EFFECTS OF OXYGEN TENSION ON CERTAIN PHYSIOLOGICAL RESPONSES OF RICE, BARLEY, AND TOMATO

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(WITH NINE FIGURES)

The relation of oxygen pressure to some metabolic processes of plants has been examined in recent experimental work with particular reference to the energy requirements of active cells. The reactions studied under the most carefully controlled conditions are accumulation of salt and respiration by excised roots and storage organs. This is particularly true of the work by HOAGLAND and his associates at the University of California, and STEWARD at the University of London.

Other investigations have been concerned with the response of entire plants to oxygen deficiency in the roots. Earlier studies compared plants grown in submerged soils with those grown in well-drained soils. It is evident that the control over such experiments is complicated by the influence of several variables such as carbon dioxide pressure, accumulation of anaerobic products, and the intervention of microbiological activity. Greater control has been possible when water cultures have been employed, exposing the root systems to continuous streams of air or relatively pure nitrogen. The influence of oxygen supply on tomato, buckwheat, barley, lupine, and other plants has been studied in this way.

The oxygen relations of the rice plant present a particularly interesting and important problem since as a crop it is grown under conditions (apparently anaerobic) which inhibit the growth of many field plants. This ability of rice to thrive in submerged soil has been observed and confirmed by some research workers and denied by others. A rather large number of diverse experiments on a qualitative scale has left suspended a final estimation of the capacity of rice, real or apparent, to grow in an anaerobic root environment.

This investigation has proceeded in several directions with the hope of providing additional information on the above problem. The response of rice roots to different oxygen tensions has been studied, especially with respect to respiration and mineral absorption. Barley and tomato were employed as comparison plants; the former because of its non-critical oxygen requirement, and the latter on account of its high need for this element. As shown generally in many greenhouse experiments, barley may be considered as occupying an intermediate position between rice and tomato in this respect.

These experiments are presented in three parts. The first concerns the salt accumulation and respiration of excised roots under different oxygen pressures, and the accumulation of salt by roots of entire plants under aerobic and anaerobic root exposure. The second covers the growth and salt accumulation by entire plants in drained and submerged soil. The third part deals with the growth and salt accumulation by plants in culture solutions exposed to streaming air, nitrogen, carbon dioxide, and methane.

Methods and results

Accumulation of salt and respiration by excised and intact roots

Accumulation of salt by excised roots.—The use of salt accumulation and respiration as an index of metabolic response to oxygen pressure was employed by Steward (**18**) using potato discs under controlled conditions. He found a great increase in the accumulation of bromide and in respiration, measured by carbon dioxide production, as the oxygen content was raised from low partial pressures up to twenty per cent. Above this value and up to pure oxygen there was little increase in salt accumulation or respiration. In subsequent work Steward and his coworkers (**19**) obtained the same type of curve for carrot and artichoke storage tissue. On the other hand, excised potato roots showed a critical value in oxygen limitation at ten per cent. This is approximately the curve described by Hoagland and Broyer (**13**) on excised barley roots for bromide, potassium, and nitrate accumulation, sugar loss, and carbon dioxide production.

Before proceeding to a comparison of the behavior of excised rice, barley, and tomato roots under different oxygen pressures, several preliminary experiments were conducted on barley roots to clarify some interesting questions raised by previous workers. Hoagland and Broyer showed the importance of the salt and sugar content of tissues involved in salt accumulation over short time intervals. The tissues used in their oxygen-variable experiment were of the low salt-high sugar type.

The influence of salt status on the response of excised barley roots to various oxygen pressures was first examined. The technique with minor elaborations is that described elsewhere (**13**). Seeds were germinated on wire screens between cheese-cloth and after a week placed in paraffined corks. Twenty-four such corks were placed in 4-liter enamel pans containing Hoagland's solution. The high-salt plants received full strength Hoagland's solution every five days, the low-salt plants were given one-quarter concentration of this solution every two weeks.

After four weeks the roots were excised and placed in 2-liter jars containing 0.005 M KBr for twenty-four hours; each jar was aerated with a different mixture of oxygen and nitrogen ranging from 0.2 to 100 per cent. oxygen. At the end of the absorption period the roots were centrifuged, frozen at 0° F., then thawed and the sap expressed for analysis. The determination of oxygen in the gas mixtures was by the Winkler method (**21**) for the range 1 to 100 per cent., and by a colorimetric method (**9**) based on oxidation of metallic copper to blue cupric ion, for the range 0.02 to 2 per cent.

Analysis of the expressed sap of low-salt and high-salt roots showed that salt status affected the absolute level of bromide accumulation without influencing the shape of the curve. That is, the inflection of the curve for both types of roots occurred at the same partial pressure of oxygen, about 3 per cent.

The effect of time upon the rate of bromide absorption was also examined. Intervals of 1, 4, 12, 24, 36, and 48 hours magnitude for each oxygen pressure disclosed the steepest gradient within 12 hours exposure, with only slight increases thereafter. For this reason the 24- and 36-hour periods were chosen as suitable intervals.

Experiment 1. Comparison of rice, barley, and tomato.—By the procedure outlined above excised roots of rice, barley, and tomato were obtained.

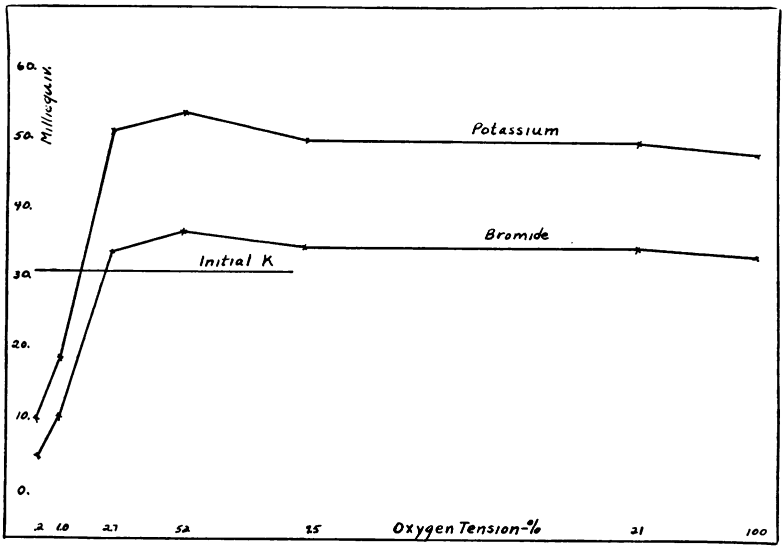

FIG. 1. Bromide and potassium content of expressed sap from excised roots of barley 24 hours in 0.005 M KBr solution.

Three varieties of rice were employed; Caloro variety from California, Hambas lowland, and Carreon upland varieties from the Philippines. Sacramento barley and Santa Clara tomato were the varieties of these plants used.

The roots were exposed to 0.005 M KBr and aerated for 24 hours with oxygen-nitrogen mixtures covering the range mentioned above. The original salt content was not uniform for the three sets as shown by the differing initial potassium concentrations at the time of excision. This accounts for the different maxima of bromide accumulation obtained with each of the root systems (figs. 1, 2, 3).

The bromide accumulation curve of all three sets of roots has the same general shape with the greatest rate of bromide absorption up to 3 per cent. oxygen and very slight increase at higher partial pressures of oxygen. It is interesting to note that as the oxygen pressure falls below 1 per cent. the

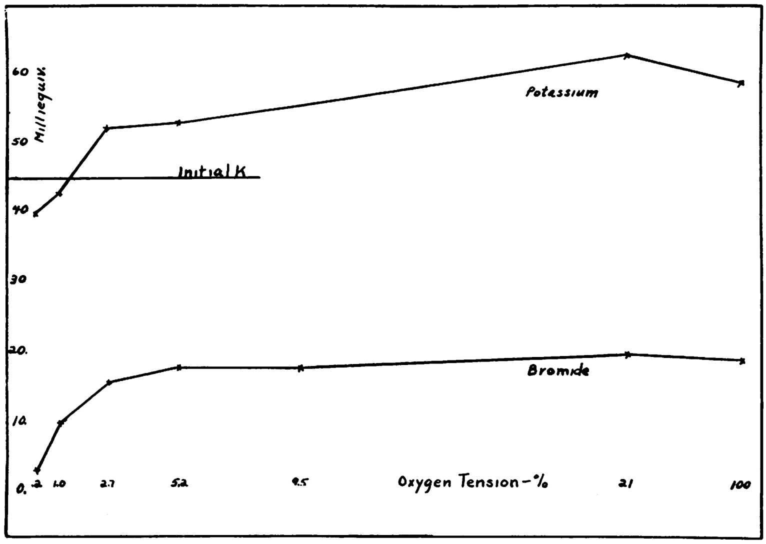

FIG. 2. Bromide and potassium content of expressed sap from excised roots of rice 24 hours in 0.005 M KBr solution.

bromide concentration of the sap fails to rise above that in the culture medium. The curves approximate those for barley given by HOAGLAND and BROYER (**13**). An examination of their data as read from a graph in their publication, shows that the bromide uptake at 4.1 per cent. oxygen was almost as high as at 21 or 100 per cent.

For potassium the picture is complicated by the different initial amounts present. In spite of this, the potassium curves likewise show an inflection

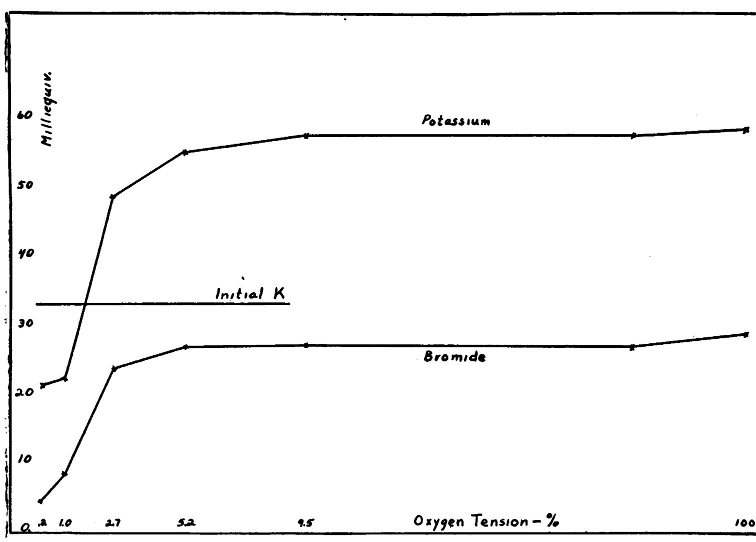

FIG. 3. Bromide and potassium content of expressed sap from excised roots of tomato 24 hours in 0.005 M KBr solution.

at 3 per cent. oxygen. Below this pressure there is a loss of potassium from all roots, a great leakage from barley and tomato, but very slight from rice. The significance of this remains to be determined.

Experiment 2. Accumulation by intact roots.—In the absence of any

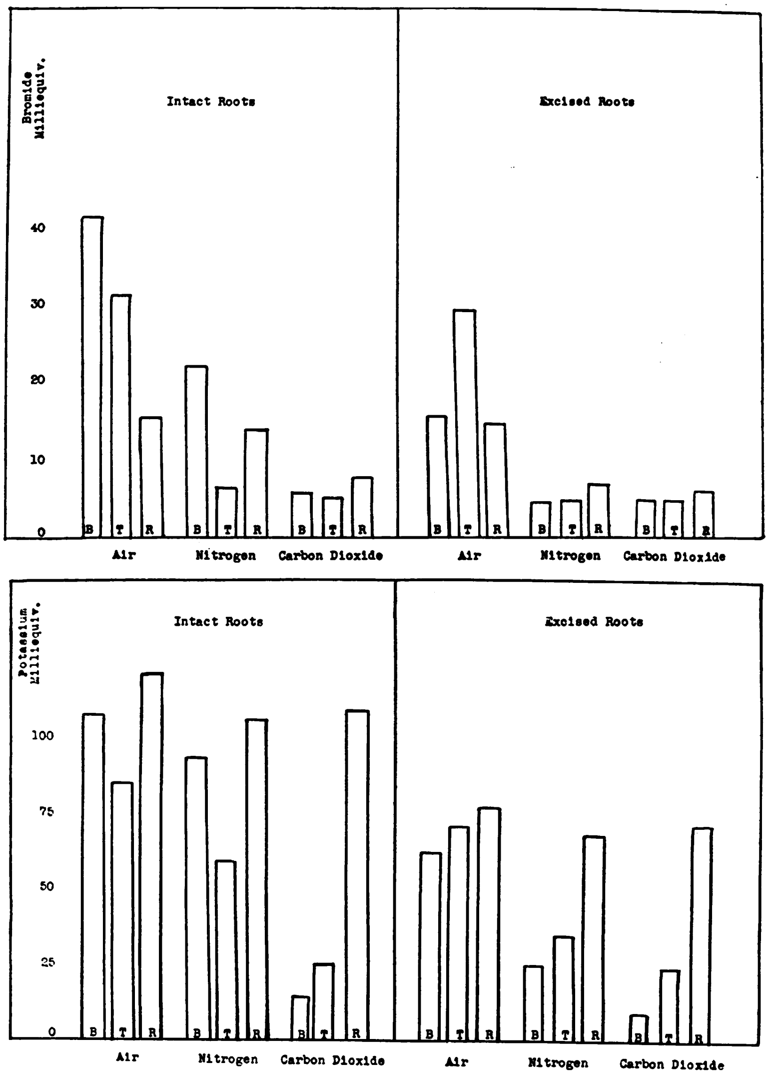

FIG. 4. Bromide and potassium content of excised and intact roots 36 hours in 0.005 M KBr solution. Barley, B; tomato, T; rice, R.

decisive differences in oxygen sensitivity by excised roots of the three plants under investigation, tests were undertaken to determine the influence of the shoot on the behavior of roots exposed to air or nitrogen.

Root systems of entire plants were placed for 36 hours in 2-liter jars containing 0.005 M KBr solutions aerated with nitrogen, air, and carbon dioxide. The nitrogen contained 0.2 per cent. oxygen, and the carbon dioxide about the same. Excised roots from similar plants were included in the solutions along with the intact roots. The results are shown in figure 4.

Bromide accumulation did not occur in excised or intact roots of any plants with the carbon dioxide atmosphere. All excised roots of the nitrogen series likewise failed to accumulate.

The roots of entire plants exposed to nitrogen showed great differences. The tomato failed to accumulate bromide. Barley roots, however, showed a fairly high bromide content and rice relatively more, compared to the respective accumulation occurring with the aerated treatment, which serves as the standard of comparison for each root system.

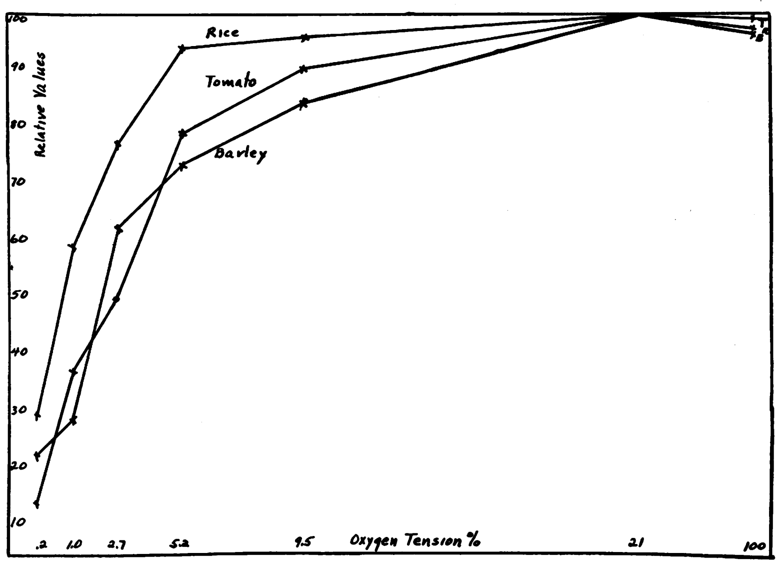

FIG. 5. Relative oxygen consumption by excised roots of barley, tomato, and rice.

The relations for potassium are not as uniform but indicate leakage under anaerobic conditions, especially in the presence of pure carbon dioxide. The aerated roots of all plants again show the highest potassium content.

Another experiment was conducted to determine the effect of a 7-day treatment with the three atmospheres of gas prior to the KBr exposure to air, nitrogen, and carbon dioxide. The resulting data did not alter in any material way the relationships indicated in the preceding paragraphs.

Experiment 3. Respiration of excised roots.—The inter-connection of accumulation and respiration (carbon dioxide production) has been demonstrated by STEWARD (**18**), and by HOAGLAND and BROYER (**13**). In their studies these responses were measured simultaneously and showed an approximately parallel sensitivity to oxygen tension. In the present case this procedure was difficult to follow and it was necessary to make separate measurements of respiration as oxygen consumption.

The respiratory behavior of excised roots from plants grown aerobically in solution was followed with the FENN apparatus (**7**). The oxygen uptake was determined over short time intervals until an equilibrium rate was established. This was achieved within one hour for each partial pressure of oxygen. The data are plotted in figure 5 with values at 21 per cent. oxygen taken as 100. While some differences appear in the curves, they are not significant.

The preceding experiments measured the effects of several partial pressures of oxygen on excised roots and the roots of whole plants previously grown in the usual unaerated culture solutions. The excised roots of the three plants behaved similarly in different oxygen pressures as measured by bromide content and oxygen consumption. On the other hand, the root behavior of whole plants showed the extreme dependence of the tomato on root aeration, as compared to the relative indifference of rice and barley. The latter two plants apparently derive a considerable portion of root oxygen by transporting it through the shoot. This is indicated by the ability of these roots with attached shoots to accumulate under anaerobic root conditions, when an aerobic atmosphere is provided to the shoots.

These tests were all conducted over short absorption periods. Furthermore, they have no direct bearing on growth but only on respiration and salt uptake. The continued effect of several types of root aeration on plant growth was therefore investigated. Experiments were undertaken to determine the growth of these plants in drained and submerged soils, and in culture solutions exposed to different gases for a major part of the vegetative cycle.

GROWTH OF PLANTS IN DRAINED AND SUBMERGED SOIL

Recognition of the importance of oxygen supply of roots in the growth of plants is shown by many investigations utilizing soil and water cultures. The inability of many crop plants to tolerate root submergence when grown in soil is usually attributed to oxygen deficiency. Several other important considerations have been neglected, however, especially the increased concentration of carbon dioxide and the products of fermentation; both inevitable corollaries to anaerobiosis characteristic of water logged or heavy soils.

The literature is comprehensively surveyed in a recent paper on oxygen deficiency in soils (**5**). Only the more pertinent references are reported here. BERGMAN (**2**) obtained no deleterious effects due to submergence of potted *Ranunculus abortivus, R. sceleratus,* and *Cyperus alternifolius,* whereas *Impatiens balsaminea* succumbed to such treatment. He suggests the presence of aerenchyma in the first group of plants. CANNON (**3**), in an extensive study of a large number of species, concluded that plants possess different sensitivity to low oxygen tension; but his results were indecisive, possibly due to inexact control of conditions and inadequate sampling. WEAVER and HIMMEL (**22**) found that submergence improved the growth of certain hydrophytes.

Harrison and Aiyer (**11**) analyzed the gases of swamp rice soils and discovered large amounts of methane with some nitrogen, carbon dioxide, and hydrogen. Further studies (**12**) convinced them of the importance of surface algae as sources of molecular oxygen for the roots of the submerged rice plant. The data on which they base this conclusion, however, are subject to a contrary interpretation in the presence of a large experimental error. Sethi (**16**) as a result of pot and field experiments identified two types of roots in rice, one resembling the structure in wheat and the other containing air spaces characteristic of aquatic roots. He obtained superior shoot and root growth under waterlogged conditions but the fruit yield was

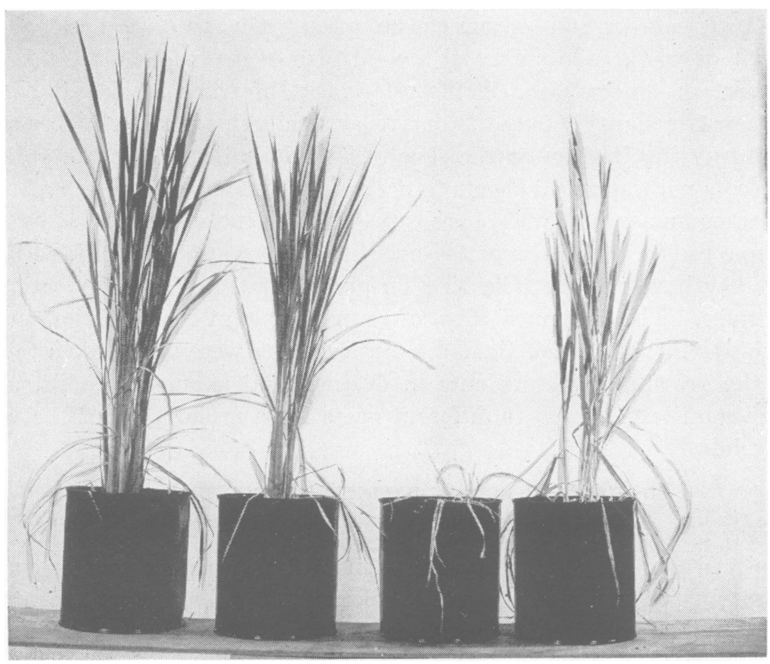

Fig. 6. Plants grown for six weeks in clay soil, drained and submerged. Left to right: rice submerged, rice drained, barley submerged, and barley drained.

only equal to the control. Conway (**5**) using *Cladium mariscus* showed a high oxygen content in intact roots, while excision resulted in a very low oxygen value. van Raalte (**15**) found a similar situation to exist in the roots of rice.

Experiment 4.—The growth of most plants in soil, potted or in the field, is predicated on adequate drainage to maintain an aerobic environment around the roots. Rice forms a notable exception to this in agriculture, and is grown almost universally under a standing water depth varying from a few inches to several feet.

A comparison of the growth of rice (3 varieties), barley, and tomato was made under similar conditions of soil drainage and submergence in a first effort to isolate the specific factors responsible for that behavior. Seeds were germinated by the method referred to previously; after growing to a length of about 15 cm., they were transferred carefully to gallon cans filled with loam or clay soil. One-half the number of cans were drained by means of numerous perforations. Protection of the plants from direct radiation was given for 48 hours to allow recovery from transplanting. All plants retained their apparent vigor during this period.

The submerged plants were gradually exposed to increasing soil moisture until one inch of free water stood above the soil surface and was kept at that level. The drained set received additions of water daily. At the end of

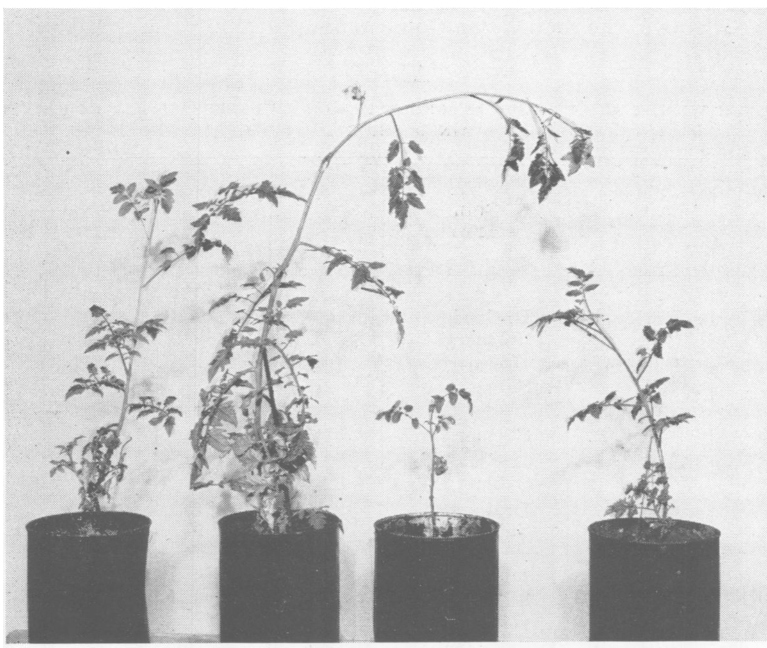

FIG. 7. Tomato plants grown for six weeks in clay and loam soil, drained and submerged. From left to right: loam submerged, loam drained, clay submerged, and clay drained.

six weeks the soil was easily washed away from the roots, photographs taken (figs. 6, 7), fresh weights measured, and after freezing the tissues the sap was expressed and analyzed for potassium. The results are given in table I.

Rice consistently excelled under submerged conditions, particularly with respect to the root system; barley and tomato failed to develop at all in the submerged clay, and grew only slightly in the submerged loam.

The potassium content of the sap of rice shoots was about equal under both growth conditions, but the drained roots of rice averaged twice the potassium concentration of the submerged roots. This difference may be due in a large measure to the dilution effect exercised by the excess water on the salt concentration of the flooded soil. No measurable sap could be extracted from submerged barley and tomato.

The improved growth of rice in a saturated soil provides a sharp contrast to the need of drainage exhibited by barley and in particular the tomato. This difference is much greater than that obtained in the preceding experiment with aerobically grown plants subsequently exposed to anaerobic KBr solutions for short periods of time. From both these experiments it appears that the advantage of intact over excised roots is derived from the presence of a shoot system having access to an aerobic atmosphere. This advantage is greatest in the rice plant, much less in barley, and negligible in tomato; it suggests the quantity of oxygen which may be transported from the shoots to the roots by anatomical adaptation. The evidence further

TABLE I

FRESH WEIGHT AND POTASSIUM CONCENTRATION OF EXPRESSED SAP OF PLANTS GROWN 6 WEEKS IN DRAINED AND SUBMERGED CLAY AND LOAM

SOIL	PLANT	SOIL TREATMENT	SHOOTS		ROOTS	
			FRESH WEIGHT	K CONC.	FRESH WEIGHT	K CONC.
			gm.	*m.eq.*	*gm.*	*m.eq.*
Clay	Barley	Drained	18.0	167	2.2	57
		Submerged	2.0	....	0.2	......
	Tomato	Drained	12.2	....	1.2	......
		Submerged	2.7	....	1.4	......
	Upland rice	Drained	25.7	171	11.1	65
		Submerged	50.5	162	50.0	35
	Lowland rice	Drained	20.0	196	9.2	55
		Submerged	34.5	209	38.6	32
	Caloro rice	Drained	20.6	167	6.3	71
		Submerged	34.8	159	27.5	37
Loam	Barley	Drained	35.1	246	13.4	32
		Submerged	18.5	125	8.0	18
	Tomato	Drained	55.5	....	9.4	......
		Submerged	9.1	....	4.5	......
	Caloro rice	Drained	44.7	187	17.5	15
		Submerged	58.8	208	27.6	11

suggests this as the most reasonable explanation of the activity of intact and excised roots. Of course, this in no way suffices to explain the increase in rice growth as a consequence of soil submergence.

An important distinction between drained and flooded soil arises from the relative availability of oxygen which affects the plant directly; and indirectly by its influence on the activity of soil bacteria and on the chemical constitution of the soil solution. This difference may be expressed by a number of interacting variables of which the more important are oxygen deficiency, carbon dioxide accumulation, products of fermentation, and the presence of reduced ions.

An improved control of these factors was attained by the use of water cultures through which several gases were passed separately.

GROWTH IN WATER CULTURE

The literature on oxygen relations in water culture media is extensive and a recent survey of it is given by DURELL (**6**). HALL, BRENCHLEY, and UNDERWOOD (**10**) noticed a 50 per cent. increase in total dry weight of lupine and barley upon aerating the nutrient solution. FREE (**8**) found buckwheat indifferent to air, oxygen, or nitrogen gases forced through a culture solution, whereas carbon dioxide proved lethal after a short exposure. CLARK and SHIVE (**4**) showed that aeration gave large increases in both shoots and roots of tomato.

ARRINGTON and SHIVE (**1**) doubled the yield of tomato by aerating, and found some carbon dioxide accumulation in the solution to be without effect.

TABLE II

FRESH WEIGHT AND POTASSIUM CONCENTRATION OF EXPRESSED SAP OF PLANTS GROWN 6 WEEKS IN CULTURE SOLUTIONS WITH GAS EXPOSURE

PLANT	ROOT TREATMENT	SHOOTS	ROOTS	
		FRESH WEIGHT	FRESH WEIGHT	K CONC.
		gm.	*gm.*	*m.eq.*
Barley	Control	24.0	10.9	101
	Air	24.6	13.5	99
	Nitrogen	13.2	7.9	102
	Carbon dioxide	1.5	2.7	....
Tomato	Control	49.2	15.1	88
	Air	120.0	34.5	94
	Nitrogen	7.1	1.9	....
	Carbon dioxide	3.5	1.2	....
Upland rice	Control	38.1	13.7	82
	Air	34.6	14.9	86
	Nitrogen	37.2	13.8	81
	Carbon dioxide	3.1	1.0	....

DURELL (**6**) likewise obtained a distinct increase in fruit and vegetative growth of tomato under aerated conditions. STILES and JØRGENSEN (**20**) improved barley and balsam growth by aeration, while buckwheat was unaffected.

Recently SHIVE (**17**) discovered different oxygen requirements for soybean, oat, and tomato as reflected by yield. Absorption of nitrate ions over a short interval was high at low oxygen pressures and decreased at higher pressures, and the absorption of ammonium nitrogen varied inversely as the nitrate.

The effect of root exposure to various gases passed rapidly through the culture solution was studied. The medium consisted of HOAGLAND'S solution and the plants were grown in 2-quart jars. Air, carbon dioxide, and nitrogen (0.2 per cent. oxygen as impurity) were used separately in addition to the unaerated control. The rate of aeration was maintained at 3 liters per hour and the gases forced through sintered glass aerators for dispersion.

Experiment 5.—Seedlings were started aerobically and when the shoots reached about 10 cm. in length were set out in the jars on paraffined corks; seven seedlings of barley and rice per jar and one of tomato. The number of individuals used was small, but due to technical obstacles it was preferred to repeat experiments entirely rather than to use larger populations in a more limited number of tests. The extreme and consistent differences that occurred also tend to reduce the difficulties inherent in variability or imperfect control of conditions.

The results shown in table II support most of the observations presented from the soil experiments. The unaerated control is taken as the standard of comparison. Aeration increased tomato shoot and root growth almost

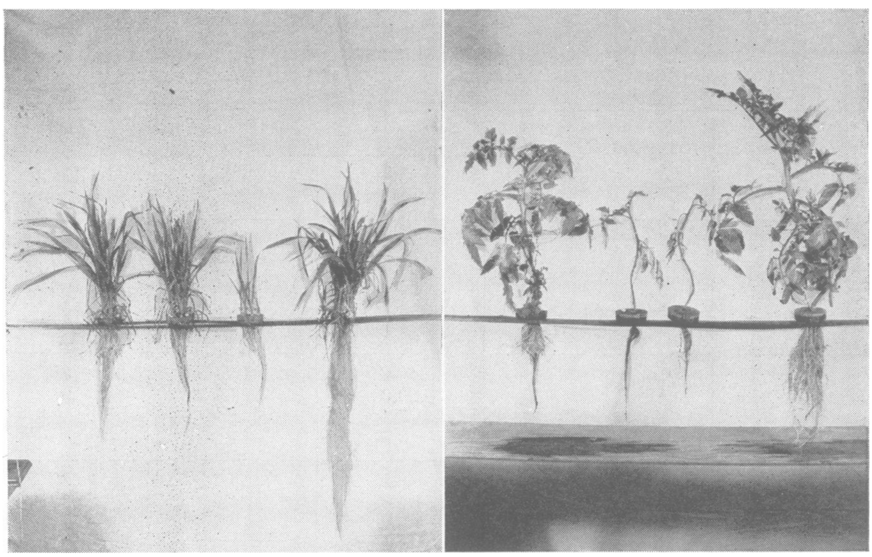

FIG. 8. Barley and tomato plants grown for six weeks in culture solutions with various gas exposures to the root system. From left to right in both series: control (unaerated), nitrogen, carbon dioxide, and air.

equally by over 100 per cent. in fresh weight. Barley consistently gave a small increase in shoot and root growth. The effect of aeration on rice fresh weight was negligible, the experimental error being larger than the treatment variations of any one experiment.

Exposing the roots to a nitrogen atmosphere reduced tomato shoots and roots by about 90 per cent. Barley shoots were 45 per cent. and the roots 30 per cent. smaller than the control. Rice root and shoot growth were of the same order of magnitude as the control. The plants at the time of harvesting are shown in figures 8 and 9.

Carbon dioxide was immediately lethal to all plants. This can hardly be purely the influence of oxygen limitation in view of the foregoing evidence; it more likely represents a specific lethal action of the carbon dioxide superimposed on oxygen deficiency. Other experiments with barley have indicated that a 20 to 30 per cent. partial pressure of carbon dioxide is toxic even if the entire residual pressure of 70 to 80 per cent. consists of oxygen.

Analyses of expressed sap were impossible for those treatments which yielded insufficient material. The sap analysis of shoots was also omitted in the face of evidence of great uniformity in virtually any treatment yielding a measurable amount of growth. Where possible the sap of roots alone was analyzed for potassium.

The tomato root sap had a high concentration of potassium in the control and that of aerated roots was slightly higher. The difference between

FIG. 9. Rice plants grown for six weeks in culture solutions with various gas exposures to the root system. Left to right: control (unaerated), nitrogen, carbon dioxide, and air.

the two, however, is trivial compared to that expressed by fresh weight. Barley roots gave no significant difference in potassium whereas fresh weight differences did occur. This indicates that whatever restricted growth is made by barley and tomato roots under adverse oxygen conditions, the character of that growth proceeds without impairment of the salt accumulating mechanism in those particular tissues. Rice roots likewise showed uniform potassium concentrations, which are in accordance with the uniformity of fresh weight values for this plant.

Inasmuch as the nitrogen contained a small amount of oxygen (0.2 per cent.), an experiment was designed to reduce this impurity as much as pos-

TABLE III

COMPOSITION OF METHANE GAS

CONSTITUENT	PERCENTAGE
Methane	85.0
Ethane	8.7
Propane	5.0
Butane and higher hydrocarbons	1.3
Oxygen (less than 0.02 per cent.)	
Total	100.0

sible. For this purpose commercial methane gas was selected, the gas having the composition given in table III, with no carbon dioxide or monoxide present, or oxygen within the limits of sensitivity of the method used.

Experiment 6.—The procedure followed was similar to the preceding one substituting methane for nitrogen. The plants were grown out of doors instead of in the greenhouse to avoid any danger from the methane. The experiment continued for six weeks.

The data appear in table IV giving fresh weight of shoots and roots, and the potassium concentration of expressed root sap. Aeration improved tomato considerably, and barley slightly; it exerted no influence over rice, however, except for a discoloration at the root tips indicating some form of injury. This apparent damage to the rice root has been observed in several subsequent experiments.

Methane gas caused an absolute cessation of tomato growth immediately on exposure. Barley was reduced by about 60 per cent., a greater loss than

TABLE IV

FRESH WEIGHT AND POTASSIUM CONCENTRATION OF EXPRESSED SAP OF PLANTS GROWN OUTDOORS 6 WEEKS IN CULTURE SOLUTIONS WITH GAS EXPOSURE

PLANT	ROOT TREATMENT	SHOOTS	ROOTS	
		FRESH WEIGHT	FRESH WEIGHT	K CONC.
		gm.	*gm.*	*m.eq.*
Barley	Control	49.2	22.6	103
	Air	53.5	25.3	98
	Methane	20.2	10.2	58
Tomato	Control	41.8	17.4	102
	Air	64.5	31.0	101
	Methane	3.2	1.2	....
Upland rice	Control	25.5	11.1	100
	Air	25.0	15.3	101
	Methane	24.0	24.7	97
Lowland rice	Control	33.6	19.3	91
	Air	31.2	15.4	88
	Methane	33.7	29.2	90

in the presence of nitrogen. The methane treatment resulted in a fresh weight increase of rice roots amounting to about 70 per cent. over the control. The nature of this stimulation of rice from methane exposure has not been determined, but may have the same origin as the beneficial effect of soil submergence on this plant.

The potassium content of the roots assumed the general proportions obtained in the preceding experiment with one exception. The barley roots exposed to methane showed a severe reduction in potassium content which was almost proportional to the decrease in fresh weight.

The apparent stimulation of rice roots by methane offers abundant room for speculation. The use of this gas was suggested by its frequent and abundant occurrence in swamp rice fields (**14**). For the purpose of these experiments it had one advantage over nitrogen in its lack of oxygen. From the effect of methane on barley its behavior would seem to be that of any

TABLE V

COMPOSITION OF NITRATE, AMMONIUM, AND AMMONIUM NITRATE SOLUTIONS

NITRATE		AMMONIUM		AMMONIUM NITRATE	
SALT	CONC.	SALT	CONC.	SALT	CONC.
	M		*M*		*M*
$Ca(NO_3)_2$	0.005	NH_4Cl	0.015	NH_4NO_3	0.015
KNO_3	0.005	KCl	0.005	KCl	0.005
$MgSO_4$	0.002	$MgSO_4$	0.002	$MgSO_4$	0.002
KH_2PO_4	0.001	KH_2PO_4	0.001	KH_2PO_4	0.001
NaCl	0.015	$CaCl_2$	0.005	$CaCl_2$	0.005
Fe	Trace	Fe	Trace	Fe	Trace

Micro-elements added as Hoagland's supplementary A–Z solution.

inert oxygen-free gas. The present data indicate that it inhibits the growth of barley roots to only a slightly greater extent than nitrogen with a 0.2 per cent. oxygen impurity, although the effect on potassium content was more drastic. It remains to be seen whether methane has a specific stimulating influence on rice, or if its superiority rests on purely anaerobic qualities. The rough similarity between rice grown in submerged soil and in water culture exposed to methane offers the prospect of further elucidation of the anaerobic preference exhibited by entire rice plants.

As the culture solution used contained considerable amounts of nitrate, the possible rôle of this anion as a source of respiratory oxygen was examined. It has been shown with soybean plants (**17**) and excised barley roots (**13**) that nitrate is absorbed in large quantities and reduced by the root cells. This creates the possibility that in an anaerobic environment roots may absorb large amounts of nitrate, while maintaining relatively low internal concentrations of this ion by subsequent reduction. The oxygen so released presumably would be utilized in oxidative processes. A test was designed to provide salt nutrients of nitrogen in three forms: nitrate, ammonium, and ammonium nitrate in the presence of air and methane forced through the root medium with the shoots exposed to the air.

Experiment 7.—Plants of barley and rice were grown in unaerated 2-quart jars containing quarter strength HOAGLAND'S solution for three weeks and then transferred to the experimental conditions. The composition of the three solutions containing nitrate, ammonium, and ammonium nitrate, respectively, is given in table V. The osmotic value of the solutions was the same and the nitrogen concentration was also equal for each form of ion. Each solution was treated with the usual aeration and with methane, requiring outdoor growth as before. After two weeks the plants were harvested and the sap of roots expressed and analyzed for potassium. The results are presented in table VI and they do not assign any importance to

TABLE VI

FRESH WEIGHT AND POTASSIUM CONCENTRATION OF EXPRESSED SAP OF BARLEY AND RICE PLANTS GROWN OUTDOORS FOR 2 WEEKS

PLANT	SOLUTION	ROOT TREATMENT	SHOOTS	ROOTS	
			FRESH WEIGHT	FRESH WEIGHT	K CONC.
			gm.	*gm.*	*m.eq.*
Barley	Nitrate	Air	24.5	15.1	85
		Methane	17.0	9.4	70
	Ammonium	Air	21.9	13.3	95
		Methane	14.0	8.6	79
	Ammonium nitrate	Air	27.0	12.2	99
		Methane	18.6	6.4	72
Upland rice	Nitrate	Air	24.0	12.9	114
		Methane	23.1	14.6	104
	Ammonium	Air	22.9	12.1	100
		Methane	23.8	13.7	122
	Ammonium nitrate	Air	23.6	12.7	93
		Methane	21.4	14.2	105

nitrate as a source of oxygen for the growth of rice or barley under anaerobic root conditions.

The fresh weight values of barley indicate a fairly uniform inhibition due to the exclusion of molecular oxygen from the roots regardless of the form of nutrient nitrogen employed. Rice likewise fails to derive any benefit from nitrate over other sources of nitrogen when an anaerobic atmosphere prevails in the root system.

With regard to the potassium content of the expressed sap, in the case of barley the aerated roots all have slightly higher values than methane-treated roots of corresponding solutions. For rice the effect of aeration was not noticeably different from that of methane. With neither plant does the presence of nitrate offer any advantage over ammonium under anaerobic conditions with respect to the accumulation of potassium, or to the amount of growth as noted above.

Summary

The accumulation of bromide over a 24-hour absorption period by excised roots of rice at different oxygen pressures is similar to that of barley and tomato. The inflection in the accumulation curve of the three plants is reached at approximately 3 per cent. oxygen; at higher partial pressures of oxygen only small increases in bromide accumulation occur.

The net accumulation of potassium is of a similar character. Below 1 per cent. oxygen, however, there is a rapid and appreciable loss from the initial potassium content of barley and tomato, and rice releases potassium to a certain extent.

Under anaerobic conditions that prevent bromide accumulation by excised roots, rice roots with attached shoots accumulate appreciable amounts of bromide. The roots of entire barley plants show a smaller relative uptake of bromide, while intact tomato roots do not accumulate and respond like excised roots.

An atmosphere of pure carbon dioxide prevents accumulation by excised roots of all plants, and for whole plants it causes a similar inhibition accompanied by wilt of the shoot systems.

Plants grown aerobically through the seedling stage and transferred to soil culture show diverse response to drainage and submergence over a six-week period. With both loam and clay soils, submergence is lethal to the tomato, retards the growth of barley, but improves that of rice.

Plants grown aerobically through the seedling stage and transferred to water culture with continuous root exposure to air, carbon dioxide, nitrogen, and methane for six weeks follow the general relations indicated by soil cultures.

Compared to a control plant (unaerated), aeration of the root system improves the growth of tomato appreciably and of barley to a small degree. The growth of rice is not affected by aeration, but the root tips of this plant suffer a discoloration indicating injury.

The passage of carbon dioxide through the root medium results in an immediate wilting of all plants and no growth occurs.

Nitrogen gas reduces tomato shoot and root growth by about 90 per cent., while barley is decreased by 45 per cent. Rice is comparable to the control within the limits of experimental error.

Methane inhibits the growth of the tomato plant completely, while it reduces that of barley to a greater extent than does nitrogen. The root growth of rice in methane is superior to that of all other treatments, and 70 per cent. larger than the control.

Nitrate as a source of nutrient nitrogen and potentially of oxygen, does not increase the growth or potassium uptake of rice and barley compared to ammonium or ammonium nitrate, under anaerobic conditions of methane exposure to the roots.

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