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FORMATION AND TRANSPORT OF 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID IN PEA PLANTS

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Key Word Index—*Pisum sativum*; Leguminosae; pea; decapitation; ethylene biosynthesis; free and conjugated ACC; ACC transport; accumulation of conjugated ACC; stress indicator; axillary buds.

Abstract—The formation and transport of free 1-aminocyclopropane-1-carboxylic acid (ACC) and conjugated ACC [1-(malonylamino)cyclopropane-1-carboxylic acid; M-ACC] was studied in pea plants. Excision and dark incubation induced ACC and M-ACC synthesis in stem segments, including the second node. At similar rates as in segments, ACC and M-ACC were formed near the cut surface in stems after decapitation, leading to a transient increase in both compounds in the node adjacent to the cut. The maximum level of M-ACC at 6 hr exceeded that of ACC at 3 hr. Seven days after decapitation, total M-ACC in the shoot returned to the level in the control plants. Over the same period of time, M-ACC accumulated in the roots in amounts comparable to those previously observed in the shoot. It is concluded that M-ACC formed near the cut is transported basipetally, and that the roots act as a sink. Both the increase in ACC and M-ACC in the node after decapitation and the degree to which growth of lateral shoots was inhibited by ACC applied to the cut end increased with advancing age of the plant.

INTRODUCTION

Wounding, noxious chemicals and a variety of other stresses induce the formation of ACC by ACC synthase [1–3]. ACC is the immediate precursor of ethylene [4]. Another metabolite of ACC is 1-malonyl-ACC (M-ACC), a stable, non-volatile conjugated form of ACC. M-ACC was not identified until recently [5, 6], although the possibility of it existing was suggested earlier [3]. Because of its stability, M-ACC could be an indicator for an activation of the ethylene biosynthetic pathway which may have occurred before the plant is analysed. Both ACC and ethylene production indicate this activation only during a relatively short period of time [3]. In the case of stresses which are able to stimulate ACC synthesis, the occurrence of M-ACC at elevated levels would be a possible indicator of the stress history of a plant or part of a plant. This feature of M-ACC was suggested in the case of detached, wilting wheat leaves [7]. For the intact plant, however, it would be necessary to know whether or not M-ACC is translocated between different plant parts (as is, for example, ACC [8]), and to identify organs possibly acting as a sink for M-ACC.

We have examined the question about ACC and M-ACC transport using pea plants from which the shoot was decapitated. Decapitation is known to lower ethylene production in the adjacent node after an initial, short increase [9]. This may be important with respect to the possible involvement of ethylene in the release of lateral buds from apical dominance. In this context it seemed important to know if ACC formed near the cut at the top of the stem would be transported basipetally, thus leading to a transient increase in ethylene production in the node shortly after the cutting.

In this paper we describe (i) the conversion of wound-induced and exogenously applied ACC to M-ACC and ethylene by segments excised from pea stems, (ii) temporal changes in the content of ACC and M-ACC in the second node and in the root of intact plants after decapitation at the third node, and (iii) the effect of age on the capacity of pea stems to synthesize and transport ACC and M-ACC.

RESULTS AND DISCUSSION

The conversion of either exogenous or wound-induced ACC to ethylene and M-ACC by segments excised from pea stems was investigated. The data summarized in Table 1 show that segments including the second node contained both free and conjugated ACC (= initial value). Excision and incubation in darkness induced additional ACC synthesis which, in turn, led to ethylene production and an increase in M-ACC. The increase in M-ACC largely exceeded that in free ACC. This agreed with the observation made in other systems, e.g. in cotyledons of peanut after 48 hr of imbibition [10]. When 0.75 mM ACC was added to the incubation solution during the first 3 hr, ethylene production and M-ACC formation were stimulated. Increasing the external ACC concentration to 2 mM resulted in a further increase in ethylene production, but the formation of M-ACC was not significantly higher than that at 0.75 mM. This indicated that the system forming M-ACC from ACC was saturated in the presence of 1–2 mM ACC. A similar observation was made with buckwheat hypocotyl segments incubated on ACC concentrations in the range 0.5–1 mM [11].

From the data in Table 1 describing the increase in M-ACC, the rate of M-ACC formation *in vivo* was calculated and compared with the rate reported for an *in vitro* system with extracts from mung bean hypocotyls [12]. The rate of M-ACC formation in the absence of exogenous ACC was 0.74 ± 0.03 nmol/hr per g fr. wt (equivalent to $0.24 \pm$ nmol/hr per mg protein). Application of ACC

Table 1. Ethylene production and ACC content in segments excised from stems of 15-day-old pea plants at the beginning and after an 8-hr incubation period in the dark with or without application of exogenous ACC during the first 3 hr (expressed on a fr. wt basis)

	Ethylene* (nmol/g per 8 hr)	ACC (nmol/g) Free	ACC (nmol/g) Conjugated
Initial		0.48 ± 0.14	1.25 ± 0.20
−ACC	0.27 ± 0.05	1.89 ± 0.25	7.19 ± 1.10
+ACC (0.75 mM)	6.72 ± 1.12	19.60 ± 2.13	48.40 ± 5.69
+ACC (2.0 mM)	9.62 ± 1.53	57.40 ± 3.18	54.70 ± 6.36

*The value given is the sum of the amount produced between 0 and 3 hr and after changing the solution between 3 and 8 hr of incubation. Means ± s.e. ($n = 5$).

at a concentration which seemed to be saturating for M-ACC formation *in vivo* (see above) led to a rate of 6.68 ± 0.52 nmol/hr per g fr. wt (equivalent to 2.15 ± 0.30 nmol/hr per mg protein). This rate was about half of the rate determined *in vitro* (5 nmol/hr per mg protein [12]), where M-ACC was formed enzymatically by the transfer of malonic acid from malonyl-coenzyme A to ACC. The difference between the rates measured in the two systems could be due to the absence of ACC conversion to ethylene in the *in vitro* assay.

It was shown earlier by Yeang and Hillman [9] that ethylene production by pea stem segments (second and third internodal segments and second node section) increases during the first 2–3 hr after the decapitation of the plant at the third node, and that ethylene production decreases afterwards to rates lower than in non-decapitated plants. On the basis of the data presented in Table 1, it was to be expected that this initial ethylene production after the cutting would be accompanied by the synthesis of ACC and M-ACC. When the ACC level in the second node was determined, a rapid increase to a maximum between 3 and 4 hr was observed (Fig. 1A). This increase could account for the reported pattern of ethylene production in the nodal section [9]. According to experiments by Saltveit and Dilley [13], nodal sections from pea stems have a rather low capacity to form wound ethylene as compared to internodal sections. Therefore, ACC in the node as observed in the present study could have been formed near the cut and not in the node, thus suggesting basipetal ACC transport. The decrease in ACC in the node, on the other hand, could be due to its conversion to ethylene (as observed by Yeang and Hillman [9]) and M-ACC, or its basipetal transport.

The level of M-ACC in the second node started to increase together with that of ACC, but the maximum was reached later, about 6–8 hr after the cutting (Fig. 1A). There was a substantial difference between the maximum levels in ACC and M-ACC, which agreed with the data given in Table 1 showing that M-ACC accumulation in excised segments exceeded the increase in ACC more than four-fold. The M-ACC level in the node of decapitated plants increased from 0.07 to 0.56 nmol/segment after 8 hr. From this increase, an apparent rate of M-ACC formation of 0.061 nmol/hr per segment (equivalent to 0.82 nmol/hr per g fr. wt) was calculated. This rate agreed reasonably well with that derived from the data in Table 1

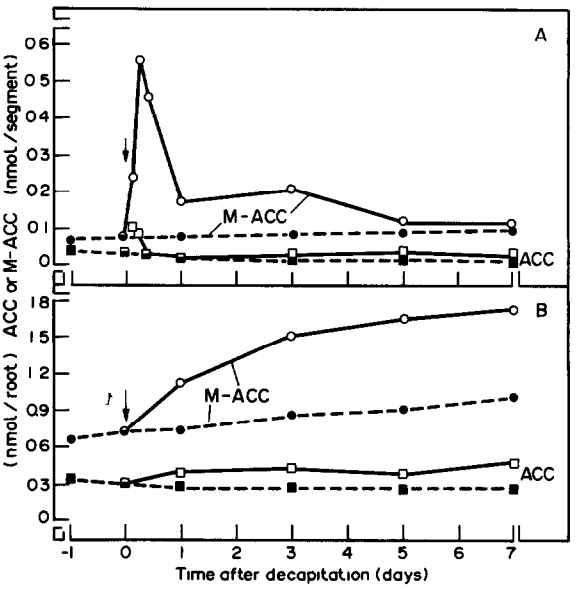

Fig. 1. Change in the levels of ACC and M-ACC in the second node (A) and in the root (B) of 8-day-old pea plants after decapitation near the third node. Decapitated plants: (□) ACC, (○) M-ACC. Controls: (■) ACC, (●) M-ACC.

for excised segments in the absence of exogenous ACC.

The decrease in M-ACC in the second node after the peak was slower than the decrease in ACC. Unlike ACC, M-ACC is not metabolized [7]. Therefore, the behaviour of the stable M-ACC in this particular part of the plant suggested its translocation to another plant part. In the case of young, decapitated pea plants without lateral shoots, the transport should occur basipetally, possibly to the roots. Some indication for basipetal M-ACC transport was found earlier by analysing phloem exudate from *Cucurbito pepo* after the application of labelled ACC to the leaves [11]. In order to investigate further the possibility of basipetal transport of M-ACC after decapitation, the M-ACC content in the roots was determined at different times. The change in M-ACC over a 7-day period is shown in Fig. 1B. A steady, marked increase in M-ACC occurred in the roots up to a more or less constant level

which was reached after 5–7 days (on a per root basis). The total increase in M-ACC was *ca* 0.7 nmol/root (determined as the difference in M-ACC between decapitated and non-decapitated plants at day 7). This increase was comparable to the amount of M-ACC previously formed in the shoot. From the area under the curve for the M-ACC level in the node at various times (Fig. 1A), a total amount of 0.75 nmol M-ACC was calculated. Thus it can be concluded that M-ACC was formed near the cut surface as a consequence of wound-induced ACC synthesis, and that it was transported basipetally to the roots where it accumulated. The roots can therefore be considered a sink for M-ACC.

On the basis of the data presented here, the possibility of M-ACC formation in the roots as the reason for the observed M-ACC accumulation after decapitation cannot be ruled out completely. There was a change in free ACC in the roots during the experiment (Fig. 1B), i.e. ACC increased at the beginning and remained constant thereafter. The higher level of free ACC in the roots after the cutting could have been associated with M-ACC formation resulting in an increased level of M-ACC. However, from the similarity in the amounts of M-ACC seen in the node and later in the root it would have to be expected that part of the M-ACC observed in the node would be retained in the shoot. Therefore, the total content of M-ACC in the shoot was determined 7 days after decapitation. At this time, the level of M-ACC in the node had returned to that in control plants (Fig. 1A), and the M-ACC level in the root had reached the maximum (Fig. 1B). The content of M-ACC in shoots of non-decapitated plants was found to be 0.98 ± 0.16 nmol/shoot. This was not significantly lower than that in decapitated plants where 1.12 ± 0.18 nmol/shoot was observed. Based on this measurement, it seems likely that M-ACC synthesized in the shoot was transported to the root, and that possible M-ACC formation in the roots contributed little to the observed increase in M-ACC.

Basipetal ACC transport after the synthesis near the cut surface seemed also possible. This could have accounted for the transient increase in ACC in the second node which eventually led to ethylene production in this section as observed by Yeang and Hillman [9]. Such ethylene production in the nodal area could affect the release of lateral buds from apical dominance [9]. It is not clear from the data presented here whether some ACC reached the roots or was completely converted to ethylene within the shoot, although a small change in ACC was observed in the roots after decapitation. However, Amrhein *et al.* [11] observed that substantial amounts of labelled ACC can be found in the roots of tomato plants after the application of labelled ACC to the leaves. This demonstrated a possible ACC transport from the shoot to the root in intact plants. The extent of this transport, however, could depend on environmental conditions, the plant species, or the age of the plant.

In order to investigate the effect of age on the behaviour of ACC and M-ACC, the maximum levels of both ACC and M-ACC occurring in the second node after decapitation of 8- and 15-day-old plants were measured and compared (Table 2). In younger plants (8 days old), the initial levels of ACC and M-ACC were not different from those in older plants (15 days old), but the maximum levels reached shortly after decapitation were markedly lower. This suggested that the synthesis and/or the transport of the two compounds were less in younger plants.

When segments were excised from stems of plants of different age and incubated in darkness, the production of wound-ethylene was highest in the oldest tissue (Table 3). Similarly, ACC-dependent ethylene production increased with age. The higher rate of ethylene production in older tissue was associated with higher internal ACC levels. There was no difference in the ratio between the release of ethylene, the formation of M-ACC and the residual ACC content among tissues of different age (not shown). Therefore, not only the capacity to synthesize ACC and ethylene, but also the capacity to absorb ACC from the incubation solution increased with age. Consequently, it seems likely that the higher levels of ACC and M-ACC in older stems after decapitation as compared to younger stems (Table 2) were the result of both a higher rate of

Table 2. The effect of age on the increase in ACC and M-ACC observed in the second node of pea stems after decapitation. Initial contents are given together with the maximum contents observed at the indicated times*

		8-Day-old plants (nmol/segment)	15-Day-old plants (nmol/segment)
ACC	Initial	0.03 ± 0.02	0.03 ± 0.01
	After 3 hr	0.07 ± 0.02	0.16 ± 0.03
M-ACC	Initial	0.06 ± 0.03	0.07 ± 0.03
	After 6 hr	0.23 ± 0.05	0.55 ± 0.07

*Means $\pm$ s.e. ($n = 5$).

Table 3. The effect of age on ethylene production and ACC content in segments excised from pea stems after an 8-hr incubation in the dark, in the presence (+ACC) or absence (−ACC) of 0.75 mM ACC in the incubation solution during the first 3 hr (expressed on a fr. wt basis)*

	−ACC		+ACC	
Age (days)	Ethylene (nmol/hr per g)	ACC (nmol/g)	Ethylene (nmol/hr per g)	ACC (nmol/g)
5	0.025 ± 0.004	1.15 ± 0.31	1.48 ± 0.21	9.22 ± 0.82
8	0.027 ± 0.005	1.25 ± 0.21	2.07 ± 0.28	12.65 ± 0.95
15	0.037 ± 0.008	1.89 ± 0.25	2.51 ± 0.25	19.62 ± 2.34

*Means $\pm$ s.e. ($n = 5$).

ACC synthesis near the cut surface and a higher capacity to transport ACC and M-ACC from the cut surface to the adjacent node. Thus it appears that the level of M-ACC determined to indicate previous stress would not only depend on the severity of the stress but also on the age of the plant.

The age-dependence of ACC (and M-ACC) transport may also be of importance with respect to the effect of ethylene on physiological processes in the plant. To examine this possibility, the effect of ACC applied to the cut end of the stem on the growth of lateral shoots released from apical dominance at the second node was determined in both 8- and 15-day-old plants. Ethylene is known to inhibit the longitudinal expansion growth of lateral shoots [9]. If the transport of ACC is different in plants of different age, a difference in the degree of inhibition due to ACC-derived ethylene should result. The dose–response curves plotted in Fig. 2 show that the growth of lateral shoots from older plants was more inhibited by the ACC treatment than that of lateral shoots from younger plants. A 50%-growth inhibition was observed in the presence of 8.5 mM ACC applied to older plants and of 19 mM ACC applied to younger plants. While growth was completely inhibited by 25 mM ACC in the case of older plants, 50 mM ACC still did not completely inhibit growth of shoots released from nodes of younger plants. This observation is in agreement with the finding previously described suggesting that the transport capacity for ACC in pea stems increases with age.

In conclusion, the findings presented here suggest that ACC and M-ACC formed by pea stems in response to decapitation are transported basipetally. The level of the stable M-ACC in the roots can thus possibly be used to indicate previous stress situations. ACC transported from the cut surface to the adjacent node can affect the growth of lateral shoots released from apical dominance. However, the capacity to synthesize and to translocate ACC and M-ACC depends on the age of the plants. This could be important with respect to the importance of ethylene for the control of developmental processes, and with respect to the use of M-ACC levels in the roots to indicate stress.

EXPERIMENTAL

Plant material. Pea (*Pisum sativum* L. cv Vatters Frühbusch) seeds were soaked in aerated H_2O for 12 hr and germinated on several layers of wet tissue paper in the dark for 3 days. Seedlings were transferred to pots with wet vermiculite and grown in a growth chamber with a 14-hr photoperiod. 8- or 15-day-old plants were routinely used for the experiments.

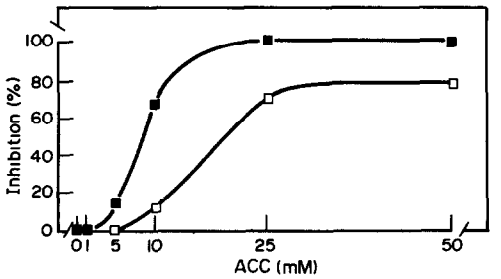

Fig. 2. Effect of various concentrations of ACC on the growth of lateral shoots released at the second node of pea plants. The length of the lateral shoot grown in the absence of ACC was taken as the control (14.1 mm/7 days for 15-day-old plants and 29.4 mm/7 days for 8-day-old plants). (□) 8-Day-old plants; (■) 15-day-old plants.

Treatments. (a) For experiments with stem segments, stems were cut 1 cm above and below the second node. The segments were transferred to flasks containing water or an aq. soln of ACC. The flasks were flushed with air, sealed with a rubber serum cap, and incubated at 25° in the dark. (b) Shoots were decapitated 1 cm above the second node. A small wad of cotton wool was wrapped around the cut end of the stem. H_2O or an aq. soln of ACC was applied to the wad (50 μl). The wad was sealed with adhesive tape to inhibit evapn (procedure according to ref. [9]).

Determination of ethylene. Gas samples were withdrawn from the sealed flasks at the end of the incubation period and analysed for ethylene by GC as described before [3].

Determination of ACC and M-ACC. All tissues used were ground in a mortar and extracted in hot 80% EtOH. The extract was centrifuged at 10000 *g* for 10 min. The pellet was re-suspended in EtOH and re-centrifuged. Supernatants were combined and EtOH was evapd *in vacuo* at 40°. Residues were dissolved in 2 ml H_2O. Free ACC was determined in a 1-ml aliquot of the aq. soln according to ref. [14], but with 8 μmol $HgCl_2$ instead of 1 μmol and with a mixture of KOCl (14%, v/v) and NaOH (10 M) (2:1, v/v). M-ACC was quantified according to ref. [7] by hydrolysing a 1 ml aliquot of the aq. soln in 2 M HCl at 100° for 3 hr. After neutralization with 10 M NaOH, the soln was assayed for ACC as described above. The difference between the ACC content before and after HCl hydrolysis was taken as the amount of conjugated ACC (M-ACC).

Protein determination. Soluble protein content was measured according to the method of ref. [15].

Growth of axillary buds. After decapitation, the longitudinal growth of axillary buds released from the second node was determined by measuring the increase in length of the first and second internodes of the lateral shoot over a 7-day period.

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