

# AUXIN RELATIONSHIPS OF THE RICE COLEOPTILE<sup>1</sup>

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It has long been known that the rice coleoptile is not only capable of growth under water but that this organ actually grows more extensively under water than in air. This property of the rice coleoptile has an important practical significance for rice culture. Thus, in the United States, rice seeds are usually sown by airplane in water which is kept 5-6 inches deep over the field. The seeds germinate under water and the coleoptiles grow up to the surface of water within a few days. In the northern part of Japan, the seed bed, after seeding, is frequently flooded relatively deeply in order to prevent cooling of the soil during cold weather. In this case also, the rice coleoptile grows under water.

That the rice seed is capable of germination in an atmosphere of reduced oxygen concentration as is the case under water has been shown directly by Yokoi (21), Takahashi (15), Sasaki (14), Edward (6) and Morinaga (9, 10). In these reports it has also been found that the growth of the rice coleoptile under water is greater than in air. Vlamis and Davis (19) have reported a detailed study of the growth of the rice coleoptile in relation to oxygen tension, and have compared rice with barley in this respect. It was found that rice shoot growth (measured by fresh weight) is at a minimum in 21% oxygen (air) and increases with decreasing oxygen tension, reaching a maximum at an oxygen tension of about 3%. Taylor (17, 18) has studied the influence of oxygen tension on the respiratory rate, fermentation, and growth of rice seedling, and has compared rice with wheat. Other attempts have also been made to correlate the capacity of rice to germinate and grow under water with such physiological characteristics as fermentation, production of alcohol, tolerance to alcohol, or particular changes in enzyme activities of rice when it is grown under water (11, 8, 7, 17, 12). No clear correlations of this kind have, however, been established, and the physiological basis of the peculiar characteristics of the growth of seedling rice have remained obscure.

This paper deals with the auxin relationships of rice coleoptile growth. The elongation of this organ depends, as shown in figure 1, on submersion, being greater when the seedling is immersed in water than when it is allowed to grow in air. It will be shown that the elongation of the rice coleoptile is auxin dependent, just as is the case with the coleoptiles of *Avena*. Rice differs from *Avena*, however, in possessing a highly active system for the destruction of auxin, a system whose activity depends greatly on oxygen tension.

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## MATERIALS AND METHODS

The rice seeds used in these experiments were of the variety "Colusa." Seeds were soaked for ten minutes in a 0.5% solution of sodium hypochlorite and were then rinsed thoroughly with distilled water. The seeds which remained floating during this procedure were discarded, and the remainder selected for uniformity in size and appearance. The selected seeds were then allowed to germinate under water in a dark room at 25 to 26°C. Various cultural procedures were used as noted below.

## EXPERIMENTAL RESULTS

The growth of the rice coleoptile was first investigated in relation to decapitation. For these experiments groups of 20 seeds were placed at the surface, at 5 cm below or 10 cm below the surface of water contained in separate beakers. The elongation of the coleoptile was followed in each instance. The results summarized in figure 1 show that vigorous elongation occurs under water, but that elongation is much lower in seedlings growing at the surface. It may be noted that the elongation of the mesocotyl, known to be more sensitive to auxin than the coleoptile in other cereals (20), was also greater under water than in air. The initiation and growth of the roots on the contrary is inhibited under water.

Coleoptiles growing in air (seeds placed on the surface of water) or under water (5 cm depth) were

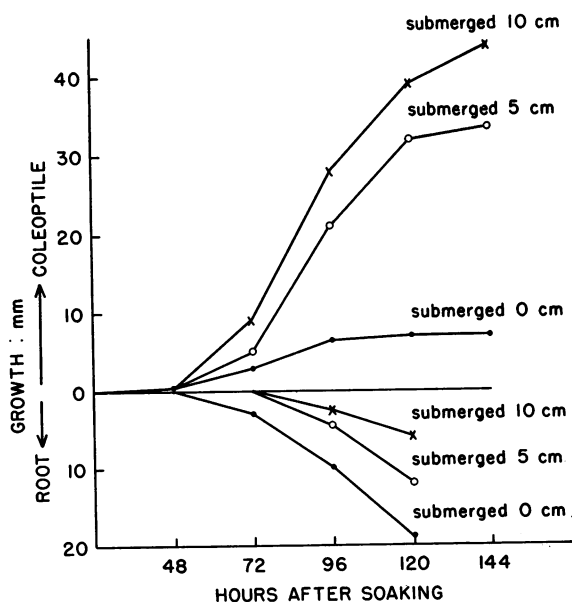


FIG. 1. Growth of rice coleoptiles in air and under water. Seeds placed at 0, 5 and 10 cm below the water surface.

decapitated by removing 2 to 3 mm tips, and after four hours a second decapitation was made. The elongation of the coleoptiles in air decreased to zero after the second decapitation. This behavior then is similar to that known for the *Avena* coleoptile (20). The coleoptile growing under water does not, however, show any appreciable decrease of elongation even after the second decapitation (fig. 2 a). A more drastic decapitation was therefore carried out in which approximately 10 mm of the upper portion of the coleoptile (total length 22 mm) was removed. After four hours a second 3 mm of the apical stump was again

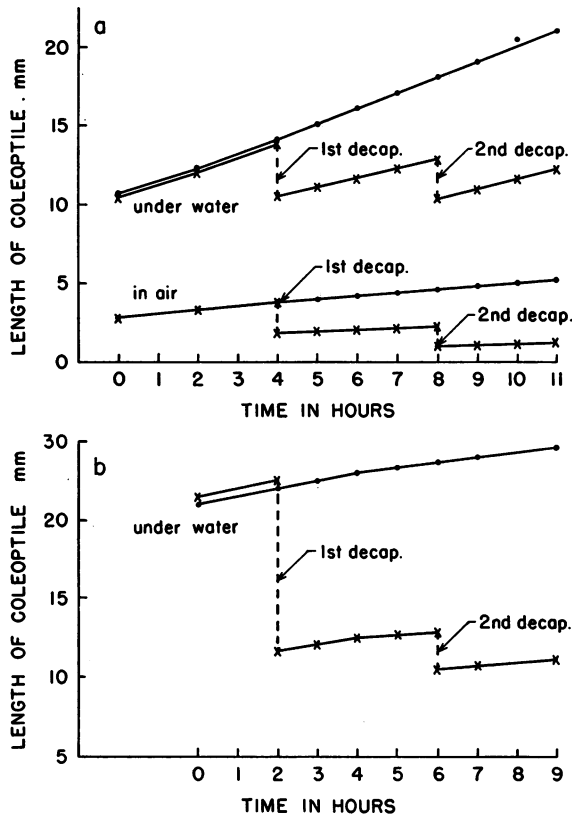


FIG. 2. Effect of decapitation on growth of rice coleoptiles in air and under water. C: control coleoptiles, D: decapitated coleoptiles.

removed. The remaining tissue continued to grow, with no decrease in rate (fig. 2 b). The facts summarized above indicate the possibility that rice coleoptile tissue grown under water may contain such large amounts of auxin, that the removal of the top does not result in any immediate deficiency of auxin in the remaining portion. Coleoptiles grown in air appear on the contrary, to be limited by auxin after decapitation, much as are the coleoptiles of *Avena*.

It will now be shown directly that the growth of rice coleoptiles does depend on auxin. Seeds were soaked and germinated under water 6 cm in depth and contained in large (24 × 40 cm) trays in a standard *Avena* dark room. Seedlings with coleoptiles 1–1.5 cm in

length were selected, and sections 5 mm long were removed from the portion lying between 3 mm and 8 mm below the top of coleoptile. For this purpose use was made of a special double bladed cutting tool. These sections were placed in lots of 20 in 20 ml of indole acetic acid (IAA) solution of each of a series of concentrations placed in glass vials (2 cm diameter and 8.5 cm high). Each group of 20 sections was either floated on the surface or submerged at about 5 cm below the surface of the solution. This was achieved by use of screen made of nylon net which restrained the sections at the desired depth.

Preliminary experiments showed that the growth response of rice coleoptile sections to IAA decreases with the age of the seedling. As a standard practice, relatively young coleoptiles 1 to 1.5 cm in length were therefore used exclusively. Certain of the early experiments also yielded irregular results owing to the growth of microorganisms in the culture solutions.

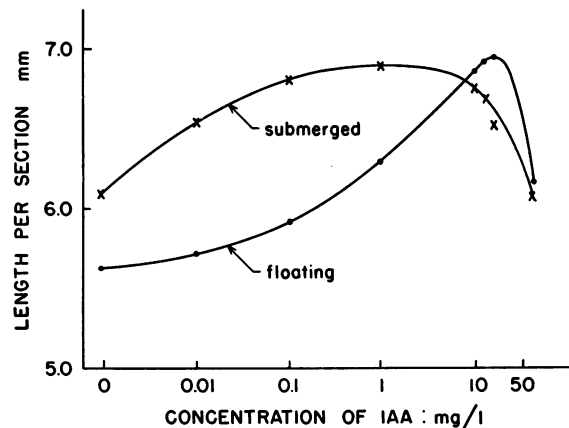


FIG. 3. Growth response of coleoptile sections to IAA solutions of varied concentration. 48 hours incubation in dark. Initial length: 5.0 mm. Each point based on average of forty sections.

This was readily controlled by the use of penicillin G in a concentration of 16,000 units/100 ml.

In the experiments reported below, coleoptile sections were incubated in IAA solutions either in a floating or in a submerged condition. After 48 hours of incubation in a dark room at 26°C the growth of the sections was measured under a dissecting microscope. The results of a typical experiment are shown in figure 3. The submerged sections are more sensitive to the lower concentrations of IAA than are the floating sections, and reach a maximum growth at an IAA concentration of 1 mg/l. The floating sections, on the contrary, although they are less sensitive to lower IAA concentrations, do grow to approximately the same final length as do submerged sections in sufficiently high concentrations of IAA (20 to 30 mg/l). These results suggest the possibility that the superior growth of rice coleoptiles under water may depend on a decreased auxin destruction as compared to coleoptiles in air.

It may be noted that there is a considerable differ-

TABLE I

EFFECT OF THE AUXIN ANTAGONIST, 2,4-DICHLOROPHENOXY-ISOBUTYRIC ACID ON THE GROWTH OF RICE SEEDLINGS. SEEDS SUBMERGED IN EXPERIMENTAL SOLUTIONS TO A DEPTH OF 5 CM. INITIAL LENGTH OF COLEOPTILE IS 1-2 MM. 48 HOURS INCUBATION

	CONCENTRATION (MG/L) OF ANTI-AUXIN							SEEDS PLACED ON MOIST FILTER PAPER
	0.0	0.01	0.1	1	10	25	50	
Length of coleoptile, mm ...	25.3	20.9	21.3	20.7	3.2	2.0	1.7	10.3
Length of mesocotyl, mm ...	1-3	1-3	1-3	1-3	0	0	0	0
Length of root, mm .....	3.2	3.2	3.4	1.4	0	0	0	27.6
Number of roots .....	1	1	1	1	..	..	..	2.8

ence in growth between submerged and floating sections placed in water alone. This is true even though the same initial plant material was used for the two conditions.

This growth as well as the growth of decapitated rice coleoptiles under water is, however, undoubtedly due to endogenous auxin since it is completely inhibited by competitive auxin antagonists. For these experiments the anti-auxin, 2,4-dichlorophenoxy-isobutyric acid (4) was used. Germinating seeds with coleoptiles 1 to 2 mm long were submerged 5 cm deep in 2,4-dichlorophenoxy-isobutyric acid solutions of various concentrations. The anti-auxin in a concentration of 10 mg/l retards growth under water completely (table I).

EFFECT OF SOME RESPIRATORY INHIBITORS ON GROWTH UNDER WATER: It is known that the IAA induced growth of *Avena* coleoptiles is dependent on aerobic respiration and is inhibited by such inhibitors as KCN (1). This is true also of the growth of rice coleoptiles. Growth even of submerged coleoptiles may be totally inhibited by low concentrations of KCN.

For these experiments coleoptile sections prepared as described above were incubated with IAA solution (10 mg/l) containing 0,  $10^{-5}$ ,  $10^{-4}$ , or  $10^{-3}$  M KCN. Each solution was adjusted to pH 7.3. Both floating and submerged sections were used. After 24 hours incubation the length of the sections was measured. It was found as shown in table II that KCN at  $10^{-3}$  M

TABLE II

INHIBITION OF GROWTH OF RICE COLEOPTILE SECTIONS BY KCN. ALL EXPERIMENTAL SOLUTIONS CONTAIN IAA (10 MG/L). pH=7.3. 24 HOURS INCUBATION

CONDITIONS OF INCUBATION	EXPT. No.	PERCENT INHIBITION AT KCN CONCENTRATION (M) OF		
		$10^{-5}$	$10^{-4}$	$10^{-3}$
Floating	1	25.0	43.7	86.2
	2	30.4	61.0	89.3
	3	38.1	35.7	90.0
	Av.	34.5	46.8	88.5
Submerged	1	30.3	36.9	88.2
	2	35.4	53.7	90.7
	3	34.6	41.3	89.8
	Av.	33.4	44.0	89.6

causes 90% inhibition of growth both of submerged and floating sections. The concentration of KCN which causes 50% inhibition of growth is approximately  $10^{-4}$  M and is the same for sections in air and under submerged conditions. This suggests that the aerobic respiration may participate in the growth under submerged conditions as well as in the growth carried out in air.

2,4-Dinitrophenol (DNP) is known to be a most effective inhibitor of auxin-induced *Avena* section growth (2, 3) and to act by uncoupling the linkage of plant respiration to phosphorylation (4). DNP exerts its effect by blocking the aerobic synthesis of adenosine triphosphate (ATP) but is without effect on anaerobic, fermentative, ATP production. To test the effects of DNP on rice coleoptile growth, the inhibitor was added in various concentrations (0, 2, 5 and 10 mg/l) to IAA solutions (10 mg/l) adjusted to pH 5.0. The results (table III) show that 2 mg/l of DNP gives 80-90% inhibition of growth both with the submerged and with the floating materials. No difference in sensitivity to DNP was observed between submerged and floating sections. Again, the growth under water appears to depend on aerobic respiration and on the energy released by the respiratory oxidations. It appears quite unlikely that it can depend in any important manner on energy released by fermentation.

IAA DESTROYING ACTIVITY OF THE RICE COLEOPTILE: The fact that submerged coleoptiles are much more sensitive to applied auxin than are floating coleoptiles suggests the possibility that in the latter auxin destruction may play a prominent role. To test this possibility the effect of floating and submerged coleoptiles on the disappearance of exogenous auxin was first tested. Solutions of IAA were incubated with

TABLE III

EFFECT OF 2,4-DINITROPHENOL ON GROWTH OF RICE COLEOPTILE SECTIONS. ALL EXPERIMENTAL SOLUTIONS CONTAIN IAA (10 MG/L). pH=5.0. 24 HOURS INCUBATION

CONDITIONS OF INCUBATION	PERCENT INHIBITION AT DNP CONCENTRATION (MG/L) OF		
	2 MG/L	5 MG/L	10 MG/L
Floating .....	91.6	100	100
Submerged ...	88.2	100	100

TABLE IV  
RESIDUAL IAA REMAINING IN SOLUTIONS AFTER INCUBATION WITH RICE COLEOPTILE SECTIONS.  
INITIAL CONCENTRATION OF IAA SOLUTION IS 20 MG/L

CONDITIONS OF INCUBATION	RESIDUAL AMOUNT OF IAA AS PERCENT OF INITIAL							
	EXPT. 1			EXPT. 2		EXPT. 3		
	No.*	2 HRS	5 HRS	No.*	24 HRS	No.*	24 HRS	48 HRS
Sections submerged .....	96	100	100	2	47.8	2	65.9	32.8
Sections floating .....	96	80.6	58.0	2	18.1	2	49.6	2.1

\* Number sections per ml.

coleoptile sections under floating or submerged conditions and the residual IAA was determined by the colorimetric method of Tang and Bonner (16), using Salkowski's reagent. In each case 40 sections were incubated with 20 ml of IAA (20 mg/l) solution. As shown in table IV the floating sections destroy more IAA than the submerged sections. In a short period experiment, in which 2400 coleoptiles were incubated with 25 ml of IAA solution (20 mg/l) for 2 and for 5 hours, similar results were obtained.

Rice coleoptiles are also able to destroy rapidly IAA which has been infiltrated into the tissue and this destruction depends on the aeration of the sections. Thirty gm (fresh weight) of coleoptiles were prepared, and IAA solution (500 mg/l) was infiltrated into them. The material was rinsed with water and divided into four portions. Two lots were left between wet filter papers for 5 and 10 hours respectively. One lot was used for the determination of IAA at initial time, while the last lot was incubated under nitrogen for 24 hours. The samples were lyophilized and residual IAA in the tissue determined colorimetrically on the ether extract obtained in each case from 150 mg of lyophilized tissue. The data of table V show that the IAA initially present in the tissue (120  $\mu\text{g}/100$  mg dry weight) was almost completely destroyed after 10 hours incubation of the coleoptiles in air. The amount of IAA destroyed was however greatly decreased in the coleoptiles which had been incubated under conditions of reduced oxygen tension.

#### DISCUSSION

The results described above suggest that the elongation of rice coleoptile under water is not a particularly

peculiar type of growth, but that the mechanisms involved are not qualitatively different from those already known for the *Avena* coleoptile. Both auxin and aerobic respiration are required for growth of the rice coleoptile under water. An aberrant feature of the rice coleoptile does however appear to reside in its ability to destroy IAA at the oxygen tension which obtains in air while, at the lower oxygen tensions which obtain when the coleoptiles are immersed in water, auxin destruction is reduced, and the growth responses to both endogenous and to added auxin are increased. At the same time, however, aerobic respiration is limited under water by the amount of oxygen

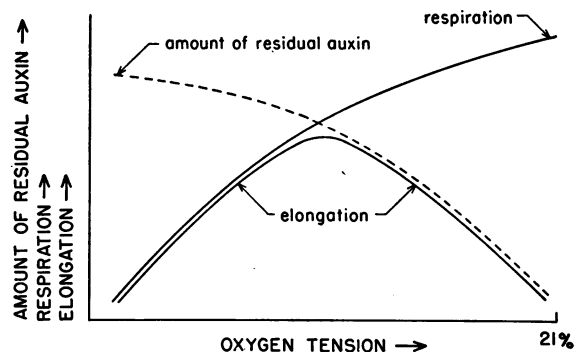


FIG. 4. Proposed scheme showing the hypothetical relationships between respiration, auxin destruction and elongation of the rice coleoptile. Auxin destruction is expressed in terms of residual (undestroyed) auxin which would remain in the tissue after a measured time and conditions which are uniform except in oxygen tension.

available to the tissue as has been shown by Taylor (17). The growth of the rice coleoptile under water is then determined by a compromise between at least two opposing factors, namely the lowered auxin destruction and the lowered aerobic respiration (fig. 4).

It has been suggested (17) that the energy released anaerobically by fermentation may be more efficiently used by the rice plant in its growth than is the energy liberated in oxidative respiration. This hypothesis was based on the fact that over the range of low oxygen concentrations in which rice exhibits greater germination and growth than wheat, rice also carries on a more active fermentation than does wheat. The results presented in this paper indicate, however, that

TABLE V

DESTRUCTION OF IAA AFTER INFILTRATION OF THIS MATERIAL INTO RICE COLEOPTILE TISSUE. RESIDUAL IAA IS GIVEN AS PERCENT OF INITIAL AMOUNT (120  $\mu\text{g}/100$  MG TISSUE DRY WEIGHT)

	PERIOD OF INCUBATION			
	0	5 HRS	10 HRS	24 HRS
In air .....	100	33.1	5.9	...
In nitrogen ...	...	...	..	50.1

fermentation cannot take the place of aerobic respiration in promoting growth of the rice coleoptile and that on the contrary growth is entirely dependent on cyanide sensitive oxidative energy coupling systems mediated in all probability through ATP.

Not only the coleoptile, but the stem and leaf sheath of the rice plant as well are known to elongate more rapidly under water than in air. This is frequently observed in the field as when older rice plants are submerged by water during floods. In addition there are grown in India and Burma varieties known as "deep water" or "floating" rice. These varieties are capable of growing in such a fashion as to keep pace with rises of water level which occur during the monsoon flood season. The shoots of these varieties may grow to heights of as much as 20 feet in response to the increase in water level (13). It will evidently be of interest to determine how far mechanisms similar to that described here for the coleoptile are involved in these further growth responses of the rice plant to water level.

#### SUMMARY

The work reported here concerns the well known fact that the rice coleoptile elongates more rapidly and more extensively under water than in air. Decapitation (removal of the apical tip) experiments carried out with plants in air or under water as well as experiments done with the growth of floating or submerged coleoptile sections suggest that the superior growth of rice coleoptiles under water depends on a decreased capacity of the tissue to destroy auxin under water as compared to the extensive capacity of the tissue to destroy auxin in air. Thus, submerged rice coleoptile sections grow more in response to low concentrations of added auxin than do sections floated on the surface of solutions. Although the maximal growth rate of sections in air is equal to that of submerged sections, this maximal rate is achieved only at an auxin concentration which is 20 to 30 times as great for sections in air as for submerged sections. Rice coleoptile sections floated on the surface of an IAA solution remove IAA from the solutions much more rapidly than do similar but submerged coleoptile sections. Similarly, IAA disappears much more rapidly from coleoptile tissue infiltrated with IAA and incubated in air than from similar coleoptiles incubated under reduced oxygen tension. The growth of the rice coleoptile depends on aerobic respiration even under conditions of complete submersion in water. The inhibition of growth by KCN as well as by DNP was found to be the same for floating and for submerged sections. It is suggested as a working hypothesis that growth of the rice coleoptile is determined by the interaction of two important opposing factors: (1) decrease of auxin destruction at lower oxygen tension, and (2) decreased aerobic respiration at low oxygen tension.

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#### LITERATURE CITED

1. BONNER, J. The action of the plant growth hormone. *Jour. Gen. Physiol.* 17: 63-76. 1933.
2. BONNER, J. Limiting factors and growth inhibitors in the growth of the *Avena* coleoptile. *Amer. Jour. Bot.* 36: 323-332. 1949.
3. BONNER, J. Relations of respiration and growth in the *Avena* coleoptile. *Amer. Jour. Bot.* 36: 429-436. 1949.
4. BONNER, J. and BANDURSKI, R. S. Studies of the physiology, pharmacology, and biochemistry of the auxins. *Annual Rev. Plant Physiol.* 3: 59-86. 1952.
5. BONNER, J. and MILLER, A. Oxidative phosphorylation of plant mitochondria. *Arch. Biochem.* 42: 135-148. 1952.
6. EDWARDS, T. I. The germination and growth of *Peltandra virginica* in the absence of oxygen. *Bull. Torrey Bot. Club* 60: 573-581. 1933.
7. ERYGIN, P. S. Changes in activity of enzymes, soluble carbohydrates, and intensity of respiration of rice seeds germinating under water. *Plant Physiol.* 11: 821-832. 1936.
8. MORINAGA, T. Catalase activity and the aerobic and anaerobic germination of rice. *Bot. Gaz.* 79: 73-84. 1925.
9. MORINAGA, T. Germination of seeds under water. *Amer. Jour. Bot.* 13: 126-140. 1926.
10. MORINAGA, T. The favorable effect of reduced oxygen supply upon the germination of certain seeds. *Amer. Jour. Bot.* 13: 159-166. 1926.
11. NAGAI, I. Some studies on germination of the seed of *Oryza sativa*. *Jour. Coll. Agr. Tokyo Imp. Univ.* 3: 109-159. 1916.
12. PHILLIPS, J. W. Studies on fermentation in rice and barley. *Amer. Jour. Bot.* 34: 62-72. 1947.
13. RAMIAH, K. and RAMASWAMI, K. Floating habit in rice. *Indian Jour. Agr. Sci.* 11: 1-8. 1941.
14. SASAKI, T. On an abnormal type of germination of rice seeds under reduced air supply. *Jour. Sci. Agr. Soc. Japan* 288: 101-102. 1927.
15. TAKAHASHI, T. Is germination possible in the absence of air? *Bull. Coll. Agr. Tokyo Imp. Univ.* 6: 439-442. 1905.
16. TANG, Y. W. and BONNER, J. The enzymatic inactivation of indoleacetic acid. 1. Some characteristics of the enzyme contained in pea seedlings. *Arch. Biochem.* 13: 11-25. 1947.
17. TAYLOR, D. L. Influence of oxygen tension on respiration, fermentation and growth in wheat and rice. *Amer. Jour. Bot.* 29: 721-738. 1942.
18. TAYLOR, D. L. Effects of oxygen on respiration, fermentation and growth in wheat and rice. *Science N.S.* 95: 129-130. 1942.
19. VLAMIS, J. and DAVIS, A. R. Germination, growth and respiration of rice and barley seedlings at low oxygen pressures. *Plant Physiol.* 18: 685-692. 1943.
20. WENT, F. W. and THIMANN, K. V. *Phytohormones*. Macmillan. 1937.
21. YOKOI, T. On the development of the plumule and radicle of rice seeds with various quantities of water in the germinating medium. *Bull. Coll. Agr. Tokyo Imp. Univ.* 3: 482-487. 1898.