ブドウ'デラウェア'の休眠枝ざしの発根における光合成の関与

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The Involvement of Current Photosynthesis in Rooting of Hardwood Cuttings of 'Delaware' Vines'

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Summarv

Distribution of current photosynthates was studied in hardwood cuttings of 'Delaware' vines which were exposed to \$^{14}CO_2\$ at different stages of rooting to clarify the involvement of photosynthesis in rooting. The \$^{14}C\$ activity in the ethanol soluble fraction of the new shoot, upper stem, lower stem and roots were measured 6, 24 and 72 hours after \$^{14}CO_2\$ feeding.

At the early stage of rooting, nearly all of the fixed ¹⁴C remained in the new shoots, while after root appearance the amount of ¹⁴C translocated from new shoots into roots increased with time after ¹⁴CO₂ feeding. This suggests that the developing roots may become a significant sink for current photosynthates. Separation of ethanolic extracts into sugar, organic acid and amino acid fractions revealed that over 90% of the ¹⁴C in each extract was found in sugars 6 hours after ¹⁴CO₂ feeding, and that the percentage of radioactivity in sugars declined with time in new shoots and roots, but changed little in the upper and lower half of stems. Organic acids in the roots showed a sharp increase in the level of ¹⁴C but there was little increase in amino acids. The increased incorporation of ¹⁴C into organic and amino acids in roots seemed to be involved in the active growth of roots.

The present result that a considerable amount of current photosynthates is supplied to the developing roots confirms our previous assumption that photosynthesis in hardwood cuttings of 'Delaware' vines plays an important role in root development during the latter half of the propagation period.

Introduction

Light is one of the important environmental requirements for the successful rooting of cuttings. In numerous attempts to obtain a better understanding of the relationship between light and rooting, the contribution of photosynthesis to rooting has been studied. Most investigators agree that photosynthesis in leafy cuttings plays an important role in root formation (1, 2, 3, 7, 8). It is also generally accepted that the rooting of hardwood cuttings depends upon stored carbohydrates (5). However, in previous studies with hardwood cuttings of grapevines, we observed that the rooting was

influenced by various treatments such as different light levels, CO₂ enrichment and CO₂ removal during the propagation period(10, 11, 12, 13).

In the present study, the involvement of current photosynthesis in rooting of hardwood cuttings of vines was examined in detail using $^{14}\text{CO}_2$.

Materials and Methods

Uniform hardwood cuttings of 'Delaware' vines, 10 cm long with one bud, were prepared in mid-March. Each cutting was inserted into sand in individual plastic pots and placed in an open plastic house.

 $^{14}CO_2$ exposure Nine cuttings were randomly selected 33, 43 and 62 days after planting. Cuttings left potted were exposed to $^{14}CO_2$ released from a reaction of adding

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one ml of 25% lactic acid to 250 μ Ci of NaH¹⁴CO₃ for 2 hours at 22°C with an irradiance of approximately 12 Klx.

Sampling and 14C assay Three 14C-labelled cuttings were sampled 6, 24 and 72 hours after the release of ${}^{14}\mathrm{CO_2}$ in each treatment. Immediately after sampling, cuttings treated after 33 and 43 days were divided into 3 parts, i.e., new shoot, upper half and lower half of stem. Cuttings treated after 62 days, in which roots had begun to emerge, were divided into 4 parts including a root portion in addition to the above parts. Each part was weighed, fixed in boiling 80% (v/v) ethanol, extracted for one hour with 80% ethanol after grinding in a porcelain mortar, and the homogenate was centrifuged. supernatant was made up to 100 ml with One ml of solution was distilled water. evaporated to dryness at 40°C and used to determine radioactivity with a gas flow counter.

The solution was separated into sugar, organic acid and amino acid fractions by ion exchange resins. An aliquot of each fraction was used to determine ¹⁴C activity.

Results

Total leaf area per cutting determined 33, 43 and 62 days after planting was 7.2, 18.2 and 46.6 cm², respectively. Roots began to emerge after 43 days and grew to 5.5 in number and 237 mg in fresh weight per cutting after 62 days.

Total amounts of radioactivity fixed 6 hours after ¹⁴CO₂ exposure on the 33 rd, 43 rd and 62 nd day were 405, 11,947 and 37,886 cpm per cutting (Table 1). That is, uptake of ¹⁴CO₂ increased more than 90 times in one month from the 33 rd to 62 nd day, along with a 6-fold increase in leaf area in the same period. Nearly all (more than 99%) of the fixed radioactivity was recovered in new shoots on the 33 rd and 43 rd day. On the 62 nd day, 3.9% of the fixed ¹⁴C was exported from the new shoots, with 96.0% and 2.6% of the exported ¹⁴C being found in upper and lower half of stems, and the remainder in roots.

Changes in the distribution ratio of radio-

Table 1. Distribution of ¹⁴C in ethanol soluble fraction of different parts of 'Delaware' vine cuttings exposed to ¹⁴CO₂ at different stages of rooting.

Date of ¹⁴ CO ₂ exposure	¹⁴ C ^z (cpm/cutting)				
(Days after)	New shoot	Upper half stem	Lower half stem	Roots	Total
33	401	2	2		405
43	11,939	6	2	_	11, 947
62	36, 413	1, 414	39	20	37, 886

² Determined 6 hours after ¹⁴CO₂ exposure

Table 2. Changes in distribution ratio of ¹⁴C in ethanol soluble fraction of new shoot, upper half stem, lower half stem and roots of 'Delaware' vine cuttings after ¹⁴CO₂ exposure.

Days after cutting	Hours after ¹⁴ CO ₂ exposure	Distribution ratio of ¹⁴ C			
		New shoot	Upper half stem	Lower half stem	Roots
43	6	99.93%	0.05%	0.02%	- %
	24	99. 90	0.07	0.03	_
	72	99. 36	0.60	0.04	
62	6	96. 11	3. 73	0. 10	0.06
	24	67. 17	12.65	7.70	12.48
	72	66.00	10.44	8.69	14.87

Table 3. Distribution ratio of ¹⁴C in sugars, organic acids and amino acids extracted from new shoot of 'Delaware' vine cuttings exposed to ¹⁴CO₂ at different stages of rooting.

Days after cutting	Distribution ratio of ¹⁴ C ^z					
	Sugars	Organic acids	Amino acids			
33	56.0%	24.9%	19.1%			
43	81.4	13.0	5. 6			
62	91.5	5. 5	3. 0			

^z Determined 6 hours after ¹⁴CO₂ exposure

activity in each part after ¹⁴CO₂ exposure are shown in Table 2. The changing patterns of ¹⁴C distribution on the 33 rd and 43 rd day were similar, and therefore data on the 33 rd day are not shown in the table. On these days, more than 99% of radioactivity remained in the new shoots even after 72 hours of exposure, although the ¹⁴C imported into upper and lower half of stems showed a slight increase with time. On the

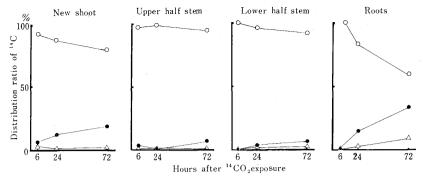


Fig. 1. Chages in distribution ratio of ¹⁴C in sugar (○), organic acid (●) and amino acid (△) fractions of different parts of 'Delaware' vine cuttings after ¹⁴CO₂ exposure.

62 nd day, the percentage of radioactivity in new shoots declined rapidly within 24 hours. After 72 hours, 34% of the ¹⁴C was exported from the new shoots, with 30.7%, 25.6% and 43.7% of the exported ¹⁴C being observed in the upper half of stem, lower half of stem and roots, respectively.

Table 3 shows the distribution ratio of ¹⁴C in sugar, organic acid and amino acid fractions extracted from new shoots 6 hours after ¹⁴CO₂ exposure. The highest percentage distribution of ¹⁴C was in sugars regardless of the stage of rooting, and became higher as the cuttings aged. As ageing occured, the ¹⁴C activity in organic acids and amino acids declined equally.

Separation of ethanolic extracts into sugar, organic acid and amino acid fractions revealed that over 90% of the ¹⁴C was found in sugars 6 hours after ¹⁴CO₂ exposure (Fig. 1). The percentage of ¹⁴C in sugars declined with time in new shoots and roots, but changed little in the upper and lower half of stems. The level of radioactivity in organic acids increased in new shoots and roots. A slight increase in amino acids was shown in roots. In the upper and lower half of stems, the percentage of ¹⁴C in organic and amino acids was relatively constant, showing a range of 0 to 5.5% and 0 to 2.7%.

Discussion

Most of the previous work to elucidate the contribution of photosynthesis to rooting of leafy cuttings has supported its beneficial effects(1, 2, 3, 7, 8). In contrast to the great store of knowledge in leafy cuttings, few studies on photosynthesis in leafless hardwood cuttings are available(10). It has been commonly considered that rootings of hardwood cuttings depends upon carbohydrates reserves(5).

In the earlier study with hardwood cuttings of 'Delaware' vines propagated in a plastic house, we observed that the dry weight of a whole cutting increased following a gradual decrease 60 days after planting, then returned to its original weight after 80 days(6). Subsequent studies revealed that rooting of 'Delaware' vine cuttings was best under a moderate light intensity at the end of the propagation period, although rooting was initiated earlier at a higher light intensity(10). Furthermore, it was observed that rooting was actually hastened by CO2 enrichment during the latter half of the propagation period and inhibited by CO₂ removal during the same period(12, 13). Considering these results, we postulate that photosynthesis in hardwood cuttings of 'Delaware' vines plays an important role in the development of roots during the latter half of the propagation period.

In the present study, nearly all of the fixed ¹⁴C remained in the new shoots even 72 hours after ¹⁴CO₂ feeding during the earlier stage of rooting, suggesting that before the roots emerge the new shoot is a more powerful sink. Recently, it has been shown that the young shoot is a powerful sink

during the early development of the shoot in intact vines as well as in other fruit trees(4, 9, 14). Thus, it may be reasonable to assume that the moving pattern of current photosynthates in hardwood cuttings is initially the same as that in intact vines.

However, after the roots began to emerge, the distribution ratio of the fixed ¹⁴C in the new shoots declined rapidly during the first 24 hours after ¹⁴CO₂ feeding and nearly half of the ¹⁴C exported from the new shoots was translocated through the stem to the roots over a 72 hours period. This fact suggests that at this stage the roots act as a significant sink for current photosynthates. It can be assumed that the translocation of ¹⁴C into the roots may become more active as the root development progresses.

As shown in Fig. 1, the changing patterns in the distribution ratio of 14C in sugar, organic acid and amino acid fractions after ¹⁴CO₂ feeding were distinctly different in new shoots and roots and upper and lower stems. That is, while the percentage of radioactivity in each fraction remained relatively constant in upper and lower stems, in new shoots and roots the level of 14C in sugars declined rapidly and increased in organic acids. In roots, a slight increase in amino acids was also shown. We cannot discuss at present the metabolism of total labelled materials moving into roots, because the incorporation of 14C into the ethanol insoluble fraction was not determined. However, it is conceivable that the increased incorporation of ¹⁴C into organic and amino acids in roots may be involved in active growth.

In conclusion, it is evident that a considerable amount of current photosynthates is supplied to the developing roots. Although current photosynthesis may influence rooting indirectly by affecting some other factor(s) which control rooting, the present results offer sufficient evidence to confirm our previous assumption that photosynthesis in hardwood cuttings of 'Delaware' vines plays an important role in root development during the latter half of the propagation period.

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ブドウ'デラウェア'の休眠枝ざしの発根における光合成の関与

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摘 要

ブドウ'デラウェア'の休眠枝ざしにおける発根と光合成の関係を明らかにするために、さし木33、43、62日後に $^{14}CO_2$ を施し、6、24、72時間後のさし穂内におけるアルコール可溶性物質中の ^{14}C の分布を調査した.

さし木後の日数の経過とともに,さし穂内に取り込まれる ¹⁴C の総量は著しく増大したが,発根開始前の33及び43日後では,施用72時間後でもそのほとんどが新芽中に存在した。しかし,発根がみられた62日後では,施用後の時間の経過とともに新芽から新根へ転流する ¹⁴C の量が増大し,新根が光合成物質に対する sink として働くことを示した。また,62日後のさし穂について,¹⁴C の糖,有機酸及びアミノ酸物質への分布を調査したとこ

ろ,施用 6 時間後では,さし穂のいずれの部位においても 14 C のほとんどが糖中に存在した.そして,茎では上半,下半ともに, 14 Cの分布割合の変化は少なかったが,新芽及び新根では,時間の経過とともに糖中の 14 C が減少し,有機酸中の値が増大した.また,新根では,アミノ酸中の 14 C もわずかに増大した.このように新根において有機酸及びアミノ酸への 14 C の取り込みが増大したことは,それらが新根の発育に関係するものであることをうかがわせた.

本実験の結果は,ブドウ'デラウェア'の休眠枝ざし における光合成が,分化後の根の発育に重要な役割を果 たすことを確証づけた.