planted on LBD media in the presence of a Riboside-cytokinin (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^{-3} 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).

	NAA (mg/l)		
10^{-4}M	0	0.1	1.0
Control	329 ± 41	547 ± 52	898 ± 78
CAA	394 ± 36	594 ± 61	989 ± 92
CHA	517 ± 57	716 ± 81	1079 ± 89

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).

NAA (mg/l)		CHA (M)		CAA (M)	
	0	10^{-4}	10^{-3}	10^{-4}	10^{-3}
0	189	111	104	136	117
0.1	681	519	294	617	274
1.0	833	711	337	796	507

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 apparantly specific in the various tissues of different plants.

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

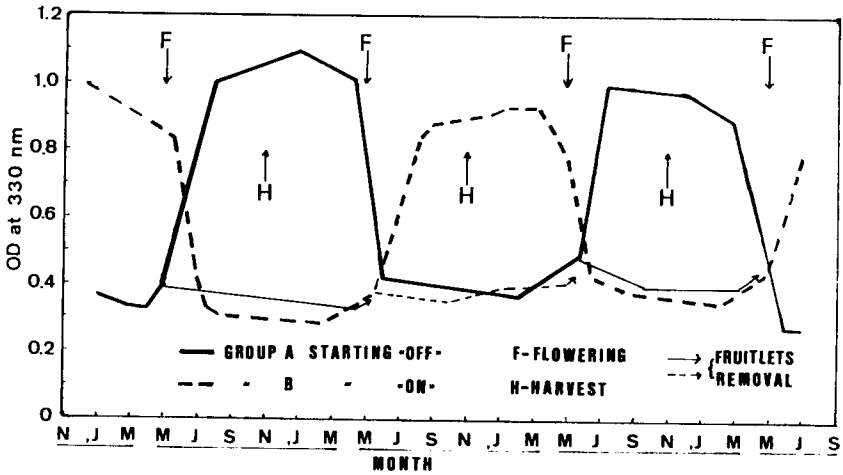

Fig. 2: Protein distribution in leaves of "on" and "off" olive trees from four cultivars. K=Koronaiki; U=Uovo de piccione; B=Barnea; M=Manzanillo.

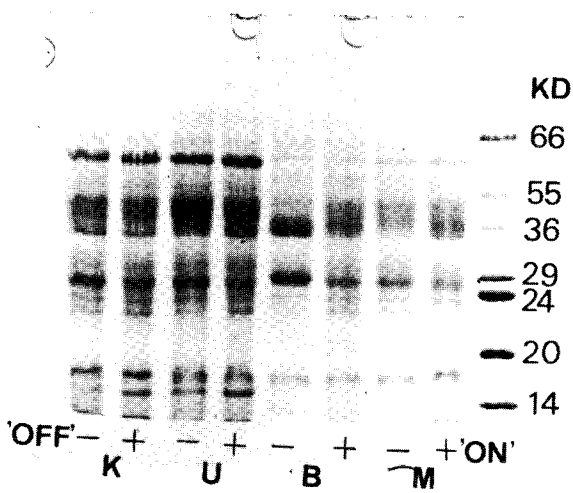

Fig. 3: In vitro growth of cv. Manzanillo olive callus on media containing tryptophan (TRY) and 4 phenolic acids of the cinnamate-lignin pathway in the presence (+) and absence (-) of IAA.

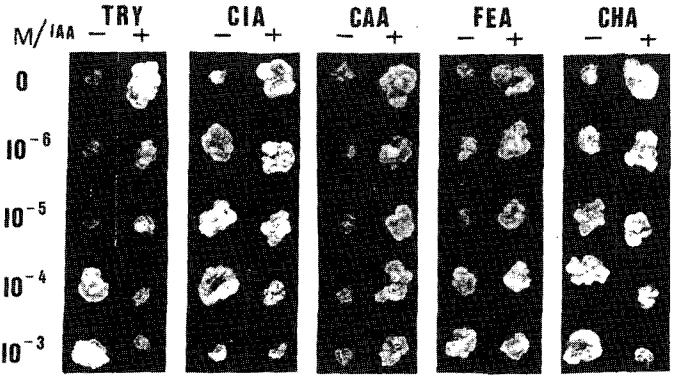

Fig. 4: In vitro oak callus growth, [Quercus robur seedling strain (J_5)], on LBD medium with a riboside cytokinin (IPA) and either NAA or phenolic acids of cinnamate-lignin pathway.

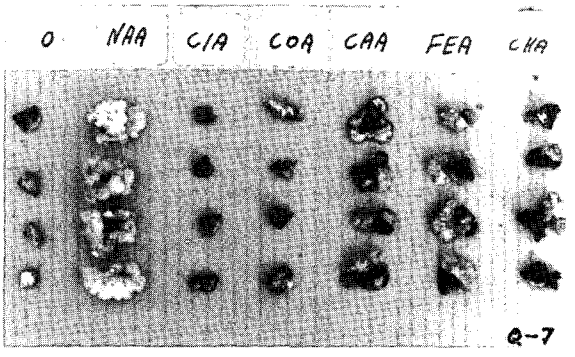

Fig. 5: The effect of phenolic acids of the cinnamate-lignin pathway on in vitro callus growth of oak strain J_5 in the presence of a high concentration of $3x10^{-5}$M NAA. (LBD medium with 1 mg/l BA.)

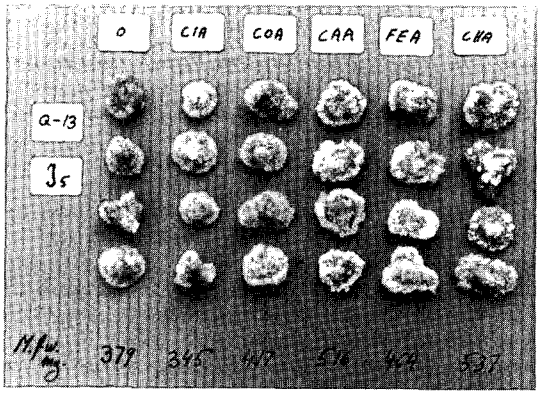

Fig. 6: The effect of phenolic acids of the cinnanate-lignin pathway in the presence of NAA on Fraxinus callus growth in vitro. (LBD medium with 1 mg/l IPA).

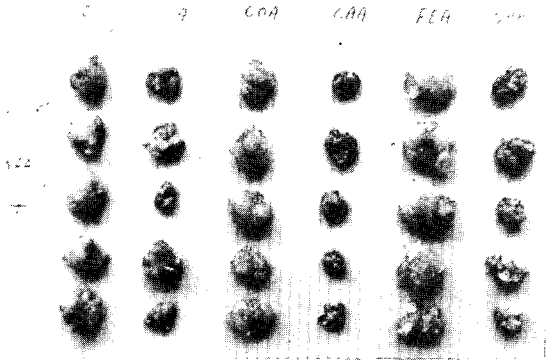

Fig. 7: The effect of CAA and CHA acids on rooting of cuttings from in vitro grown lilac rootstock A_2 shoots in the presence and absence NAA in the LBD medium .