

# STUDIES ON THE PHYSIOLOGY OF RICE. XIII. DISTRIBUTION OF FREE AUXIN IN DIFFERENT ORGANS OF THE PLANT<sup>1</sup>

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It has been reported previously from this laboratory (Sircar and Das 1954) that the endosperm of rice contains a large amount of auxin which gradually disappears with germination of the embryo. Further, Sircar, Das and Lahiri (1955) and Sircar and Lahiri (1957) have produced evidence of the presence of some factor in the endosperm which exerts a retarding effect on embryo growth in the initial stages of germination. From the results of a series of experiments in which fractions of endosperm were removed and endosperm extract or IAA substituted, they have presumed that this retarding factor in the endosperm is of auxin in nature which in supra-optimal concentration retards the growth rate of the embryo. The problem then arises wherefrom this auxin accumulates in the endosperm of rice, whether it is related with the development of the flower and what part of the flower contributes to its accumulation. The answer to these questions would obviously be of considerable importance to determine the auxin relations of rice plant.

Earlier workers, notably Muir (1942), Hatcher (1945), Lund (1956) and Nitsch (1950) have followed the changes and synthesis of auxin in tobacco, corn, strawberry and rye. Hatcher has shown that the auxin content of developing rye grain is very small upto 2 weeks after anthesis and during the next 3 weeks there is a rapid accumulation but as the grain ripens auxin disappears almost entirely. In order to locate the site of auxin formation Hatcher analysed the whole ear and concluded that in the ear of rye there are two systems of auxin production—one in the developing carpel and the other in the developing anther. In both these organs auxin first accumulates and then disappears. He also found that endosperm adjacent to the embryo is the region of auxin accumulation. The precise location of auxin in the endosperm was done by extracting separately the excised embryo, the aleurone and the endosperm. An increase in the auxin content of tobacco pistil after pollination has been noted by Muir (1942) and Lund (1956). With the background of these results the present investigation on the free auxin content of the developing stamens and carpels of rice and its distribution in the mature grain and early stages of germination was undertaken.

## MATERIALS AND METHODS

### *Method of auxin assay*

*Root inhibition test.*—Root inhibition by the application of indolyl acetic acid bears a logarithmic relationship with the concentration of the solution used. This relation has been used by Moewus (1949) and Andus (1951) to develop a method for the assay of auxin in plants. Das (1953) made a detailed study of this method for the determination of auxin in rye using the same material for culture as test plant. By the root inhibition test slight variation of auxin can be detected. In this work the method devised by Das was adopted using rice root in place of rye as test plant. The details of the method are as follows: Rice grains (var. *Rupsail*) were soaked in distilled water for 24 hours at  $25 \pm 1^\circ\text{C}$  in complete darkness. They were then sprouted on moist filter paper in petridishes for another 24 hours in

darkness at the same temperature conditions. After 24 hours, the germinating embryos just protruded from the husk. At this stage healthy seeds showing equal embryo development were selected out and transferred to sterilised agar slopes in test tubes containing auxin extract. Similarly, a set of control was prepared where no auxin extract was added. The test plants were allowed to grow for a period of 48 hours in dark chamber maintaining a temperature of  $25 \pm 1^\circ\text{C}$ . At this stage rice seedling showed only primary root which was measured upto the correct mm. The mean root length was expressed as percentage of the control root growing on aqueous agar.

*Computation of results.*—To determine quantitatively the amount of auxin present in a particular plant material, a calibration curve has been prepared. For this purpose IAA solutions ranging from 100mg/l to  $10^{-5}$  mg/l in logarithmic concentrations were prepared and the percentage inhibition of root length produced by these ranges of concentration of IAA were determined. From this calibration curve the IAA equivalent in  $\mu\text{g}$  of a plant extract was determined.

*Extraction of auxin.*—Different methods for extraction of auxin from different plant materials have been tried by workers in this field. Went and Thimann (1937) found that whereas alcohol failed to extract auxin from oat grain, water-alcohol mixture gave intermediate values, varying with the proportion of alcohol used. Again Van Overbeek (1938) used water for extracting auxin from maize grain. In this laboratory Sircar and Das (1954) adopted water as solvent for free auxin from rice grain. The method proved to be a suitable one, accordingly in the present investigation auxin has been extracted with water. The fresh plant material was washed in tap water and then in distilled water to clear off external dirt. The weighed material was cut into pieces, small quantity of water added and then stored in complete darkness for 24 hours at  $0^\circ\text{C}$  in a refrigerator. Then the extract was filtered several times after washing with water. The filtrate was then diluted to 50 c.c. and an equal volume of 3 per cent agar was added. The agar extract was then plugged and autoclaved at  $120^\circ\text{C}$  for 15 minutes and were slanted at an angle of  $85^\circ$ . Control tubes containing only aqueous agar and tubes containing IAA media were also subjected to similar sterilisation. For each determination duplicate extracts were made and the auxin content of each extract was determined with 15-20 test plants.

#### THE CHANGE OF AUXIN CONTENT IN DIFFERENT PARTS OF GRAIN DURING GERMINATION

Rice grains (var. *Rupsail*) were soaked in distilled water for 2, 4, 10, 14, 18 and 30 hours respectively. Soaked grains were then dissected under Zeiss Sterio Microscope into the following parts : (1) Embryo proper, (2) Scutellum, (3) Portion of the endosperm adhering to the scutellum, (4) Remaining upper half of the endosperm and (5) Husk.

The fresh weight of the tissues was determined accurately and their auxin content determined by the "Root inhibition test" method described previously. The concentration of auxin has been expressed in terms of the weight of air dry seeds, thus the error due to relative water absorption was eliminated. The results are represented graphically (Fig. 1) and in Table 1.

*Results.*—In air dried mature rice grain about  $50\mu\text{g}$  of auxin is present per gram of endosperm of which the lower half of the endosperm contains slightly less than the upper half. The amount of auxin present in an embryo is  $0.144\mu\text{g}$  of IAA Eq. while the scutellum contains comparatively negligible amount ( $0.002\mu\text{g}$  of IAA Eq.). With soaking, hydration of the tissue begins, as a consequence of which changes in the concentration of auxin takes place in different parts of the grain (Fig. 1). After 2 hours of soaking a sudden rise in the auxin concen-

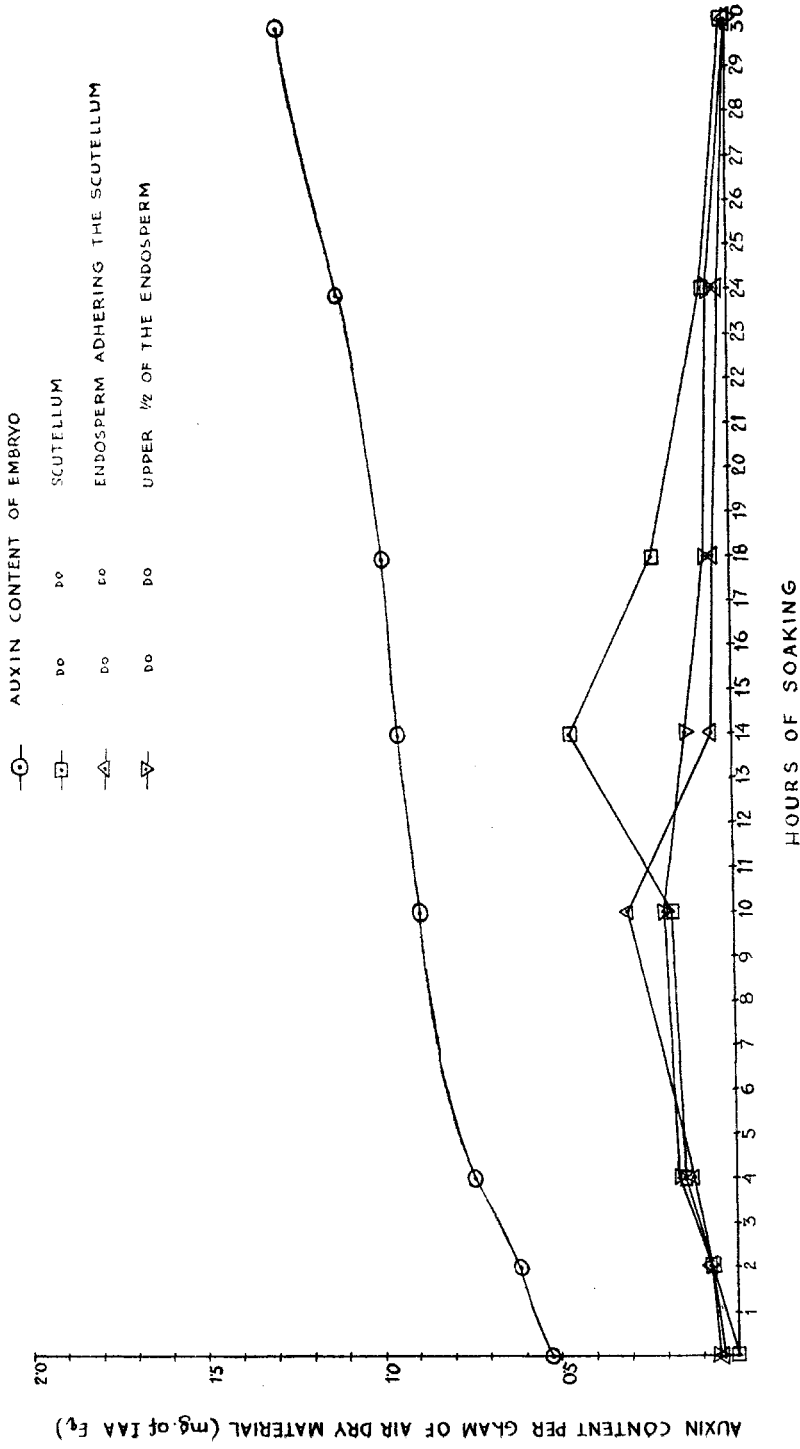


Fig. 1

TABLE I  
*Changes of auxin content in the grain during germination (Fig. 1A A Eq.)*

Treatments i.e., hrs. of soaking	Embryo			Scutellum			Endosperm adhering to the scutellum			Upper half of endosperm			Husk	
	Total auxin of 50 embryos	Auxin conc. per gm. of air dry material	(2)	Total auxin of 50 scutellums	Auxin conc. per gm. of air dry material	(4)	Total auxin of 50 endos- perm halves	Auxin conc. per gm. of air dry material	(7)	Total auxin of 50 upper halves of endos- perm	Auxin conc. per gm. of air dry material	(9)	Total auxin of 50 husks	Auxin conc. per gm. of air dry material
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)				
Dry seed	7.23	527.51	0.10	7.12	12.59	41.96	18.20	60.65	0.50	2.03				
2 hours	8.49	619.78	1.00	71.20	26.30	87.67	25.12	83.73	0.45	1.81				
4 hours	10.20	745.14	1.99	143.16	35.48	118.27	50.12	161.64	0.40	1.61				
10 hours	12.27	895.21	2.34	168.25	79.43	294.71	63.10	203.53	0.40	1.61				
14 hours	13.15	953.39	6.29	459.44	19.95	66.58	42.66	137.60	0.40	1.61				
18 hours	14.09	1028.61	3.15	226.96	17.78	59.27	31.62	102.09	0.31	1.28				
24 hours	15.81	1154.12	1.12	80.09	12.88	42.94	28.84	96.63	0.31	1.28				
30 hours	17.74	1297.11	0.16	11.38	6.31	21.03	19.95	64.38	0.31	1.28				

tration of the scutellum was noticed which increased upto 10 times the quantity present in the scutellum of dry grain. Thus a change from  $0.002\mu\text{g}$  to  $0.020\mu\text{g}$  of IAA Eq. took place after first two hours soaking. It seems that hydration of the tissue activated auxin precursor which caused such a rapid increase. After the next two hours of soaking the auxin concentration doubled and a gradual rate of increase followed upto 14 hours soaking. At this stage  $0.126\mu\text{g}$  of IAA Eq. is present in the scutellum of each grain or if expressed otherwise  $459.44\mu\text{g}$  of IAA Eq. is present per gm. of the scutellum of air dried seed. While studying the changes in the auxin content of endosperm it is evident that at the same stage i.e., after 14 hours soaking decrease in the auxin content of endosperm begins, which indicates the beginning of the utilisation of endosperm auxin by the growing embryo. After 14 hours soaking scutellum auxin is found to decrease. This decrease continues upto 30 hours when only minute amount of auxin is left ( $0.003\mu\text{g}$  of IAA Eq. per grain). At this stage the embryos are well sprouted. Auxin content of the embryo gradually increases with the onset of soaking. Rate of increase is slow and follows a more or less straight path (Fig. 1). In the course of 30 hours soaking the auxin in the embryo has increased about two and half times. Endosperm auxin increased from the beginning of soaking to 10 hours. During this period inactive precursor is presumably activated. In between 10 to 14 hours utilisation of the endosperm auxin by the embryo begins, as a consequence of which endosperm auxin begins to decrease (Table 1). This decrease after a period of 10 hours soaking is more marked in the lower half of the endosperm than the upper half. Similarly after 18, 24 and 30 hours soaking decrease of auxin content is more pronounced in the lower half of the endosperm than in the upper part. Inference from this can be drawn that auxin of the endosperm adjacent to the scutellum is first utilised. A very small amount is present in the husk and no remarkable change in the content is noticed during successive stages of germination.

#### DISTRIBUTION OF AUXIN IN DIFFERENT PARTS OF THE SEEDLING

The seedlings were grown in sand culture in July-Sept., 1955 at the experimental garden of the Department of Botany, Calcutta University. The method of sand culture and nutrients applied were the same as in the previous work (Sircar and Sen, 1941).

*Results.* (Fig. 2 and Table 2).—In mature rice grain about 80 per cent total auxin is present in the endosperm. While the embryo contains the rest, the endosperm shows the presence of  $0.81\mu\text{g}$  of IAA Eq., husks contain practically none. In the sprouted embryo the auxin level increases from  $0.14\mu\text{g}$ . of IAA Eq., it decreases to  $0.64\mu\text{g}$  in the endosperm. It should be noted that the amount of auxin decreased in the endosperm is much greater than that accumulated in the coleoptile. In the second day i.e., one day after sprouting, auxin level of the coleoptile decreases to  $0.08\mu\text{g}$ ., while the reduction to  $0.63\mu\text{g}$  is noticed in the endosperm. It thus appears that the rate of decrease of the endosperm auxin is comparatively slow at this stage. Auxin content of root at this stage has been estimated to be  $0.03\mu\text{g}$  IAA Eq., which is half the amount present in the coleoptile. On the third day, coleoptile auxin decreases to a level of  $0.01\mu\text{g}$  of IAA Eq.; by that time the endosperm loses about  $0.13\mu\text{g}$  of IAA Eq. At the age of 5 days, 1st leaf is fully developed and 57 mm. in length, the 2nd leaf with 32.3 mm. length is unfolding and the root length is 56.6 mm. Distribution of auxin in different parts of the seedlings shows that auxin accumulates in the leaves with growth. Young leaf, however, contains higher auxin which decreases as the leaf approaches maturity. At the age of 20 days the endosperm is completely exhausted and no extractable auxin is detected. Auxin content of the coleoptile is the highest in the sprouted grain and decreases with age, while the crown shows an

FREE AUXIN IN DIFFERENT PARTS OF THE SEEDLING ( $\mu\text{g. of IAA Eq.}$ )

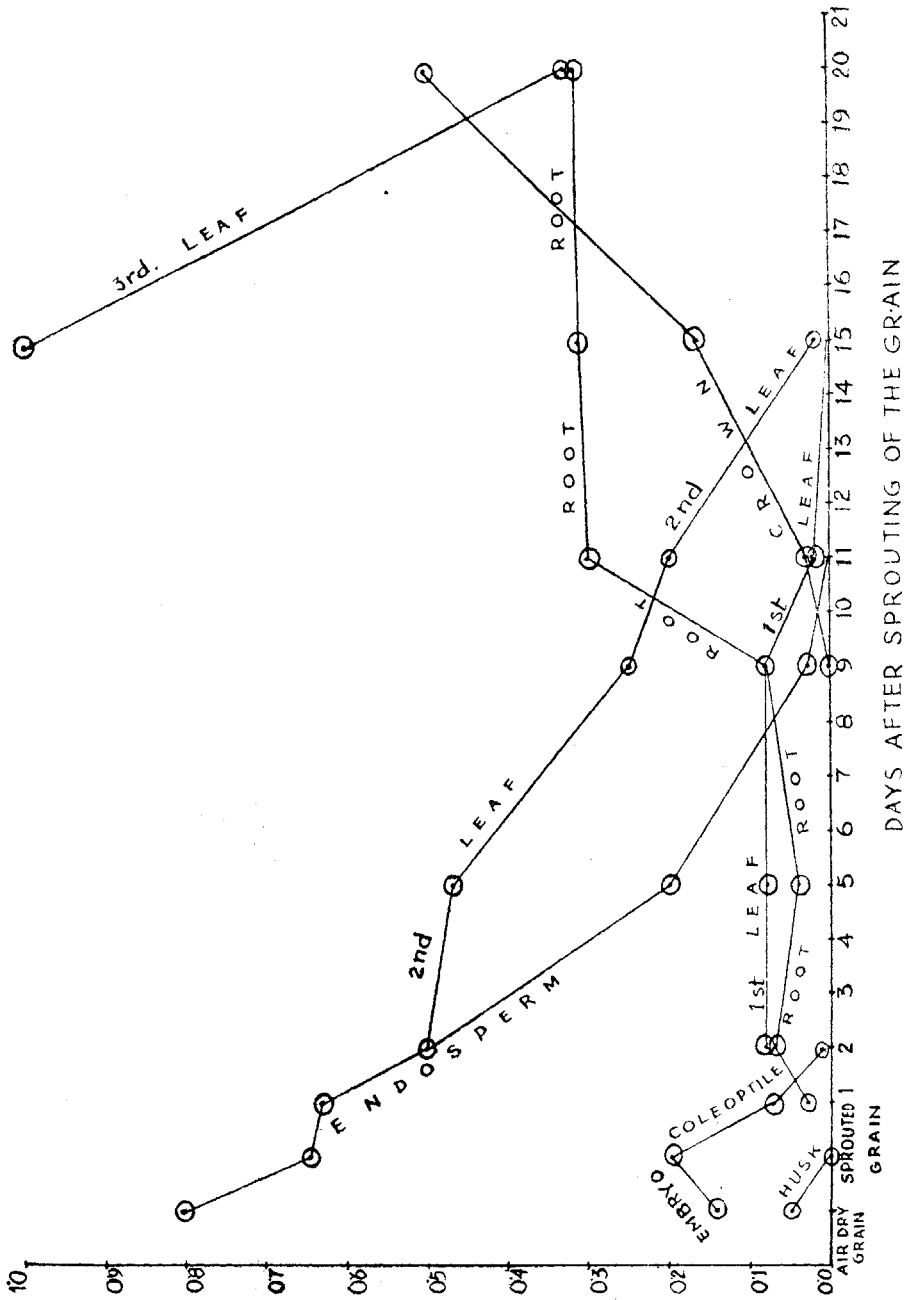


Fig. 2

TABLE 2  
Auxin content of the different parts of the seedling

Age of the seedling	EMBRYO				ENDOSPERM				ROOT				CROWN			
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)			
	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo $\mu$ g. of IAA Eq.	Free auxin conc. on fresh wt. $\mu$ g. of IAA Eq.	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo $\mu$ g. of IAA Eq.	Free auxin conc. per gm. fresh wt. $\mu$ g. of IAA Eq.	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo $\mu$ g. of IAA Eq.	Free auxin conc. per gm. fresh wt. $\mu$ g. of IAA Eq.	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo $\mu$ g. of IAA Eq.	Free auxin conc. per gm. fresh wt. $\mu$ g. of IAA Eq.	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo $\mu$ g. of IAA Eq.		
Air dry seed	0.0027	0.1445	527.4	0.119	0.8092	67.46	—	—	—	—	—	—	—	—		
Sprouted grain	0.03	0.199	66.35	0.2	0.6456	32.28	—	—	—	—	—	—	—	—		
Days after sprouting	C O L E O P T I L E															
1	0.01	0.07924	72.59	0.22	0.6308	28.67	0.015	0.0315	20.23	0.012	0.0041	0.347	0.12	0.0316		
2	0.0576	0.01101	1.74	0.222	0.5012	22.57	0.023	0.0706	30.71	0.12	0.0316	2.635	0.12	0.0316		
5				0.025	0.1995	9.24	0.149	0.0398	2.67	0.20	0.1698	8.494	0.20	0.1698		
9				0.197	0.0251	1.27	0.147	0.0794	5.40	0.20	0.1698	8.494	0.20	0.1698		
11				0.180	0.007	0.388	0.14	0.3162	22.58	0.21	0.5011	24.81	0.21	0.5011		
15							0.14	0.3162	22.58	0.21	0.5011	24.81	0.21	0.5011		
20							0.14	0.3235	23.82	0.21	0.5011	24.81	0.21	0.5011		

TABLE 2—contd.  
*Auxin content of the different parts of the seedling— contd.*

Age of the seedling	FIRST LEAF				SECOND LEAF				THIRD LEAF																														
	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo µg. of IAA Eq.	Free auxin conc. per gm. fresh wt. µg. of IAA Eq.	(15)	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo µg. of IAA Eq.	Free auxin conc. per gm. fresh wt. µg. of IAA Eq.	(16)	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo µg. of IAA Eq.	Free auxin conc. per gm. fresh wt. µg. of IAA Eq.	(17)	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo µg. of IAA Eq.	Free auxin conc. per gm. fresh wt. µg. of IAA Eq.	(18)	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo µg. of IAA Eq.	Free auxin conc. per gm. fresh wt. µg. of IAA Eq.	(19)	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo µg. of IAA Eq.	Free auxin conc. per gm. fresh wt. µg. of IAA Eq.	(20)	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo µg. of IAA Eq.	Free auxin conc. per gm. fresh wt. µg. of IAA Eq.	(21)	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo µg. of IAA Eq.	Free auxin conc. per gm. fresh wt. µg. of IAA Eq.	(22)							
1	—	—	—	(14)	—	—	—	(15)	—	—	—	(16)	—	—	—	(17)	—	—	—	(18)	—	—	—	(19)	—	—	—	(20)	—	—	—	(21)	—	—	—	(22)			
2	0.202	0.0794	4.95	—	0.0739	0.5012	67.79	—	0.0739	0.5012	67.79	—	0.0739	0.5012	67.79	—	0.0739	0.5012	67.79	—	0.0739	0.5012	67.79	—	0.0739	0.5012	67.79	—	0.0739	0.5012	67.79	—	0.0739	0.5012	67.79	—	0.0739	0.5012	67.79
5	0.196	0.0793	4.046	—	0.099	0.4677	47.24	—	0.099	0.4677	47.24	—	0.099	0.4677	47.24	—	0.099	0.4677	47.24	—	0.099	0.4677	47.24	—	0.099	0.4677	47.24	—	0.099	0.4677	47.24	—	0.099	0.4677	47.24	—	0.099	0.4677	47.24
9	0.2	0.0794	3.971	—	0.24	0.2511	10.464	—	0.24	0.2511	10.464	—	0.24	0.2511	10.464	—	0.24	0.2511	10.464	—	0.24	0.2511	10.464	—	0.24	0.2511	10.464	—	0.24	0.2511	10.464	—	0.24	0.2511	10.464	—	0.24	0.2511	10.464
11	0.14	0.0255	1.763	—	0.225	0.1995	8.78	—	0.225	0.1995	8.78	—	0.225	0.1995	8.78	—	0.225	0.1995	8.78	—	0.225	0.1995	8.78	—	0.225	0.1995	8.78	—	0.225	0.1995	8.78	—	0.225	0.1995	8.78	—	0.225	0.1995	8.78
15	0.14	0.0035	0.253	—	0.229	0.0239	1.05	—	0.229	0.0239	1.05	—	0.229	0.0239	1.05	—	0.229	0.0239	1.05	—	0.229	0.0239	1.05	—	0.229	0.0239	1.05	—	0.229	0.0239	1.05	—	0.229	0.0239	1.05	—	0.229	0.0239	1.05
20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		

Days after  
sprouting



increase with the age of the plant. Some difficulty arises in interpreting the changes in the auxin content of the root. In root, growth usually takes place at the tip and so it is probable that growth-promoting substances are present in the tips. But during quantitative estimation the total amount of root present was considered. Hence an irregularity in the auxin content of root was noticed.

*Auxin contents of developing stamens and carpels*

*Sampling*—The vegetative shoot apex of rice is very small and before floral initiation it remains completely encased within leaf primordia. Stem elongation, however, starts one to two weeks before floral initiation. Unlike rye and other temperate cereals it does not bear double ridges on the surface. The floral primordia first appear in the shape of globular protuberance on the sides of the slightly elongated shoot apex (Sircar and Sen 1953). Stamens are differentiated earlier than carpels. During emergence of flag leaf the inflorescence remains very young, about 6" long. The husks are whitish and transparent. Stamens are only developed, their colour being greenish white; carpels not yet formed. From a sample of 100 flowers, stamens and husks were carefully dissected with the help of a very fine sterilised needle. The stamens were then collected in a small tube, previously weighed with 1 c.c. water. The tube was kept surrounded with ice so as to prevent evaporation from the water surface during the time of collection. The husks were collected in another tube. The tubes were finally weighed and the net fresh weight of the material collected was thus obtained. The tubes were subsequently kept in a refrigerator. Next sampling was done 3 days after the flag leaf opened out; carpels were formed at this stage and sampled separately.

Different stages at which the samples were made are as follows :—

Nov. 2, 1955	<i>1st stage</i>	Just after the emergence of the flag leaf, only stamens were developed; carpels not yet formed.
Nov. 5, 1955	<i>2nd stage</i>	Flag leaf opened out, but the spikelets were still within the flag. Carpels were formed.
Nov. 7, 1955	<i>3rd stage</i>	During the time of anthesis.
Nov. 14, 1955	<i>4th stage</i>	7 days after anthesis. From this stage only husks and carpels were collected as stamens withered out after anthesis.
Nov. 17, 1955	<i>5th stage</i>	10 days after anthesis; milk stage.
Nov. 21, 1955	<i>6th stage</i>	14 days after anthesis, the endosperm became milky.
Nov. 25, 1955	<i>7th stage</i>	18 days after anthesis, the embryo was differentiated.
Nov. 28, 1955	<i>8th stage</i>	3 weeks after anthesis, the grain was mature.

*Results.* (Fig. 3 and Table 3).—When the flag leaves emerged the inflorescence enclosed within the flag leaf was soft, slender and transparent (3–5" in length

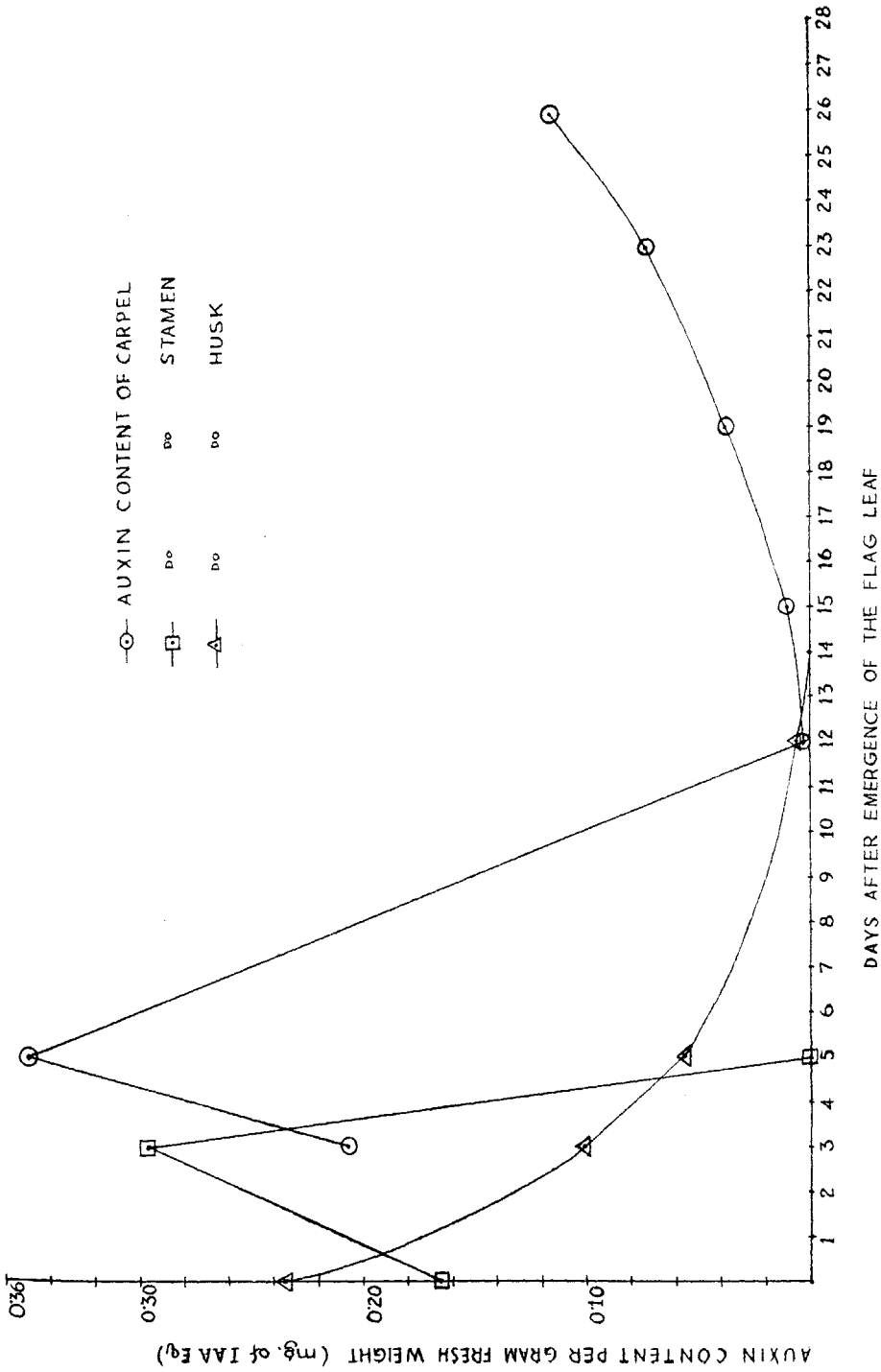


Fig. 3  
DAYS AFTER EMERGENCE OF THE FLAG LEAF

TABLE 3  
Auxin content of stamens, carpels and husk

Stages of development	CARPEL					STAMEN					HUSK	
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)		
		Fresh wt. of 100 carpels (gm.)	Total free auxin of 100 carpels $\mu$ g of IAA Eq.	Free auxin conc. per gm. fresh wt. $\mu$ g of IAA Eq.	Fresh wt. of 600 stamens gm.	Total free auxin of 600 stamens $\mu$ g of IAA Eq.	Free auxin conc. per gm. fresh wt. $\mu$ g of IAA Eq.	Fresh wt. of 100 flowers gm.	Total free auxin of 100 flowers $\mu$ g of IAA Eq.	Free auxin conc. per gm. fresh wt. $\mu$ g of IAA Eq.		
<b>FIRST STAGE</b>												
2nd. Nov. 1955— Just after the emergence of the flag leaf, only stamens are developed. Carpels have not yet formed.					0.024	3.972	165.0	0.15	35.39	235.98		
5th Nov. 1955— Flag leaf has opened out, but the spikelets are still within flag leaf. Carpels are formed.		0.006	12.418	208.0	0.026	7.74	297.69	0.22	22.334	101.51		
7th Nov. 1955— During the time of anthesis		0.014	49.097	350.67	0.026	0.012	0.482	0.2798	16.179	57.82		
14th Nov. 1955— 7 days after anthesis		0.1208	0.35	2.95	—	—	—	0.2536	1.774	7.03		
17th Nov. 1955— 10 days after anthesis		0.4334	5.012	11.56	—	—	—	0.2528	0.281	1.12		
<b>MILK STAGE</b>												
21st Nov. 1955— Milk stage		0.6520	25.12	38.5	—	—	—	0.2520	0.002	0.007		
25th Nov. 1955— Embryo differentiated at this stage		0.8022	58.88	73.42	—	—	—	0.2502	0.001	0.006		
28th Nov. 1955— 3 weeks after anthesis. The grain is mature.		1.4264	162.18	113.63	—	—	—	0.3555	0.001	0.004		

with small stamens 0.8 mm. in length). Three days after the unfolding of flag leaves, the stamens were still green and unripe ; but the size of the anthers and filaments increased and slight increase in the fresh weight was also noticed. As the inflorescence grew it became thicker and stouter and its green colour turned darker. On the 7th November, 1955 at about 10 A.M. the glumes opened at the mouth and stamens peeped through. This extrusion of the stamens signifies the time of anthesis. Six hours after anthesis the dehisced anther became yellowish and flaccid. Its size increased from 0.8 mm. to 2.4 mm. at the time of anthesis and increase was more rapid during the first half of its development. The rate of increase in fresh weight of the stamens was not parallel to the rate of increase in size. Even when the stamens were collected after anthesis their fresh weight remained the same as that found two days before. Carpels were first noticed on the 5th November, 1955 and were very small roundish, somewhat ovoid with feathery stigma. As the size of the carpel increased there was gradual increase in fresh weight. After anthesis the increase in the size of the carpel was noticeable. Seven days after anthesis the endosperm content was juicy and colourless and ten days after it became milky. This milk stage continued upto 14 days when the endosperm content became thicker. At about 2 weeks after anthesis the grain matured. Husks attained their full length, 7 days after anthesis but fresh weight was maximum at the anthesis (0.02798 gm./husk). After this the fresh weight of the husk gradually decreased though the size of the husk remained the same ; the husk gradually lost its water content and its colour became brownish after 17 days. But at an age of 3 weeks a slight increase in the fresh weight of the husks was noticed, which is possibly due to deposition of minerals. In rice (var. *Rupsail*) a large amount of auxin was present in unripe stamens i.e., at the stage when the stamens were green and unripe, largest amount (0.013  $\mu$ g. of IAA Eq. per stamen) being present at the second stage when the flag leaf just unfolded, spikelets still enclosed and the carpels just formed. As the stamens turned yellow the auxin content gradually decreased several times while after anthesis very little auxin was detectable. Auxin level of very young carpels was very high (0.125 of IAA Eq. per carpel) and it increased 4 times after anthesis which took place 3 days after the full development of the carpels. In the stamens also, an increase in the auxin content was noticed 3 days after their full development. Thus increase of auxin content of carpels and stamens in the early stages of growth are parallel to each other. This is followed by a steep fall in the carpels after 7 days of anthesis. Auxin concentration again increased with the onset of milkstage which was practically the auxin content of the endosperm. It, however, did not attain the initial level of the carpel though the total auxin content of the endosperm was more than thrice that of the young carpel. Total auxin content of young embryo was much less than the total auxin content of the endosperm at the same stage. But auxin concentration of embryo and endosperm was more or less the same. Husks in their very early stages of development contained some amount of auxin which decreased regularly and at a stage of 7 days after anthesis there was practically nothing left. In the young husks growth and development continue, so it is probable that auxin present there is consumed with maturity.

#### DISCUSSION

*Change in auxin content in different parts of the grain during germination.*—It appears that with soaking the auxin content of the embryo continues to increase. Obviously this comes from the endosperm. With different periods of soaking (upto 10 hours) diffusible quantity of auxin in the endosperm increases and upto 12 hours soaking it is also rising in the scutellum. Thereafter its presence in both scutellum

and endosperm decreases. Thus it appears that between 10-12 hours soaking endosperm auxin is translocated to the embryo through the scutellum. As the fall in the lower half of the endosperm is more sharp it is presumed that auxin of the endosperm next to the scutellum is first mobilised. With increasing periods of soaking presumably large amount is consumed by the sprouting embryo. It is very likely that this demand is met from the scutellum and the endosperm which have a decreasing level after 10-14 hours.

*Distribution of auxin in different parts of the seedling in early stages of growth.*—Diffusible amount of auxin has been estimated from endosperm, coleoptile, root and leaves of rice at different stages of germination. It has been shown that a large amount of auxin present in the endosperm gradually disappeared. Auxin level of the embryo was at first very low but with the sprouting of the grain, it increased rapidly and after reaching its peak it decreased again. Similar rise was noticed in the case of leaves and roots. Auxin is known to be present in the endosperm in large quantities as shown by Avery *et al.* (1940, 1941) in wheat, oat and maize; Hatcher (1945) in rye and Das (1953) in rye. Evidence for the movement of auxin precursor from the base to the tip of coleoptile has been presented by Skoog (1937) and a gradual disappearance from the grain during germination has been shown by Sircar and Das (1954). In the present work the gradual loss of auxin released from the endosperm is not equal to the amount gained by the embryo during growth. Das (1953) has shown that without any participation of the endosperm some amount of auxin may be synthesised independently in the coleoptile during growth. This possibility together with that of Funke and Soding's (1948) observation that during transport auxin of the endosperm becomes inactive in the coleoptilar node and activated again in the coleoptile tip, may explain the discrepancy with the change of auxin balance in the embryo and endosperm; as the amount of activated auxin may not be equal to the amount released from the endosperm. The results of the auxin relations of the growing embryo suggest that auxin is closely associated with its growth. The maximal rate of its production has been found to occur in the embryo just prior to the period of maximal growth rate i.e., the rate of auxin production follows a course parallel to the growth rate. It should be borne in mind that the amount which is available in the tissue at any instant is the balance between the amount of production and consumption during growth. It is possible that at the initial stage of growth the rate of production is much higher than that of the consumption, hence large accumulation resulted which helped the growth rate to reach its peak but after this the production probably stopped and the level of accumulated auxin gradually decreased by consumption. This possibility would go far to explain the fact that a large accumulation occurs prior to the grand period of growth and that the rate of auxin production follows a curve parallel to the growth rate.

*Auxin content of developing stamens and carpels.*—Wittwer (1943) found that an appreciable amount of auxin accumulated in the carpel of *Zea* after anthesis and he emphasised the phenomenon of reduction division as a cause of such accumulation. In this work a detailed study has been made on the relation of auxin to the development of different parts of the rice flower. Auxin was found to accumulate in the developing carpel upto the stage of anthesis. Such an accumulation possibly helps the growth of the carpel. After anthesis within 4-7 days there was a rapid fall in the concentration; over 90 per cent was found to disappear from carpillary tissue at that period. Such a reduction was not observed by Wittwer. But Hatcher (1945) showed that the auxin content of winter rye rapidly increased after fertilisation and subsequently fell during the ripening of the grain. Das (1953) found that the auxin is gradually accumulated in the ear of vernalized or unvernallized winter rye under long day condition and decreased after the grand period of ear elongation. Again after the ear emergence concentration rises up. In rice such a rise of auxin concentration has been noticed after initial rapid fall. This

rise is presumably due to the accumulation of auxin in the developing endosperm tissues. Auxin content of the stamens of rice also presents an interesting picture. It gradually increased in the stamens and reached the peak during anthesis and then rapidly disappeared with withering of the tissue which indicates that it is closely associated with the growth of the stamen. Similar phenomenon was also observed in the case of the developing husk in which concentration of auxin was maximum at the beginning of growth and at maturity it almost disappeared.

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#### SUMMARY

Distribution of auxin in different parts of rice grain during germination and its production in the stamens and carpels have been investigated using the root inhibition test. The test materials in these experiments were also the primary roots of rice seedling. The auxin content of the embryo increases gradually until sprouting. On the other hand the auxin content of the scutellum and the endosperm (both lower and upper half) rises to its peak value in between 10-14 hours and then a fall is noticed. In the sprouted grain amount of auxin in the embryo increases while a decrease in the endosperm is noticeable. But the amount decreased in the endosperm is greater than that accumulated in the coleoptile. Coleoptile auxin is highest in the sprouted grain and decreased with age. Young leaves show a high auxin content which is destroyed or inactivated at maturity. An increase in the amount of auxin along with the age of the plant is noticed in the crown. Root shows an increase which is maintained more or less constant. Auxin content of stamens gradually reaches a peak during anthesis which rapidly disappears with the withering of the tissues. Auxin level of carpels is also high, which increases about 4 times during the time of anthesis. After anthesis it rapidly decreases which again shows an increase with the onset of milk stage. Husks in their early stages of growth contain some amount of auxin which decreases at maturity. Total auxin content per young embryo is much less than the total auxin content of the endosperm but their auxin concentration is more or less the same.

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