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Gaseous exchange between the developing cotyledons, hypocotyls of *Avicennia* pericarp in relation to outside atmosphere

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Abstract

Study on the gaseous exchange between the developing cotyledons, hypocotyls in the pericarp of the *Avicennia* in relation to its outside atmosphere was conducted. The relative proportions of pericarp and the cotyledon tissue was determined. The structure of the pericarp and its relationship to the cotyledons was also studied. Internal gas content of *Avicennia* seedling was examined. Permeability and exchange of the gas with respect to the pericarp and seedling of *Avicennia* was also assessed. Acetaldehyde, Ethanol and Lactate Content in the seedlings *Avicennia* was also measured. The results showed the continuous decrease in the relative weight of the pericarp. The gas bubbles from immersed intact seedlings were found to be larger. The intercellular gas was found to be high CO_2 than those found in air.75 % and 87% reduction in the levels of lactic acid and acteladehyde was noticed, when the cotyledons tissue was exposed to the atmosphere by the fruit walls; whereas the ethanol disappears completely

Keywords: *Avicennia* Pericarp, *Avicennia* cotyledon, *Avicennia* Hypocotyls, Gaseous exchange, Gas permeability, Diffusion resistance

Introduction

“There is evidence that complete or partial anaerobiosis may occur in seeds before the testa is ruptured” (Barker & El Saifi, 1952). This has been suggested to be the relative impermeability of the seed coat to gases. This, together with the high diffusion resistance of the cotyledons of bulkier seeds, may induce fermentation in some peas (Seaman & Turner, 1956), and may be reflected in heightened RQ values or by the presence of anaerobic end-products.

It has been suggested that the transient accumulation of lactic acid often found in germinating seeds related to either “a limiting supply of oxygen” (Barker & El Saifi, 1952) or “to a high internal carbon dioxide concentration” (Turner, 1951; Thomas, 1925).

Sherwin and Simon (1969), found that the removal of the testa from soaked seeds prevented any subsequent accumulation of lactic acid. It accumulated under normal conditions for about 30 hours in *Phaseolus* seeds then disappeared slowly as the radical began to emerge. This suggested that conditions within the intact seed were anaerobic.

A high concentration of carbon dioxide and a low concentration of oxygen within a seed may lead to an inhibition of germination (Thomas, *et al.,* 1958). Kidd and west (1917, 1920) found that *Brassica alba* seeds in an atmosphere of carbon dioxide at concentrations between 2 and 4% did not germinate, but upon removal to a normal atmosphere, they did. The tissues could tolerate even very high concentrations of carbon dioxide and still germinate on return to air. This was "attributed to an effect of carbon dioxide in depressing anaerobic processes which given rise to toxic products". Kidd and West found that concentration of carbon dioxide necessary to inhibit germination is correlated with both temperature and oxygen supply. The higher the temperature, or the higher the percentage of oxygen, the higher is the concentration of carbon dioxide necessary to produce inhibition. Morinaga (1926), found that the removal of the seed coat improved the germination of white clover. It may be that a lowered oxygen concentration is more significant than a high carbon dioxide concentration. The lowering of oxygen below a certain level (the extinction point) may induce the production of toxic end-products.

The *Avicenna* embryo passes through no resting stage but continues to grow. The developing seedling is surrounded by a tough hairy coat, the pericarp, formed from the ovary wall. Guppy (1912) reported that the *Avicenna* plant is viviparous. Here, however, germination is associated with the rupture and death of the fruit envelopes. "The possibility cannot be overlooked that the pericarp of *Avicenna* imposes a barrier between the developing seedling and the atmosphere, restricting gaseous exchange and causing carbon dioxide to build up around the seedling (Macgregor Skene, 1959). In *Bruguiera*, on the other hand, continued development of the hypocotyledons on the tree may be due to the absence of auto-nacrcosis induced by respiratory carbon dioxide (Kidd, 1914).

Bharucha and Shirke (1947) reported the effect of the pericarp on the germination of *Avicenna officenalis* observed that it "has an inhibiting effect on the germination of the 'Unripe' seeds" and that this "gets less pronounced as the testa (sic) matures." They found that the carbon dioxide output from seeds removed from their pericarps was 34% greater than that from seeds with their pericarps intact. Chapman (1962) confirmed that the presence of the intact fruit wall in *Avicennia* result in a lower respiratory carbon dioxide output and attributed this to the restruction of gaseous exchange imposed by the pericarp.

Brown (1940) worked on the seed coat of *Cucurbita pepo* observed that forms an important vehicle for transport of gases across the testa, and that, as carbon dioxide is more soluble, it diffuses more quickly. It is possible that high concentrations of carbon dioxide do not build up in *Avicennia* tissues. The young *Avicennia* seedling falls into the sea, its pericarp soon loosens and falls off, but until this time, the seedling remains relatively dry. Although the lower parts of the mangrove trees are periodically submerged, the tide rarely reaches the fruit-branches.

It was decided to look more closely at the *Avicennia* pericarp in an attempt to see whether it imposed a restriction on gaseous exchange between the developing cotyledons and hypocotyledons and the outside atmosphere.

Materials and Methods

Morphology of the Seedlings

Relative proportions of pericarp and cotyledon tissue

A range of tree-borne seedlings from very small to large (hence from young to those joust lprior to dropping from the parent tree) was selected. The pericarps were carefully separated from the cotyledons. Each was weighed separately and expressed as a percentage of the whole.

Structure of the pericarp and its relationship to the cotyledons

Cross sections were cut from *Avicennia* seedling of a range of sizes in order to study the relationship of the pericarp to the cotyledons and the structure of both.

The pericarp is a fleshy tissue. As it ages the cells lose water and become compressed. In general, the pericarp is between 35-45 cells wide. It is made up of two zones:

1. An exocarp bearing numerous, usually multicellular, thick-walled hairs:
2. A mesocarp of thin-walled, parenchymatous cells. The tissue is at first dense with no intercellular space but these develop towards the center as the cells become larger. For example, in a 4 mm. diameter seedling, of which 1 mm was pericarp tissue, there were no intercellular spaces in the mesocarp until 10-12 cells in from epidermal layer.

There is o distinct endocarp. At all stages of development apart from an occasional small gap, the percarp are closely adherent to the cotyledon tissue. The cotyledon is made up of a dense mesophyll with small intercellular space comprising only 8.5% of the whole (Outred, 1966).

Analysis of the Internal Gas Content of *Avicennia* Seedling

Gas from the intercellular space of *Avicennia* seedlings was collected under reduced by displacement of water from a tube over a filter funnel. This simple collection device was placed in a large beaker of degassed water in a desiccators attached to a vacuum-pump. The gas was collected through water for convenience in preference to saturated salt solution.

When intact seedlings were gas bubbled from air became trapped at the hypocotyls end of the cotyledon and in the folds of the cotyledon itself. Attempts to brush these away by hand or with an initial high vacuum before collecting the intercellular gas were only partially successful. It was found necessary to break up the tissues under water and to shake the pieces up and down vigorously for a few seconds before beginning to collect the gas. Pericarps were remove immediately before gas extraction. Gas samples were analyzed on a mass spectrometer after 15-minutes collection times.

Gas permeability (Diffusion Resistance) of the Pericarp

A simple apparatus (fig. 5) was devised to measure the permeability of the *Avicennia* pricarp. A similar type of apparatus was used by Kidd and West (1917) for testing the permeability of pea tested to oxygen.

A disc of pericarp tissue out cleanly with a cork-borer was held between the ground-glass top of a glass tube (smeard with a little Vaseline) and the rim of the metal cap using a tight spring to obtsin a good seal. Pressure was established either by sucking air out with a vacuum-pump or by blowing air in, causing the fleshy pericarp to swell inward or outwards. Changes of pressure were followed on the manometer and corrected for atmospheric variations using a control manometer-flask assembly of the same volume. The apparatus was tested for leaks using 1-3 layers of thick plastic instead of pericarp tissue. Plastic is permeable so there was a slowe lead and normal pressure was re-established over several hours.

Gas Exchange of *Avicennia* Seedlings

The gas exchanges of small, whole seedlings, both with and without their fruit-walls, was measured under humid conditions using the direct manometric method (Umbreit, Burris & Stauffer, 1957).

Acetaldehyde, Ethanol and Lactate Content of *Avicennia* Seedlings

Within 30 minutes of their collection of *Avicennia* seedlings were rinsed in distilled water, removed from their branchelts and stem-distilled or else the cotyledons and pericarps were distilled separately either immediately after their separation or after standing for 24 hours in a humid atmosphere at room temperature. Extracts were analyzed for acetaldehyde, ethanol and lactic acid (i.e. the common end-products of fermentation).

Results

Relative proportions of pericarp and cotyledon tissue

The result is in fig. 2. There is a continuous decrease in the relative weight of the pericarp as the cotyledons continue to grow. Fig. 2. Show that in seedling of approximately 0.6 g. fresh weight pericarp and cotyledon tissue make up equal proportions of the total structure. As the seedling matures the cotyledons become the dominant tissue.

Structure of the pericarp and its relationship to the cotyledons

The structure of the pericarp and cotyledon is shown in fig. 3 and 4.

Both pericarp and cotyledon tissues has gas and possibly some gas is held between the two. It was noticed that gas bubbles from immersed intact seedlings were larger than those from or salt water. Those from which the pericarps have been removed usually sink in fresh water, but only rarely sink in salt water.

Analysis of the Internal Gas Content of *Avicennia* Seedling

Table 1. Percentage composition of gas extract from *Avicenna* cotyledons.

Gas	Air Blank	Cotyledon Sample No			
		1	2	3	4
N_2	78.87	79.48	78.80	82.95	79.41
O_2	21.00	17.03	18.18	11.43	18.01
CO_2	0.03	3.48	3.03	5.62	2.58

The result show that the intercellular gas is composed of amounts of carbon dioxide higher, and of oxygen lower, than those found in air. A more sophisticated technique of gas extracted might reveal differences more marked than those found here. spectrometer after 15-minutes collection times.

Gas permeability (Diffusion Resistance) of the Pericarp

As the pericarp dried the cells collapsed and it became parchment-then. It became permeable and the mercury column leveled off after 18-20 hours. As the process could not be hastened by artificially drying the tissue it was thought to be caused by the pericarp disc shrinking away from the wall of the metal cap. If the whole apparatus was kept in an atmosphere of high humidity , drying of the pericarp was retarded and the pressure could be maintained for long periods (6 weeks or more) even thought the became paper-thin.

It was necessary to use pericarp from medium to large-sized seedling to prevent splitting of the tissue discs. Thus it was not possible to say whether there was any change in permeability with age.

The pericarp seems to present a barrier of high diffusion resistance between the developing seedling and the outside atmosphere.

Gas Exchange of Avicennia Seedlings

The result are shown in table 2. Table 2. respiration rates of small whole Avicennia seedlings, with and without their pericarps, and of the pericarps alone, measured under Warburg conditions in a hind atmosphere at 25degree centigrade. Seedling were used in which cotyledon tissue made up only 38%of the whole. Gas exchanges are expressed as µ1.g-1dwt.

Table 2. respiration rates of small whole Avicennia seedlings

	Whole Seedlings (Intact pericarps)			Seedlings without Pericarps			Pericarps alone		
Min	O_2	CO_2	RQ	O_2	CO_2	RQ	O_2	CO_2	RQ
60	793	1247	1.6	1422	1456	1.0	1085	977	0.9
60	683	1135	1.6	1238	1284	1.0	886	942	1.1
120(Total)	1476	2382	1.6	2660	2740	1.0	1971	1919	1.0

The RQ of unity indicates that cotyledon and pericarp probably have a carbohydrate metabolism. That the pericarp restricts gaseous exchange Is indicated by the lower respiration rate of intact seedlings. The oxygen uptake is 45% lower in intact seedlings than in those without a pericarp. When seedlings are intact the RQ value of 1.6 indicates that carbon dioxide is being produced by both aerobic and anaerobic processes. Calculated from a basal RQ unity, 38% of the carbon dioxide is being contributed from anaerobic sources. Both oxygen and carbon dioxide values were after the second 60-minutes period as compared to the first by approximately 12% in all cases, probably due to factors inherent in the experimental method.

Acetaldehyde, Ethanol and Lactate Content of Avicennia Seedlings

The results are shown in table 3. Table 3.Levels of acetaldehyde, ethanol and lactic acid in *Avicennia* seedlings removed straight from the parent tree and either distilled intact or pericarps and cotyledons separately. Pericarps and cotyledons were distilled immediately or after standing for 24 hours in a humid atmosphere.

Table 3. Levels of acetaldehyde, ethanol and lactic acid in *Avicennia* seedlings

Sample	No.per sample	Approx f.wt of each (g)	µg.g-1dwt.		
			Acetaldehyde	Ethanol	Lactate
Small intact Seedlings	65 36 18	<0.1 0.5 0.7	9 8 12	23 0 0	371 410 1113
Medium seedlings: Intact	8	5.0	23	125	648
Cotyledons	8	5.0	15	88	580
Cotyledons after 24 hours	8	5.0	2	0	148
Pericarps	14	0.8	5	25	117
Pericarps after 24 hours	14	0.8	0	0	0

Avicennia seedlings contain lactic acid but negligible amounts of acetaldehyde and ethanol. The bulk of each (75% of ethanol and 83% of lactic acid) occurs in the cotyledons. Except in small seedlings (where the value is 16), 5-6 times more lactic acid than ethanol is producer. When the cotyledons tissue is exposed to the atmosphere by the fruit walls, the levels of lactic acid drops by approximately 75%, acetaldehyde by 87% and ethanol disappears completely. The levels in pericarp tissue separated from cotyledons drop to zero.

Discussion

The *Avicennia* seedling in its development follows the rules formulated by Guppy (1912) that " there is a continuous decrease in the proportion by weight of the perricarp, and a continuous increase in that of the seeds", until at the stage just before the seedling drops from the parent tree the pericarp makes up only 12% of the total fresh weight of the seedling.

Anatomical study shows that the pricarp fits snugly against the cotyledons with only the occasional inter-tissue space between the two.

An investigation of the permeability of the pericarp reveals that is capable of retarding free gaseous diffusion between the cotyledons and the outside atmosphere, and the composition of the intercellular gas indicates that respiratory processes are building up an atmosphere of high carbon dioxide and low oxygen concentration around the developing seedling. Atmospheres of nearly 6% carbon dioxide and 11% oxygen are recorded.

The gaseous environment of the seedling surrounded by the pericarp is such that aerobic respiration processes are lowered and processes of fermentation are initiated, i.e. the tissue is respiring in a concentration of oxygen below the extinction point. If the gas analysis figures are correct the extinction tissues (Stiles & Leach, 1960).

If a simple EMP pathway of glycolysis was being followed, levels of ethanol equivalent on carbon dioxide measured would be expected. However, an analysis of the common end-products of fermentation reveals that the seedlings contain lactic acid but only small quantities of ethanol. While the production of lactic acid is generally thought to involve no simultaneous production of carbon dioxide, the acid may also arise from the dismutation of pyruvic acid with the production of carbon dioxide; the acid may also arise from the dismutation of pyrvic acid with the production of carbon dioxide.The peak of lactate production occurs in 0.7g. Seedling seedlings at a stage of development when the ratio of pericarp: cotyledon is approximately 1:1 (see fig. 2). It may be that from this stage on the pericarp becomes increasingly permeable. The greter amount of lactic acid produced in small seedlings is probably also related to the fact younger tissues have a higher respiration rate.

It seems that a partial anaerobic, at least, occurs in *Avicennia* seedlings before the pericarp is ruptured. The lowering of oxygen tensions leads to an initiation of the reactions of fermentation in the cotyledons. That the pericarp tissues also contain small amounts of ethanol and lactic acid may be a reflection of the intimate association of the two tissues.

There must also be effects of increased carbon dioxide concentration on the developing seedling. There are indications that if carbon dioxide is allowed to build up around tissues, a much slower metabolic rate is established. In experiments using a range of carbon dioxide concentrations, a concentration of 3% was found to depress substantially the oxygen uptakes of small, whole *Avicenna* seedlings under humid conditions. Kidd and West (1917) comment on the effect of carbon dioxide in depressing anaerobic processes. In experiments where *Avicennia* seedlings were kept either under sealed anaerobic conditions or under conditions where nitrogen gas was blow continuously over the tissue (see page 84) lactate production was found to be substantially lowered when carbon dioxide was allowed to accumulate.

On the removal of the cotyledons from their percarps the levels of the end-products of fermentation drop, indicating that the presence of the pericarp and the accumulation of the end-products are related. That levels in the pericarp drop to more quickly than those in the cotyledons on the separation of the two tissues may be related both to lower levels in the pericarp and to the thinness of the pericarp tissues of the volatile end-products.

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