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STUDIES ON RESPIRATORY QUOTIENT AND GAS EXCHANGES IN AVICENNIA PERICARP

Dr K. Suresh
Nalanda Degree and PG College , Jagtial, Karimnagar, INDIA.
suresh45883@gmail.com

Abstract—**In Avicennia pericarp ,the seeds resists diffusion higher rate which results in fermentation with its end produets i.e lactic acid etc. Fermentation in this germinating seeds is due a limited supply of oxygen or high concentration of co_2. Less concentration of o_2 is more consitive than high co_2 for inhibition.**

The analysis of internal gas content of avicennia seedling for different somples showed that the amount of co_2 was higher and o_2 lower then these found in air.gas exchanges and respiration rates of small whole seedling, with and without pericarp was measured by direct manometric method and under Warburg conditions.o_2 and co_2 values were decreated after 60 minates period as compared to the first by approximately 12% in total cases. In that seedling pericarp restrict, gases exchange that lowered the respiration rate. By removal of the fruit walls the cotyledon tissue is exposed by approximately 75%,acetal dehyde by 87% and ethanol disappears completely, zero levels of formentation products is obscrved when pericarp tissue is separated.

Index terms- **Respiratory Quotient and Exchanges.**

I. Introduction

"There is evidence that complete or partial anaerobisi may occur seeds before the testa is ruptured" .this has been suggested to be due to the relative impermeability of the seed coat to gases. This,together with the high diffusion resistance of the cotyledone of bulkier seeds,may induce fermentation in some and may be reflected in heightened RQ vslies or by the presence of anaerobic end-products.

It has been suggested that the transient accumulation of lactic acid often found in germinating seeds is related to either "a limiting supply of oxygen "or to a high internal carbon dioxide concentration .

Sherwin and simon (1969) found that the removal of the testa form soaked seeds any subsequent accumulation of lacti acid. It accumulated under normal conditions for about 30 hours in phaseolus seeds then disappeared slowly as the radicle 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 found that Brassica alba seeds in an atmosphere of carbon dioxide at concentrations between 2 and 4 % did not germination, but upon removal to a normal atmosphere ,they did. The tissues could tolerate even very high concentration of carbon dioxie and still germinate on return to air. Even after exposure of the seeds to 50% corbon dioxie in the absence of oxygen they all germinated. This was 'attributed to an effect of carbon dioxide in depressing anaerobic processes which give rise to toxic products'. Kidd and West found that the concentration of carbon dioxide necessary to inhibit germination is correlated with both temperature and oxygen supply. The higher is the temperature,or the higher the percentage of oxygen, the higher is the concentration of carbon dioxide necessary to inhibition. Morinaga(1926).Found that 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 carbon dioxide from anaerobic respiration and cause the accumulation of toxic end-products.

The Avicennia 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) asserted "where the embryo grows quicker than the fruit-case, as in Avicenna, the plant is viviparous. Here, however, germiration 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, restrioting 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 hypocotyI on the parent tree may be due to the absence of auto-narcosis induced by respiratory carbon dioxide (Kidd, 1914).

Bharucha and Shirke (1947) writing of the effect of the pericarp on the germination of Avicennia officinalis observed that it "has an inhibiting effect on the germination of the 'Unirpe' seeds' and that this "gets less pronounced as the testa (sie) 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 results in a lower respiratiory carbon dioxide output and attributed this to the restriction of gaseous exchange imposed by the pericarp .He concluded: "The presence of the fruit wall, IN maintaining a low respiration rate, may impose a barrier to rapid seedling development while it remains attached to the parent tree."

Brown (1940) working on the seed coat of Cucurbita pepo observed that water 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 pericarp is a fleshy tissue which loses 75./. Of its weight on drying. As the tissue ages its moisture content drops. When the Avicennia seedling 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-bearing 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 hypoctyI and the outside atmosphere.

II. METHCDS AND RESULTS

A. Morphology of the seedlings

(a) Relative proportions of pericarp and cotyledon tissue

A range of tree-borne seedlings from very small to large (hence from young to those just prior 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. The result s are shown in fig.2.

There is a continuous decrease in the relative weight of the pericarp as the cotyledons continue to grow.Fig.2. Shows that in seedlings 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.

(b) Structure of the pericarp and its relationship to the cotyledons

Cross sections were cut from Aviceenia seedlings 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 spaces but these develop towards the centre as the cells become large. For example, in a 4mm. diameter seedling, of which I mm. was pericarp tissue, there were no intercellular spaces in the mesocarp until 10-12 cells in from the epidermal layer.

There is no distinct endocarp.

At all stages of development, apart from an occasional small gap, the pericarp is closely adherent to the cotyledon tissue.

The cotyledon is made up of a dense mesophyll with small intercellular spaces comprising only 8.5. /. Of the whole (Outred, 1966).

The structure of the pericarp and cotyledon is shown in fig.3 and 4.The diagrams were traced from camera Lucida projections.

Both pericarp and cotyledon tissues contain 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 the cotyledons alone. Intact seedlings always float in fresh or salt water. Those from which the pericarps have been removed usually sink in fresh water, but only rarely sink in salt water.

B.Analysis of the Internal Gas Content of Avicennia Seedlings

Gas from the intercellular spaces of Avicennia seedlings was collected under reduced pressure 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 desiccator attached to vacuum-pump. The gas was collected through water for convenience in prefecence to saturated salt solution.

When intact seedlings were used gas bubbles from air became trapped at the hypocetyI end of the cotyledon and in the folds of the cotyledon it self. 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 removed immediately before gas extraction. Gas samples were analyzed on a mass spectrometer after 15-minute collection times. The results are shown in table-1.

Table-1. Percentage composition of gas extracted from Avicennia cotyledons

Gas	Air blank	Cotyledon sample no.			
		1	2	3	4
N2	78.48	79.80	78.95	82.95	79.41
O2	21.00	17.03	18.18	11.43	18.01
Co2	0.03	3.48	3.03	5.62	2.58

The results show that the intercellular gas is composed of amounts of carbon dioxide higher, and of oxygen lower, than these found in air. A more sophisticated technique of gas extraction might reveal different more marked than those found here.

C. Gas Permeability (Diffusion Resistance) of the Pericarp:-

A simple apparatus (fig.5) was devised to measure the permeability of the Avicennia pericarp. A similar type of apparatus was used by kidd and west (1917) for testing the permeability of pea testas to oxygen.

A disc of pericarp tissue cut cleanly with a cork –berer was hold between the groud-glass top of a glass tube (smeared with a little Vaseline) and the rim of the metal cap using a tight spring to obtain a good seal. Pressure was established either by sucking air out with a vacuum-pump or by blowing air in, causing the fleshy percarp to swell inwards 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 slow leak and normal pressure was re-established over several hours.

As the pericarp dried the cells collapsed and it became parchment- thin. It became permeable and the 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 though the tissue became paper-thin.

It was necessary to use pericarps from medium to large sized seedlings to prevent splitting of the tissue discs. Thus it rus 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.

D. Gas Exchanges 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).The results 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 humid atmosphere at 25ºc. Seedlings were used in which cotyledon tissue made up only 38./. Of the whole. Gas exchanges are expressed as ul.g^{-1}dwt.

	Wholeseedlings (intact pericaps)			Seedlings without pericarps			Pericarps alone		
Mins.	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(tot)	1476	2382	1.6	2740	2740	1.0	1971	1919	1.0

The RQ of unity indicates that both 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 up take 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 of unity, 38. /. Of the carbon dioxide is being contributed from anaerobic sources.

Both oxygen and carbon dioxide value were lower after the second 60-minute period as compared to the first by approximately 12./. in all cases, probably due to factors inherent in the experimental method (see page 76.).

Avicennia seedlings contain lactic acid but negligible amouts of acetaldehyde and ethanol .The bulk of each (75./. of asetaldehyde, 78./. of ethanol and 83./. of lactic acid) accours in the cotyledons. Except in small seedlings (where the value is 16), 5-6 time more lactic acid than ethanol is produce. When the cotyledon tissue is exposed to the atmosphere by the removal of the fruit walls, the level 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.

III. DISCUSSION

The Avicennia seedling in its development follows the rule formulated by Guppy (1912) that "There is a continuous decrease in the proportion by weight of the pericarp, 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 pericarp 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 it 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. Atmosphere 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 point occurs at an oxygen concentration not uncommon for bulky tissues (Stiles & Leach, 1960).

If a simple EMP pathway of glycolysis was being followed, level of ethanol equivalent on a molecular basis to the amount of fermentation 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 though to involve on simpultaneous production of carbon dioxide, the acid may also rise from the dismutation of pyruvic acid with the production of carbon dioxide (see page 296)

The peak of lactate production occurs in 0.7g. Seedlings at a stage of development when the ration of pericarp: cotyledon is approcimutelly 1:1 (see fig.2). It may be that from this stage on the peridarp become increasingly permeable. The greater amount of lactic acid produced in small seedlings is probably also related to the foot that younger tissues have a higher respiration rate (see page 230.)

It seems that a partial anaerobiosis, at least, occurs in Avicennia seedlings before the pericarp is ruptured. The lowering of li mentanion 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 concentrations 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 uptake of small, whole Avicennia seedling under humid conditions (see page82.) kidd and last (1917) comment on the effect of carbon dioxide in depressing anaerobic processes. In experiments where Avicennia seedlings were kept sither under sealed anaerobic conditions or under conditions are nitrogen gas was blown continuously over the tissue (see page 84). Lactate production was found to be substantially lowered then carbon dioxide was allowed to accumulate.

On the removal of the cotyledons from their pericarps the levels of the and –products of fermentation drop, indicating that the presence of the pericarps and the accumulation of the end-products are related. That levels in the pericarps drop to zero more quickly than those in the cotyledons on the separation of the two tissues may related both to lower levels in the pericarp and to the thinness of the pericarp tissue allowing for more rapid diffusion out of the tissues of the volatile end-products.

IV. CONCLUSION

The pericarp is suggested to present a barrier to free gaseous diffusion and to induce a partial anaerobiosis in the developing seedling

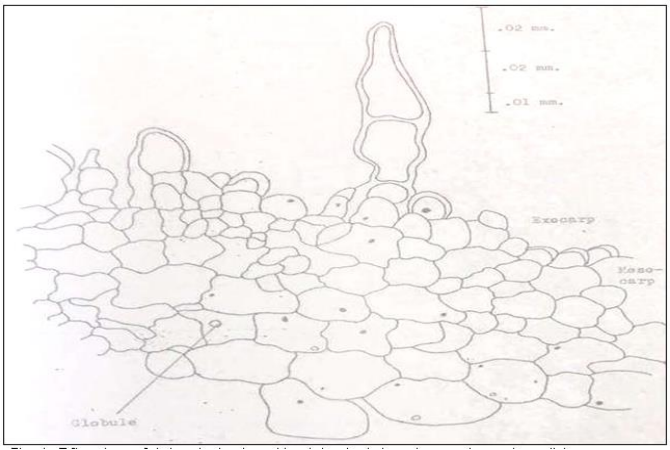
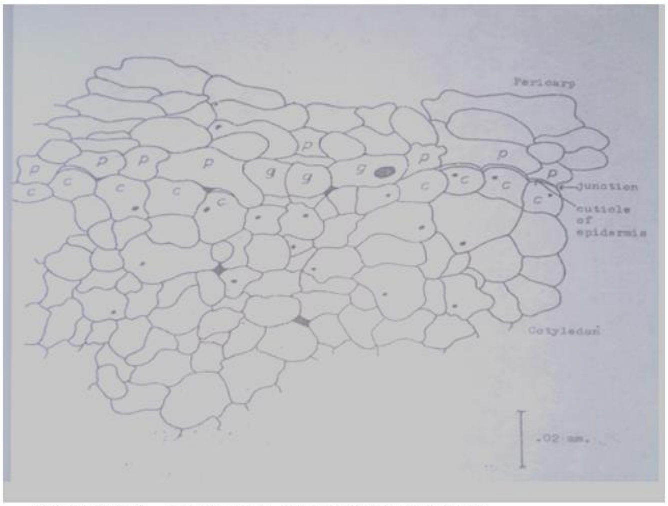


Fig:- 1 T.S pericarp of Aviccenia showing epidermis bearing hairs and outer region no intercellular speces.



C= cotyledon tissue, P= pericarp tissue, g =salt-secreting glands of cotyledon

Fig:-2T.S Aviccenia seedling showing junction between pericarp and cotyledon tissue. Intercellular spaces are shaded.

REFERENCES

[1] Sherwin and simon (1969) found that the removal of the testa form soaked seeds any subsequent accumulation of lacti acid

[2] Guppy (1912) asserted “where the embryo grows quicker than the fruit-case, as in Avicenna, the plant is viviparous.

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