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PHYSIOLOGICAL FACTORS LIMITING GROWTH AND YIELD OF *ORYZA SATIVA* UNDER UNFLOODED CONDITIONS

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INTRODUCTION

The origin of rice culture dates back over 5000 years and is lost in antiquity. With passing centuries rice has acquired a wide diversity of cultural methods. These vary from unflooded or upland culture, where the soil is kept moist and aerated, to flooded or lowland culture, where the crop is grown under partial or complete submergence. The growth of all varieties of rice is generally favored by flooding and yields have been increased as much as 60 per cent over unflooded culture [14]. It appears that no variety of rice, either upland or lowland types, has the capacity to overcome the disadvantages encountered when grown in a well-aerated soil.

Much has been written to explain why rice, a morphologically non-aquatic plant, should germinate and flourish under flooded conditions. In contrast, no substantial effort has been made to investigate the process or processes limiting the growth of rice under unflooded conditions. Several benefits of flooding have been determined, but the physiological basis for the superior growth of rice is not agreed upon. Available evidence indicates that rice benefits from an abundance of water, yet does not have a water requirement greater than other common field crops. Standing water, apart from weed control and micro-climate regulation, appears to have no beneficial physiological role. The flood water may supply some nutrients to the crop, and may enhance nitrogen

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fixation by blue-green algae and other organisms. These may be important factors in areas where soil fertility limits production. Soil submergence also brings about changed oxidation–reduction conditions which alters the soil gases, the form and availability of plant nutrients, and other soil constituents.

Evidence suggests that variations in the availability and form of nitrogen, iron, manganese, and phosphorus, either singly or as a result of some interactions, may determine the physiological basis for the superiority of flooded rice culture. The approach of experiments in this investigation has been to evaluate the effect of these variables and some of their interactions on the mineral nutrition and related physiology of the rice plant.

METHODS

The experiments reported in this study were conducted under typical field conditions and where greater control was necessary, under greenhouse conditions. The procedures used were designed to critically evaluate: (1) the effect of flooded and unflooded culture on the growth and nutrient uptake of *Oryza sativa, japonica* var. Caloro and, (2) the various interrelations of nitrogen, phosphorus, iron, and manganese in controlled nutrient cultures.

Field experiments

Rice was grown on a Stockton clay soil, pH 4.7, in both unflooded and flooded culture. In the unflooded treatment, seed was drilled 1 inch deep in a prepared seedbed in rows 6 inches apart and irrigated. The flooded treatment was sown into standing water after land preparation and flooding. The water level in this treatment was maintained at a height of 6″ throughout the growing season. The seeding rate was 100 pounds per acre in both treatments.

Whole-plant samples were collected at weekly intervals during most of the growing season. Plants were collected at random in the plots between 8 and 10 a.m. The collected materials were carefully washed, dried in a forced-air oven at 70°C and then saved for growth measurements. For purposes of discussion, the coleoptile was considered the first leaf and the bladeless leaf sheath, the second leaf. All other leaves were numbered from 3 to 15 according to the time of exertion, leaf 15 being the "flag" leaf. After elongation had taken place, the culm of the main stem was separated, internode by internode. Length and weight of leaves, culms, roots, and inflorescence, if any, were recorded for the main stems.

Plant height and root length were measured on fresh material representing 15 plants per treatment. Leaf area was determined on fresh tissue by taking prints on sensitized paper. These prints were cut out and weighed, and the area of the leaves was then calculated. Moisture content was determined on a separate, freshly harvested sample.

The progress of tillering was studied on 50 plants taken at random from each treatment. Plants were marked and each tiller was tagged as it was produced.

Plants materials were dried and analyzed for nitrogen, by the Kjeldahl method, and phosphorus, sulfur, iron, magnesium, and manganese by methods described by Toth *et al.* [26]

Plant materials collected from the field and soil treatments in large tubs maintained in the greenhouse were sampled 45, 60, 80, and 110 days, respectively, after sowing. A composite sample of blades from the 3rd and 4th leaves, as well as culms, were frozen in dry ice and maintained at 17°C for a short period prior to analysis. The frozen tissues, separated before freezing into 2- and 5-gram lots, were used for the determination of "soluble" iron and manganese by methods described by Sommers and Shive [24], for catalase activity as determined by Haskins [8], and peroxide, as outlined by Siegel and Galston [6]. Assays of the samples for 3-indoleacetic acid were not successful because of interfering substances in the tissue extracts.

Nutrient-culture experiments

The nutrient-culture experiments were conducted with both ammonium and nitrate as a source of nitrogen. For convenience they are referred to as the ammonium and nitrate cultures. The nitrogen cultures were made up from either $Mg(NO_3)_2$ or $(NH_4)_2SO_4$ with additional $CaCl_2$, $MgSO_4$ and a micro-nutrient supplement. The nutrient solutions were made with distilled water and had the following composition at full strength.

Composition of nutrient solutions (Concentration in ppm)											
Nitrogen series	N	K	Mg	Ca	S	Cl	Mn	Mo	B	Zn	Cu
NH_4–N	56	23	48	20	128	35	0.5	0.01	0.5	0.05	0.02
NO_3–N	56	23	73	20	32	35	0.5	0.01	0.5	0.05	0.02

It was not possible to use identical anion and cation concentrations in the two series of cultures. Magnesium and sulfur were considered less critical elements in this study and they were varied.

With each of these ammonium- and nitrate-nitrogen series, iron was varied using ferrous sulfate. The levels of iron used were: 0, 0.005, 0.010, 0.030, 0.060, 0.100, and 0.200 ppm; phosphorus was maintained constant at 9 ppm in these cultures. Similarly with each of the nitrogen sources, a phosphorus series was run using potassium dihydrogen phosphate to supply phosphorus levels of: 0, 0.10, 0.50, 1.00, 2.00, and 3.00 ppm; iron was maintained constant at 1 ppm in this series.

Reagent-grade chemicals were used throughout and no effort was made to purify these salts. The maximum amounts of iron and phosphorus carried as impurities were determined to be 0.0006 ppm and 0.00012 ppm, respectively.

Young rice plants do not tolerate high salts concentrations, therefore the seedling plants were started in culture solution maintained at quarter strength for the first week, half strength the second week and full strength thereafter.

All solutions were changed twice weekly throughout the experiment, and the pH of the solutions were maintained between pH 3.7 and 4.0. No change in the form of nitrogen in the culture solution was detected from one change of solution to the next.

Harvested plant materials were analyzed after drying by the methods described earlier.

EXPERIMENTAL RESULTS

Influence of cultural treatments on growth

Observations made by earlier investigators on the performance of rice grown under the two cultural conditions being evaluated, amounted to estimates of yield in most cases. Since yield integrates all the factors affecting the crop any attempt to analyze the factors contributing to final yield must include a detailed study of the development of the plant. Further, it was conceivable that detailed growth studies could help direct attention towards the basic physiological relations responsible for the growth differences. To make the discussion of growth developments more meaningful, a brief calendar of growth is presented in Table 1.

Results on seedling, tillering, and post-tillering stages of growth will be discussed separately.

Seedling stages. Differences in seedling growth due to treatment were evident soon after germination. Seven days after sowing, the plants grown under unflooded conditions appeared robust, weighed more, were taller, and had more roots. After 14 days the plants under flooded conditions produced enhanced shoot growth but had restricted root growth. The accelerated shoot growth and poor root development are explained on an auxin basis as influenced by standing water during germination [9]. Between the 11th and 14th day, the plants in the flooded treatments exerted their leaves through the water. At the same time it speeded up root elongation which is likewise attributed to an auxin relationship.

Apart from plant height, the seedling growth under unflooded

TABLE 1

Calendar of growth events		
Age of plants in days	Flooded rice	Unflooded rice
19	Plants through the water; leaves lying flat on water surface	Robust plants
26	First leaves standing above water	Tillering begun
33	Tillering just begun. Plants light green	50 per cent tillered. Plants dark green
40	Plants tillered, and darker green than before	Plants dark green and well tillered
54	Broad leaves as compared to unflooded. Roots thick and relatively unbranched	Narrow leaves. Roots branched and not thick
61	Plants showing new root system on surface. Plants better grown than these in other treatments	No new root system observed
68	New root system developing fast	No new root system
75	Plants green, healthy appearance. Culm elongating. Plants taller	Plants small, but green, healthy appearance. No elongation of culm
82	Two nodes of the culm elongated. Inflorescence visible	No internodes elongated. Inflorescence smaller. Plants producing small tillers
89	Inflorescence well developed and in early boot stage	Little development of inflorescence
96	Plant in heavy boot stage. Flag leaf out	Leaves distinctly yellowing. Heads still small
103	Inflorescence about to be exerted. Flag leaf dropped	Leaves continue to yellow. Inflorescence developing
110	Inflorescence exerted	Inflorescence in boot
116		Inflorescence not exerted
123		Inflorescence exerted in some plants

conditions made vigorous early growth and produced a larger amount of dry matter and a materially increased leaf area. From these studies it is evident that unflooded culture favors seedling growth. As such, if subsequent environmental conditions are equal, these more vigorous seedlings would be expected to produce better growth and yields.

Tillering phase. Tillering commences soon after seedling establishment. The importance attached to tillering, arises from the fact that it is positively correlated with yield, as shown by Rao [19] and others.

The first observable differences between cultural treatments with regard to tiller production, was the age at which the plants tillered. The plants grown under unflooded conditions tillered one week ahead of those grown flooded. The "grand period" of tillering was short-lived and reached its peak for both treatments about the 49th day. The flooded treatment produced an average of 4 tillers in addition to the main stem. In comparison, the unflooded treatments produced only 3 tillers per plant.

The plant samples collected were examined to determine the locus of tiller production. Tillers originated from a lower node in the plants grown under well-drained soil conditions. The lowest tillers on the plants grown under unflooded conditions originated from the axil of the third leaf. In contrast, the plants in the flooded treatment started tillering from the fifth node and on upwards. This confirms the work of Sekiya [22] with regard to change in tillering locus on flooding.

The high correlation between tiller order and its liability to die or remain unproductive has been reported by Rhind and Thein [21]. The plants collected from both treatments had only first-order tillers. There was, however, an appreciable difference in the rates of productive to unproductive or dead tillers between treatments. The flooded treatment produced an average of 21 per cent unproductive tillers as compared with 39 per cent for the unflooded. The tendency for the latter treatment to remain small was conspicuous.

Unflooded culture appeared to exert a growth-restraining effect on tiller development. Data showing changes in dry weight of tillers during the period of 95 to 160 days shows the increase in flooded to be almost double that of unflooded plants. These large differences in dry-matter production indicate that a physiological mechanism may be involved.

Post-tillering to harvest. The title post-tillering is not meant to indicate a total absence of tiller production but denotes the phase covering 100 days commencing after the "grand period" of tillering. This period includes over-lapping growth stages, involving the change from vegetative to the generative phase, the development and exertion of the inflorescence, ripening of the grain and senescence of the plant as a whole.

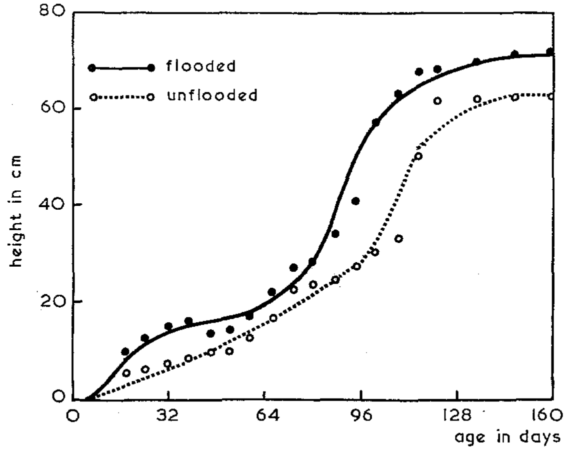

Fig. 1. Seasonal increase in plant height.

Growth in plant height is presented in Figure 1. The early rapid growth in height of the plants grown under flooded conditions was associated with emergence through water. The subsequent rapid elongation – about the 96th day, 14 days ahead of the unflooded plants – was due to culm extension a feature which will be discussed in greater detail.

Increase in leaf growth deserves close study, as it is the main avenue through which the plant elaborates carbohydrates and thus determines yield. However, the rice plant consists of a colony of tillers, some productive, others not, and it is a cumbersome unit for detailed examination. Since the main stem is less liable to variation from plant to plant, it was selected for study in making further comparisons between treatments. Detailed growth studies were made on each leaf at weekly intervals, however, only appropriate data on the 7th, 10th, and 13th leaves will be presented, since they were considered representative.

Under the consideration on seedling growth, the early advantage of the unflooded treatment in increasing leaf weight and leaf area was stressed. This is confirmed by this data, but the advantage lasted only until about the 39th day. From this day a change in relative treatment response was noticed, the flooded treatment showing quicker leaf weight gains. After reaching a maximum leaf weight about the 102nd day, a decline set in which apparently resulted from the translocation of elaborated food material out of the leaves. In comparison, the plants in the unflooded treatment did not reach maximum leaf weight until the 116th day and did not seem to translocate elaborated food out of the leaves until after the 149th day. The main difference appeared to be a restrained growth effect on the plants in the unflooded treatment.

Examination of the time of exertion and senescence of each leaf on the main stem shows that up until the 32nd day, or the time when the 8th leaf was exerted, the unflooded treatment either produced leaves ahead of the flooded treatment, or they both exerted a new leaf during the same week. From then on, the flooded treatment produced leaves faster than the unflooded.

The effect of treatments on leaf area is interesting. Using three representative leaves, it was seen that leaf areas of plants grown under flooded conditions, was greater than of the unflooded. The leaf areas of three selected leaves are shown in Figure 2. During the period when the plants were 50 to 90 days old there was a difference of 20 to 34.5 cm^2 in leaf area per main stem between unflooded and flooded treatments, respectively. When this difference is further weighed with the consideration that the longest daylengths in the year coincided with this period, one cause of depressed dry-matter production and yields of unflooded rice can easily be visualized.

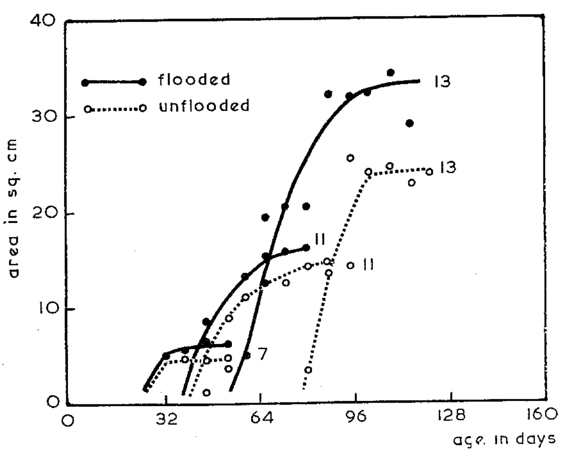

Fig. 2. Seasonal increase of area of leaves in sq. cm.

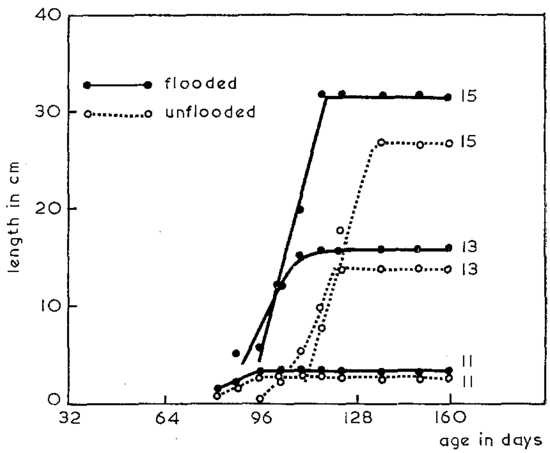

Fig. 3. Seasonal increase in length of internodes.

Internode elongation in the different culture treatments are shown in Figure 3. Internode 11, produced little growth while internode 13 and 15 both showed appreciable elongation. These internodes not only indicated a treatment difference in regard to time of elongation, but also in respect to final length. Here again depressed and delayed growth were observed in the unflooded treatments.

The final result of the delayed rate of elongation of the culm in the unflooded treatment, was the later flowering of these plants.

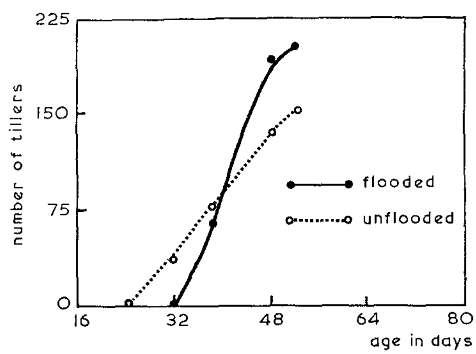

Fig. 4. Seasonal increase in number of tillers. (Based on a sample of 50 plants).

This difference in flowering amounted to about 14 days, some plants being a little further ahead of others.

The cumulative effect of poor tiller growth, shown in Figure 4 along with slow leaf exertion and smaller leaf area and delayed flowering, resulted in fewer and less plump grain on plants in the unflooded treatments. The yield of grain on the unflooded treatment was only 52.6 per cent of the flooded, which is in fair agreement with the yield data reported by Matsuo [14] who obtained 60 per cent for the same comparison. In general, yield reduction was caused by fewer grain bearing tillers, less grain per tiller and smaller kernel size.

Another interesting difference was observed in regard to the moisture content of the plants. The moisture content increased with age as similarly observed by Suzuki [25]. The important difference arises from a comparison of moisture content in plants between treatments. An average of 4 to 5 per cent higher moisture in the plants grown under flooded conditions was observed from the 46th to the 95th day. Apart from the physiological significance of this result, the studies of Murato *et al.* [15] show that increased moisture content was positively correlated with photosynthetic activity. This would thus be added advantage for this treatment from the point of view of carbohydrates elaboration.

Influence of cultural treatment on nutrient uptake

Composite sample of leaves representing every leaf position on the main stem were analyzed separately for certain mineral constituents. Only data from leaves 7, 10, and 13 are discussed since they adequately reflect the nutritional status of the treated plants.

The general trend for all the leaves showed a gradual decline in nitrogen, phosphorus, potassium, sulfur, and magnesium composition with increased maturity. This decline during early development of the leaves was associated with dilution as a result of carbohydrate elaboration and at later stages with translocation of material out of the leaves with approaching senesence. These elements in leaf 7 decreased earlier in the flooded treatment. This difference is due to the shorter life period of leaves on plants grown under flooded conditions and applies also for leaves 10 and 13. The data obtained did not show significant differences in composition of the leaves of the two treatments and no recognized symptoms of deficiencies occurred.

The iron composition showed a gradual increase in the leaves as they matured, but no significant differences were noted between flooded and unflooded plants.

As in the case of iron, manganese accumulates in the tissues and the data revealed a very constant relationship in all leaves. Under flooded culture the manganese content of leaves started low and gradually increased with leaf age. The manganese content of leaves from unflooded plants likewise increased but to a much greater extent. This difference between treatments in total manganese content ranged from 250 to 773 per cent on plants growing in unflooded treatments, as compared with the flooded.

In analytical observations made thus far discussions have been concerned with total iron and manganese in the plant tissue. The active iron and manganese in plants are considered to be the fractions which are in solution within the cells, and as such may be more directly involved in physiological activities of the plants [24]. The active iron and manganese referred to here as the soluble fraction was obtained by pressure extraction. The fraction remaining in the press cake is referred to as the inactive or insoluble fraction. The analytical results obtained on leaves from plants grown flooded, unflooded, and unflooded with subsequent flooding are shown in Table 2.

The striking similarity in the soluble, insoluble, and total iron content of the plants regardless of treatment was evident from this study. A comparison between iron content in the insoluble and soluble fractions showed that there was nearly twice as much iron in the insoluble fraction as there was in the soluble fraction. The

TABLE 2

Soluble and insoluble iron and manganese fractions of leaves, from plants grown flooded, unflooded, and unflooded with subsequent flooding

Metal fraction	Per cent iron			Per cent manganese		
	Flooded	Unflooded + flooded	Unflooded	Flooded	Unflooded + flooded	Unflooded
Soluble fraction	.0018	.0016	.0018	.0044	.0212	.0375
Insoluble fraction	.0034	.0037	.0029	.0058	.0290	.0414
Total	.0052	.0053	.0047	.0102	.0502	.0789

proportion appeared to be a little less in the plants grown in the unflooded culture.

The results obtained on manganese content of both fractions showed a wide variation between treatments. The plants grown under flooded culture contained the lowest manganese in respect to both fractions. A treatment that was started unflooded and subsequently flooded was intermediate and the continuously unflooded treatment had the highest manganese content in both fractions. Regardless of treatment, in every instance the insoluble fraction contained more manganese. The ratio of manganese in the soluble as compared to the insoluble fraction was similar for all the treatments.

In the analysis of young leaves, older but green leaves, and chlorotic leaves, it is striking that there was little difference in the iron content of the soluble fraction. However, the insoluble fraction obtained from chlorotic leaves contained more iron than the green leaves.

The manganese content of young leaves was only a little higher in plants grown in the unflooded treatment. The soluble manganese fraction obtained from the older green leaves contained more than twice as much manganese as found in the young leaves. The chlorotic leaves contained by far the largest amounts of manganese in both fractions.

Nutrient culture experiments

One of the basic differences between flooded and unflooded rice culture is the form in which the plants absorb their nitrogen. Under oxidizing conditions prevailing in an unflooded soil, nitrate nitrogen is the main source of nitrogen available to plants. Under submergence, where nitrates are rapidly denitrified, ammoniacal nitrogen predominates. The ability of rice to utilize both forms of nitrogen has been amply demonstrated but conflicting data exist concerning the suitability of each form. It therefore seemed desirable to evaluate nitrogen source together with interactions of iron and phosphorus in these experiments.

The cultural solution experiments showed that plants grown in nitrate nitrogen cultures made better growth than plants in ammonium cultures when supplied with adequate iron and phosphorus.

Results of all the interactions of source of nitrogen, iron and

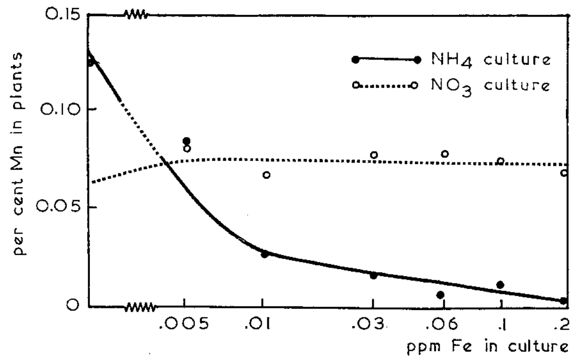

Fig. 5. Percentage manganese in plants as influenced by iron levels.

phosphorus cannot be discussed here. It is significant to note, however, the influence of iron levels in cultures of nitrate and ammonium-nitrogen on the accumulation of manganese. As shown in Figure 5, the plants grown in ammonium culture accumulated manganese when no iron was added. With increasing iron supply, the manganese levels in the tissue decreased steadily, to about 0.01 per cent with adequate iron. In the nitrate nitrogen cultures the level of iron supply had no influence on manganese content, within the range of iron levels used in this experiment. At adequate levels of iron, plants in nitrate cultures had 6 to 7 times as much manganese as those in ammonium cultures.

The response of plants to increasing phosphorus levels in nitrate and ammonium nitrogen cultures is shown in Figure 6. With

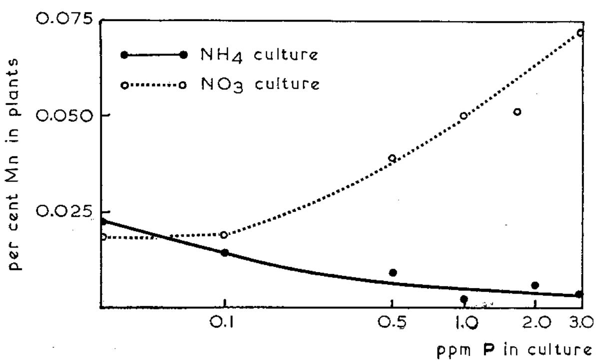

Fig. 6. Percentage manganese in plants as influenced by phosphorus levels.

ammonium nitrogen, the response of rice to increasing phosphorus was one of gradual depression of manganese content. At adequate phosphorus levels of 1 ppm or more the plants contained only traces of manganese. In sharp contrast, with nitrate nitrogen and increasing phosphorus supply, the manganese content increased likewise. Within the range of phosphorus levels studied from 0 to 3.0 ppm, the manganese content increased nearly 400 per cent.

In these solution culture experiments, variations in kind of nitrogen, phosphorus, iron, and manganese were evaluated. These systems exist under flooded and unflooded rice culture and offer additional understanding of rice nutrition, growth and ultimately yield effects. It has been demonstrated that the source of nitrogen interacting with iron and phosphorus help determine the manganese content of rice plants.

Since auxins could not be determined directly in extracts from plants grown under flooded and unflooded conditions, enzyme systems known to influence auxin metabolism in other plants were investigated. Inasmuch as catalase and peroxidase activity have been directly linked to iron and manganese variations, the activities of these systems in plant extracts were evaluated.

The data on variation in catalase activity between treatments are presented in Figure 7. The catalase activity measured from plant extracts from the flooded plants was greater than from the unflooded. In contrast the extract obtained from plants grown under unflooded conditions showed higher peroxidase activity (Figure 8). The significance of peroxidase and catalase variations between

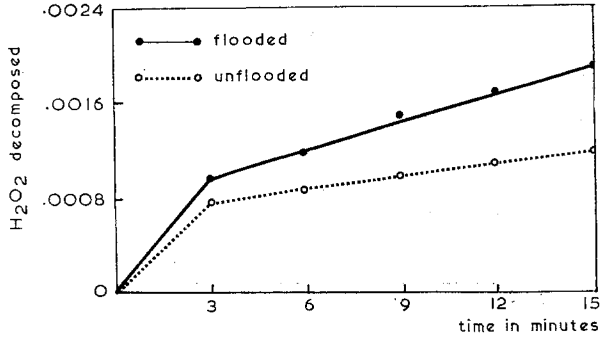

Fig. 7. Catalase activity of leaf extracts.

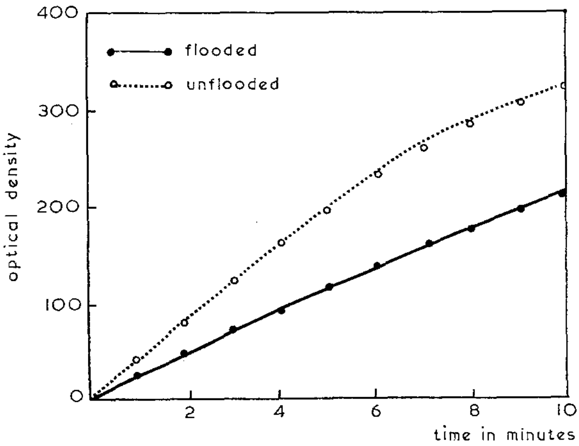

Fig. 8. Peroxidase activity of leaf extracts.

treatments, arising from high manganese accumulation, has been associated with auxin destruction in some plants [3 13]. These enzymes may also directly effect the growth response in rice. The physiological significance of these enzyme variations in relation to plant growth will be discussed subsequently.

DISCUSSION

Results of field and greenhouse experiments have shown both physiological and nutritional differences in rice grown under flooded and unflooded conditions. In evaluating the salient growth characteristics, it was shown that plants grown in unflooded culture were more robust, had a greater dry weight, and appeared in better tone than flooded plants until about 35 days after emergence. After this period, the unflooded plants appeared to suffer from some growth-retarding influence, even though soil moisture was kept near field capacity. The unflooded plants gradually became chlorotic, had a lower moisture content, tiller number was restricted, tiller development retarded, leaf area was reduced, and leaves were exerted at a slower rate. The appearance of macroscopic flower primordia occurred about the same time in both treatments, but a delay in internode elongation and flowering was observed in the unflooded treatment. Grain yields of the unflooded rice showed a 52.6 per cent reduction as compared with the flooded treatment.

In interpreting these growth responses, the plant behaviors are suggestive of differences in auxin metabolism. The experimental verification of this observation however involved prohibitive difficulties in analytical determinations and interpretation of results. Some relationship can be developed however by relating the observed plant responses to known auxin effects obtained under controlled conditions.

Several of the growth responses observed in these experiments are possibly related to plant auxin metabolism. The depressed plant growth and leaf area (Figures 1 and 2), the lower number and poorer survival of tillers (Figure 4), delayed flowering and reduced number of grains per head are all well-known auxin effects on plant growth [2 7]. The low moisture content of the unflooded plants, in spite of their growth in adequate soil moisture, is an auxin effect observed by various investigators [17 23 27 28] and reviewed by Bonner and Bandurski [4]. The more rapid and extensive internode elongation observed under flooded culture (Figure 3) may be an auxin response similar to that obtained by Liverman [12] and Koneshi [10]. Evidence would indicate that rice grown under flooded conditions has a higher auxin level, and thus internode elongation is initiated earlier. Tillering in grasses is known to be influenced by low auxin levels [11].

The analogous evidence obtained from the growth observations and related auxin response supports the contention that plants grown under unflooded conditions probably contain low auxin levels. Such low levels of auxin could arise from either a subnormal capacity for auxin synthesis or enhance auxin destruction which does not contribute to the growth processes. Variations in auxin synthesis could be genetically controlled or influenced by nutrient relationships among other environmental factors. Since the same variety was used in these experiments, the factor of genetic variability can be eliminated.

The chemical composition of plants harvested from flooded and unflooded treatments were not essentially different in total nitrogen, potassium, phosphorus, magnesium, sulfur, or iron content. There was however a markedly higher manganese content of plants grown under unflooded conditions. The presence of 0.40 per cent manganese was far in excess of values reported for rice grown under flooded conditions and also for other crops.

Since a pronounced interpendency of iron and manganese has been observed [30] and since it has been suggested that the poor growth of unflooded rice might be caused by iron deficiency [18], the iron—manganese inter-relationship was examined. Results presented in Table 2 did not reveal a constant iron—manganese relationship in the treated plants. The iron content of the soluble or "active" fraction and the insoluble fraction were nearly the same in all treatments, but the manganese content varied widely. The iron—manganese ratios were 0.41 and 0.048 respectively for flooded and unflooded plants.

Green and chlorotic leaves from plants grown under unflooded conditions were analyzed to establish a basis for their physiological condition. No differences in iron content were observed but a high manganese content was associated with the symptoms of chlorosis. The chlorosis observed in the plants was unlike iron deficiency described [1 29] and recognized locally. It seems unlikely that iron deficiency would occur in the presence of adequate levels of iron in the tissue. The possibility is not precluded however that some physiological mechanism might cause chlorosis under unflooded conditions, even in the presence of adequate iron within the plant.

Nutrient-culture experiments were conducted to evaluate the effects of ammonium and nitrate nitrogen, iron, phosphorus and some of their interactions on the mineral nutrition and related physiology of rice. Results indicated that rice could use both forms of nitrogen but that plants grown in nitrate cultures made better vegetative growth when supplied with adequate iron and phosphorus. It was shown (Figures 5 and 6) that plants supplied with ammonium nitrogen with adequate iron and phosphorus contained small amounts of manganese. Nitrate nitrogen, under similar conditions, favored the accumulation of manganese. These results agreed with data obtained from plants grown under field conditions. Rice utilizing nitrate nitrogen, such as occurs under unflooded, aerated soil conditions favors manganese accumulation. Flooded rice which uses principally ammonium nitrogen did not show manganese accumulation. This effect of nitrate nitrogen on manganese accumulation has been observed by Olsen [16]. Friedericksen [5] shows a reciprocal antagonistic effect of iron on manganese in the presence of ammonium as a nitrogen source for plants.

Consideration of the growth responses observed under unflooded conditions as typical auxin effects, together with the unusually high manganese content of these plants suggests the possibilities of manganese-induced growth regulation. A relationship between manganese and the indoleacetic acid oxidase system, involving catalase and peroxidase activity has already been established [3 13] and reviewed recently by Ray [20]. They found that high manganese in plant tissue was associated with high peroxidase and low catalase activity. The indoleacetic acid oxidase system, responsible for the destruction of the most commonly found native auxin, is expressed [6] simply as:

$$H_2O_2 + IAA \xrightarrow{\text{peroxidase}} \text{oxidized } IAA + H_2O$$

From this reaction it can be visualized that if peroxide was not limiting and other conditions were favorable, the destruction of auxin would be directly related to peroxide availability. Similarly, if peroxide was not limiting, the rate of destruction would then be determined by peroxidase activity. Since the enzyme catalase is known to destroy peroxides, the final peroxide content in plant cells will be determined at least in part by catalase activity.

The catalase and peroxidase activities of plant extracts obtained from flooded and unflooded rice are shown in Figures 7, and 8, respectively. A depressed catalase activity was observed in extracts from plants grown under unflooded conditions, while the same plants had a higher peroxidase activity than flooded-culture rice.

Considering the effect of low catalase and high peroxidase activity of plants grown under unflooded conditions, accelerated auxin destruction would be expected. In contrast, the effect of high catalase and low peroxidase activity acting together would depress auxin destruction in flooded rice. These changed enzyme relations, resulting from variations in plant manganese content appear to create wide differences in auxin levels between treatments. These auxin affects, associated with foliar chlorosis, also the result of excessive manganese, appears to be the determining factors limiting the growth and yields of rice under unflooded cultural conditions.

SUMMARY

Rice grown under flooded conditions consistently produces better vegetative growth and higher grain yields than when grown in unflooded culture. Physiological and nutritional differences in rice grown under these two conditions were determined. Growth observations showed that plants under unflooded culture made an initial vigorous start, but soon showed poor tillering, depressed leaf growth, delayed flowering, low moisture content, foliar chlorosis, and 52.6 per cent lower yield than flooded plants.

Chemical analysis emphasized the higher manganese content of plants grown under unflooded culture with no significant differences in other elements. Plants grown in nutrient cultures and under field conditions gave evidence that nitrate nitrogen nutrition, as exists for plants under unflooded conditions, favored manganese accumulation.

Growth responses suggest differences in auxin metabolism. Since auxins could not be estimated directly, some factors affecting auxin degradation were investigated. It was found that plants grown under unflooded conditions had: 1) a low catalase activity, and: 2) a high peroxidase activity, which favor accelerated auxin degradation. It is proposed that high manganese levels in plants grown under unflooded conditions affects the indoleacetic acid oxidase mechanism resulting in retarded growth and depressed grain yields.

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