Comunicaciones

Respiration in the mature embryos of coffee*

Sumario. Se estudiaron el consumo de oxígeno (QO_2) y el cociente respiratorio (RQ) en los embriones de 15 tipos de café. Los valores QO_2 mostraron una relación inversa a sus pesos. Los valores RQ más alto que se registraron pueden ser debidos a la intervención de productos intermedios del ciclo TCA como substratos respiratorios. Los datos obtenidos eliminan a la fermentación como un evento natural en la respiración del embrión.

Respiratory studies on embryos is meagre (1, 7). Though Kandler (3) and Massart and de Ketelaere (5) have studied respiration employing embryos of different plants, no work has been done on those of coffee. The present study attempts to fill this lacuna.

Method

About 7 month old fruits of some coffee species, varieties and hybrids formed the materials of this investigation (Refer Table 1 for details). The fruits were hand picked prior to experimentation. Embryos were excised by cutting open the fruits longitudinally with a scalpel. Of the two embryos thus removed from each fruit, one was used for the measurement of respiration (QO_2) and the other for the determination of respiratory quotients (RQ). They were immediately weighed and transferred to 15 ml Warburg vessels. Water was added to the flasks so that a final volume of 3 ml was maintained. The central well of each flask contained a fluted filter paper (2 x 2 cm) placed either in 0.2 ml of water or an equal volume of 10% KOH. The flasks were shaken at the rate of 100 excursions per minute in a Braun apparatus. They were equilibrated for 10 minutes at 28 ± 1°C before the readings were recorded. Two thermobarometers were run for each experiment. When it was designed to study substrate oxidations, a sodium salt of the substrate (10 μM) neutralized to pH 7 was tipped from the side arm at an appropriate time. No sparker acid was used.

Results

Table 1 shows the dry weights, QO_2 and RQ values of the coffee embryos studied. *C. congensis* and *C. excelsa* gave the highest and lowest dry weights per embryo respectively. *C. excelsa* recorded the highest QO_2 whereas it was lowest in *C. arabica* S. 288. The materials chosen for this study showed RQ values ranging from 1.2751 (*C. canephora* S. 274) to 1.7485 (*C. salvatrix*) and were thus consistently higher than unity.

Table 2 lists the QO_2 as well as the RQ values of *C. canephora* S. 274 under two different conditions: one, conditions conducive to aerobic respiration (21% O_2) and two, conditions causing fermentation (5% O_2). The QO_2 and RQ values under oxygen tension were respectively 34.92% and 188.79% of the controls. In another experiment where several Krebs cycle intermediates and fructose diphosphate were supplied exogenously, the values of QO_2 and RQ were interesting (Table 3). With the exception of succinate, all the exogenously added substrates produced QO_2 values much lower than the controls. Succinate showed the highest recorded QO_2 (2.4898) and lowest RQ (0.6983) in contrast to oxaloacetate with which a QO_2 of 1.4362 and RQ of 1.5865 have been recorded. The RQ of fructose diphosphate was near unity (1.0340).

Discussion

The QO_2 values obtained with different coffee types seem to be no more significant than bringing out a rough inverse relationship between the respiratory

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Table 1.—QO_2 and RQ values and dry weights of the mature embryos of some coffee species. — (mean of 4 replications)

Species	mg. dry wt./embryo	QO_2	RQ
1. *C. excelsa*	0.2912 ± 0.0106	2.7677 ± 0.1923	1.6235 ± 0.0980
2. *C. eugenioides*	0.3900 ± 0.0209	2.2331 ± 0.0806	1.6765 ± 0.0559
3. *C. stenophylla*	0.5521 ± 0.0210	2.0403 ± 0.0223	1.6101 ± 0.0181
4. *C. canephora* S. 274	0.5042 ± 0.0309	1.9973 ± 0.0965	1.2751 ± 0.0171
5. *C. salvatrix*	0.3308 ± 0.0166	1.7598 ± 0.1313	1.7485 ± 0.0320
6. *C. arabica* 'Kents'	0.5712 ± 0.0401	1.6351 ± 0.1868	1.4849 ± 0.1213
7. *C. bengalensis*	0.4758 ± 0.0035	1.3869 ± 0.0838	1.5871 ± 0.0590
8. *C. arabica* S. 795	0.6775 ± 0.0086	1.3690 ± 0.0650	1.3133 ± 0.0762
9. Hibrido de Timor	0.7720 ± 0.0200	1.3214 ± 0.0660	1.4552 ± 0.0552
10. *C. arabica* 'San Ramón'	0.7558 ± 0.0150	1.3053 ± 0.0212	1.1515 ± 0.0071
11. Robusta X arabica	0.7329 ± 0.0154	1.2424 ± 0.0265	1.3405 ± 0.0050
12. Devamachy	0.8720 ± 0.0234	1.1588 ± 0.0725	1.4815 ± 0.0384
13. *C. congensis*	1.1108 ± 0.0220	1.1030 ± 0.0799	1.6398 ± 0.0780
14. *C. arabica* 'Cioccie'	1.0929 ± 0.0122	1.0585 ± 0.0559	1.1570 ± 0.0914
15. *C. arabica* 'S. 288'	0.8220 ± 0.0117	0.9352 ± 0.0274	1.3847 ± 0.0274

rates and embryo weights (Table 1). The respiratory quotient also showed variation from 1.2751 to 1.7485 with the materials studied. This is in accordance with the earlier work of Kandler (3) and Massart and de Ketelaere (5) who recorded RQ values of 1.2 and 1.5 in other embryos.

The significance of this work lies in that the RQ values are considerably higher than unity. This indicates that the metabolism of embryos is not characterized by either hexose monophosphate shunt or glyoxylate bypass because these pathways would result in lowered RQ values of 1.0 and 0.67 respectively (8). It follows that in the respiration of these embryos, either substrates of higher oxidation like TCA cycle intermediates are involved or carbondioxide is anaerobically generated (8). If there had been anaerobic respiration and a consequent oxidation of the accumulated fermentation products, then oxygen debt and Pasteur effect would have been evident (9). But no such oxygen debt was observed when the system equilibrated with the ambient atmosphere (Fig. 1).

Table 2.—Respiratory behaviour in the embryos of *C. canephora* 'S. 274' under normal and low oxygen supply.

(Mean of duplicate experiments).

Conditions of the Experiment	QO_2	RQ
1. 21% Oxygen (air)	2.1064 (100%)	1.1636 (100%)
2. 5% Oxygen + 95% Nitrogen	0.6032 (35%)	2.1968 (189%)

Table 3.—Effect of some Krebs cycle intermediates and fructose diphosphate on respiration of the mature embryos of *C. canephora* 'S. 274' (Mean of duplicate experiments).

Substrate added	QO_2	RQ observed	RQ expected*
1. None	2.1677	1.2136	—
2. Citrate	1.6899	1.3421	1.33
3. Cis-aconitate	1.6503	1.3647	1.33
4. Isocitrate	1.6756	1.3670	1.33
5. Oxoglutarate	2.0476	1.1896	1.25
6. Succinate	2.4898	0.6983	1.11
7. Fumarate	1.6129	1.3326	1.33
8. Malate	1.6206	1.1128	1.33
9. Oxalacetate	1.4362	1.5865	1.60
10. Fructose diphosphate	1.8499	1.0340	1.00

* From M. Thomas (8).

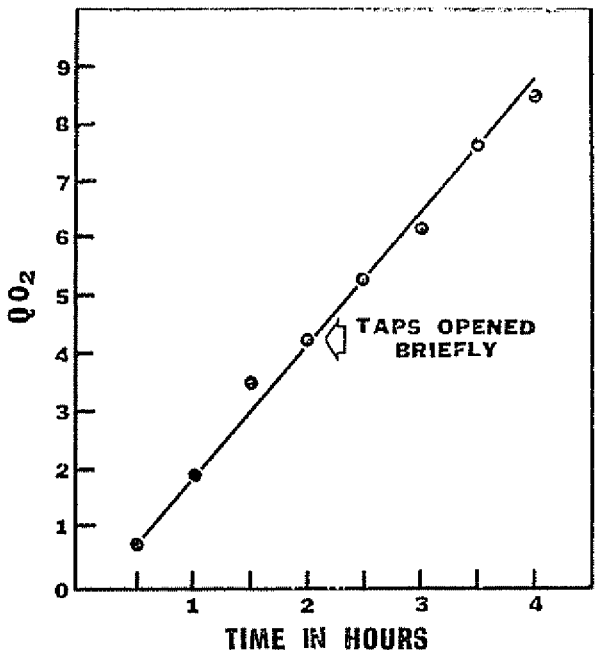

Fig. 1.—*Respiratory rates as a function of time in the embryos of* C. canephora *S 274. At 2 hours, the taps have been opened to let in air equilibrated for 5 minutes and readings continued after closing the taps.*

When the respiratory behaviour of embryos under conditions of normal air (21% O_2) and low oxygen (5% O_2) was studied, the respiratory intensity under the latter condition was one third of the control and the RQ showed a two-fold increase (Table 2). In both low and normal oxygen supply, the values of RQ should remain the same if a process like fermentation which has no requirement of oxygen had taken place. The fact that the oxygen level influenced the RQ shows that the embryos develop the faculty to ferment if conditions peculiar to this are imposed. In the light of this, the natural process of respiration in these organs appears to be the one with molecular oxygen as a final electron acceptor rather than fermentation.

If fermentation would not explain the high RQ values obtained, the possibility of TCA cycle intermediates playing a part arises. Table 3 shows a broad agreement between the theoretical and observed values of RQ for some TCA cycle intermediates and fructose diphosphate. This indicates that the embryos are capable of complete oxidation of added substrates requiring molecular oxygen as a final electron acceptor. *In vivo* studies concerning the complete oxidation of organic acids are not extensive. Krebs and Eggleston (4) recorded RQ values very close to the theoretical value of 1.2 in the pigeon breast muscle fed with pyruvate. Millerd *et al.* (6) obtained a value of 1.4 for a mixture of malate and pyruvate. The mitochondria extracted from *Arum maculatum* gave RQ values of 1.12 for malate-sparked pyruvate oxidation, 1.30 for citrate, 1.15 for oxoglutarate and 0.38 for succinate oxidation (2). The data summarized in Table 3 vividly expose the dominant role these acids have in the respiration of coffee embryos in general and the elevated RQ values in particular. More work in this direction is necessary to obtain a full picture of the respiratory metabolism of these embryos.

Summary

Oxygen consumption and RQ values in the embryos of 15 coffee types were studied. The QO_2 values showed an inverse relation to their weigts. Higher RQ values recorded might be due to the involvement of TCA cycle intermediates as respiratory substrates. The data obtained rule out fermentation as a natural event in the embryo respiration.

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