

STUDIES ON THE PHYSIOLOGY OF THE RICE PLANT XVIII.
CHANGES IN THE NITROGENOUS AND CARBOHYDRATE
CONSTITUENTS IN RELATION TO GROWTH FOLLOWING
TREATMENT WITH GROWTH SUBSTANCES*

by S. GOPALAKRISHNAN† and S. M. SIRCAR, F.N.I., *Department of
Botany, Calcutta University, Calcutta 19*

(Received December 19, 1966)

Rice plants of variety *Bhasamanik* were grown in nutrient solutions containing IAA and MH at concentrations of 10^{-1} to 10^{-4} mg/l. Plants grown without added growth regulators served as controls. The growth of root and shoot of the treated plants and their nitrogenous and carbohydrate constituents were studied at the three stages, viz. 28 days, 60 days (tillering) and 96 days (pre-flowering). An attempt has been made to relate the induced morphogenic changes with protein synthesis. An initial retardation of root length was noted with 10^{-1} mg/l of IAA and MH, followed by acceleration at subsequent stages. The root number was, however, increased significantly right from the initial stage at this concentration. The lower concentrations, viz. 10^{-2} to 10^{-4} mg/l, caused significant increase in both root length and number from the beginning. Shoot growth was very little affected at these concentrations of IAA and MH. A reduction in protein synthesis parallel to decrease in length of the root treated with 10^{-1} mg/l of IAA and MH at the early stage was noted. Increase in root number was attributed to increase in endogenous auxin level. The increased root growth at the tillering and pre-flowering stages was accompanied by increased protein synthesis. In treatments with concentrations lower than 10^{-1} mg/l, protein synthesis increased with accelerated root growth at an early stage in IAA treated plants, while with MH, increased root growth was obtained, protein content being less than that of the control.

INTRODUCTION

The significant position occupied by rice in the agricultural economy of the country has in recent years prompted a number of investigations on the relationship between auxins and growth of this plant. The effects produced on the plant by the external application of growth substances and the relation of the internal auxin levels to the physiological processes, recently reviewed by Sircar (1963, 1965), indicate a complex relationship. It appears that the various physiological processes are regulated by IAA levels in the different organs of the plant.

Sircar and Parija (1949) and Sircar and Das (1954) suggested that the internal IAA level is associated with increased tillering of the rice plant. The rapid increase in height of the plant with the rise of water level is

* The work presented in this paper comprised part of a thesis submitted by S. Gopalakrishnan for the D.Phil. degree of the University of Calcutta.

† Present address: Jute Agricultural Research Institute, P.O. Nilgunj, Barrackpur, 24-Parganas.

presumed to be due to the elongation of internodal cells by auxin synthesis at the nodal meristem (Sircar 1958). An increase in the IAA level of the crown tissue with growth and its subsequent disappearance after the shoot apex elongates has also been recorded by him. Sen and Bose (1959) reported beneficial effect of IAA on plant height, while MH had an adverse effect. When applied by seed treatment short-day variety of rice showed earliness in flowering while day neutral variety indicated a delaying effect. Spraying the plants had a delaying effect on flowering of both the varieties. Sircar and Kundu (1960) studied the root and shoot growth of the rice plant by soaking the seeds, growing the seedlings in solution for six days and the plants in water culture with added growth regulators at different levels and found no sharp distinction between the effects of IAA and MH on the growth of the plant both at the seedling and mature stages. At lower concentrations an increase in root length and number was apparent but shoot growth was very little affected. Bioassay of free auxin in the different organs of the plant growing in nutrient solutions containing the regulators revealed that there is a maximum increase in endogenous auxin level after IAA treatment and that the auxin level in root is lower in MH than in IAA treated plants. These authors attributed the stimulation of root growth to this increase in endogenous auxin level.

On the basis of these results it was felt that attempts should be made to unravel the metabolic alterations brought about by the application of IAA and MH and to relate them with the morphogenic changes. Sircar and Datta Ray (1962) made a beginning in this direction and reported that during the first seven days of germination both leaf and root growths were adversely affected initially, followed by subsequent recovery. These changes in growth were reflected in the nitrogen metabolism of the seedlings. The IAA and MH treatments retarded protein breakdown in the endosperm, especially during the first two days. Whilst this was retarded, translocation to the embryo was appreciably low, though protein synthesis there was affected only slightly. They presumed that IAA and MH retarded the action of enzymes responsible for protein hydrolysis in the endosperm. In the present investigations intact rice plants have been treated with IAA and MH and the changes in the nitrogenous and carbohydrate constituents in relation to growth at different stages have been examined.

MATERIAL AND METHODS

The experiments were carried out during the years 1961 and 1962 using pure line winter rice variety *Bhasamanik* obtained from the Rice Research Station, Chinsurah, W. Bengal.

The experimental procedures for raising the seedlings and growing them in nutrient solutions were the same as described by Sircar and Kundu (1960),

with the improvement of using culture bottles varnished with two coatings of black and one coating of white paints. Renewal of solution was done intermittently with decreasing intervals as the plants aged. IAA and MH at concentrations of 10^{-1} to 10^{-4} mg/l were added to the culture solution to expose the roots continuously to the chemical stimuli (Mitchell and Wittwer 1962). Plants grown without growth regulators served as control. The pH of the culture solution was maintained between 5.2 and 5.6. Fifty culture bottles having one plant in each were used for each treatment.

Observations from 20 plants per treatment were recorded for root length and number, shoot length, tiller and leaf numbers at stages of 14, 28, 44, 60, 78 and 96 days' growth. Samples of root and top portions were collected for chemical analysis at three stages: 28, 60 (tillering) and 96 (pre-flowering) days. The analytical methods adopted for the estimation of total, soluble, protein and amide nitrogen were those described by Sircar and Datta (1957) with the exception that in the determination of amide N, a saturated NaOH solution was used as suggested by Oland (1959). The estimation of total free α -amino N was done by the method detailed by Hawk *et al.* (1947). The α -amino N of the free amino acids was calculated by subtracting the amide N value from the total free α -amino N. The copper reduction method of Van der Plank (1936) was used for the determination of sugars. The estimation of acid hydrolysable polysaccharides was done following the method of Whelan (1955).

The nitrogenous and carbohydrate fractions in the root and shoot portions of the plant at the three different stages (28, 60 and 96 days) of growth, for the years 1961 and 1962, are expressed as percentages of dry weight and total nitrogen and also per plant. The summarized and significant data only are presented in this account.

Fisher's 'F' test was adopted to test the significance of the treatments. In order to analyse the results of chemical analysis, each fraction expressed as percentage of dry weight was considered individually. The data for both the years were subjected to pooled statistical analysis and the error was estimated from the highest order interaction. The analysis of variance was taken up as follows:

		Degrees of freedom
Treatment	..	4
Stage	..	2
Year	..	1
Interaction T \times S		8
Error	..	14
Total		<u>29</u>

RESULTS AND DISCUSSION

Treatments with 10^{-1} mg/l IAA and MH resulted in an inhibiting effect on root growth up to 28 days, after which there was recovery and stimulation of growth. However, a significant increase in root number was noted. With the lower concentrations from 10^{-2} to 10^{-4} mg/l a highly significant increase in root length and number in comparison to the control was noted at all stages of growth (Tables I, II). The retardation and acceleration in root length was

TABLE I
Effect of IAA on the growth of root of the rice plant

Treatment	28 days		60 days		96 days	
	Root length (cm)	Root number	Root length (cm)	Root number	Root length (cm)	Root number
Control	18.52	10.4	24.61	42.5	32.49	83.3
10^{-1} mg/l	16.38	15.6	27.66	67.4	35.47	110.1
10^{-2} mg/l	20.39	16.9	28.38	78.6	37.28	119.0
10^{-3} mg/l	24.79	18.7	30.03	86.0	38.74	120.8
10^{-4} mg/l	25.72	19.3	31.45	88.1	41.60	124.7
'F' test	**	**	**	**	**	**
S. Em.	± 0.14	± 0.36	± 0.15	± 0.63	± 0.25	± 0.58
C.D. at 5% P	0.40	1.02	0.41	1.81	0.69	1.65
C.D. at 1% P	0.52	1.36	0.55	2.41	0.91	2.20

** Significant at one per cent P.

TABLE II
Effect of MH on the growth of root of the rice plant

Treatment	28 days		60 days		96 days	
	Root length (cm)	Root number	Root length (cm)	Root number	Root length (cm)	Root number
Control	19.29	10.1	26.19	40.5	32.16	81.0
10^{-1} mg/l	17.81	13.4	28.28	49.7	33.35	89.8
10^{-2} mg/l	21.17	16.0	29.02	57.6	36.02	94.4
10^{-3} mg/l	22.64	17.2	31.43	63.4	37.12	100.6
10^{-4} mg/l	23.00	17.6	32.00	69.5	38.86	103.6
'F' test	**	**	**	**	**	**
S. Em.	± 0.44	± 0.29	± 0.56	± 0.66	± 0.59	± 0.65
C.D. at 5% P	1.24	0.82	1.57	1.89	1.66	1.86
C.D. at 1% P	1.64	1.10	2.08	2.52	2.19	2.48

** Significant at one per cent P.

also accompanied with corresponding decrease and increase in the root : shoot ratio of the plant (Table III). On the other hand, shoot growth was very little affected by these treatments and thus the data have not been included.

TABLE III

Effect of IAA and MH on the root : shoot ratio of the rice plant

Treatment	Age of the plant in days	Root : shoot ratio	
		IAA	MH
Control	28	0.126	0.133
	60	0.097	0.131
	96	0.176	0.123
10 ⁻¹ mg/l	28	0.095	0.094
	60	0.111	0.156
	96	0.175	0.169
10 ⁻² mg/l	28	0.144	0.139
	60	0.131	0.181
	96	0.187	0.177
10 ⁻³ mg/l	28	0.176	0.193
	60	0.140	0.199
	96	0.187	0.183
10 ⁻⁴ mg/l	28	0.201	0.205
	60	0.146	0.217
	96	0.193	0.191

These observations follow, in general, the trends discussed by Sircar and Kundu (1960), who noted increase in number of roots in both high and low auxin concentrations. Initial retardation of root length in rice seedling by 10⁻¹ mg/l IAA and MH has also been described by Sircar and Datta Ray (1962). Maleic hydrazide behaves in the same way as IAA in regulating growth; when applied in low concentrations it promotes root growth. An initial retardation of growth in tomato by 10 p.p.m. MH followed by recovery has been shown by Bose and Hamner (1960). Buis (1957) has also recorded that at certain doses it has promoting effect. Kozlova *et al.* (1960) stated that MH, depending upon its concentration and plants' age, appears to be an inhibitor as well as a stimulant of growth.

Burström (1957) recorded an initial inhibition of root elongation caused by added auxin, but after three days the inhibition was overcome and 'adaptation' occurred. He noted that elongation proceeded at an increased rate as soon as the first inhibition was overcome. The presence of IAA oxidase to

inactivate IAA to a low level which stimulates elongation has been the argument in support of this. By studying the kinetics of IAA inactivation, Pilet (1959) suggested the existence of a lag period after which inactivation takes place and to which IAA activity in retardation of root growth is limited. Audus and Das (1955) recorded that maximum response to IAA was obtained in the first few hours, thereafter the response declined. Galston (1956) proposed that a cybernetic 'feed back' system exists between IAA and IAA oxidase and that the catalytic effect of IAA on growth is due to its binding with protein. When tissues are treated with IAA their capacity to destroy IAA is augmented by the increased production of IAA oxidase. Root has a higher IAA destroying capacity than stem and hence it is more sensitive to the application of auxin. The increase in IAA-oxidase activity with age of the plant suggests that destruction of auxin by the oxidase system acts as a regulating mechanism. IAA bound to protein remains unaffected by the oxidase system. The inhibition of root growth in the rice plant noted here with higher concentrations of auxin is presumably due to more of the auxin remaining bound with protein. Manos (1961) has shown that in the intact plant auxin response to root growth is not the same as in the detached root. Reactivation occurred only in the attached root indicating that during the first seven days complex interactions occur between exogenous auxin and a substance located in parts of the plant other than root. According to him reactivation may be due to the inactivation of IAA as indoleacetylaspartic acid (IAA_{sp}) complex, as the attached root tips only are found to accumulate this substance (Andreae and Van Ysselstein 1960*a, b*). IAA has been shown to conjugate with aspartic acid to form indoleacetylaspartic acid in pea seedling by Andreae and Good (1957). Thurman and Street (1962), however, suggested a possible accumulation of IAA_{sp} and indoleacetylglutamic acid (IAG) by exposure of excised tomato roots to IAA. This evidently shows that for adaptation to auxin some interaction between exogenous auxin and endogenous factors, presumably a metabolic product (or products) translocated from the shoot is necessary.

The stimulation of root growth in wheat by *p*-chlorophenoxy-iso-butyric acid (PCIB) (Fransson 1958) and in rice by MH (Sircar and Kundu 1960) has been explained on the basis of an increase in endogenous auxin level. According to Audus and Thresh (1956) relatively small variation, in the endogenous auxin level, cannot account for the growth changes produced by MH. It is suggested that these are additional expressions of the metabolic and morphogenetic changes occurring in the plant by MH treatment.

The metabolic alterations of the rice plant at the three stages of growth influenced by the different concentrations of IAA and MH signify intricate relationship with the morphogenetic changes that have taken place (Tables IV-X).

While at a concentration of 10^{-1} mg/l IAA the roots of 28 days old plants had lower total and protein nitrogen, lower acid hydrolysable polysaccharides, total and non-reducing sugars, there was practically very little change in the soluble nitrogen. An increase in the amide nitrogen content with a corresponding decrease in the free α -amino nitrogen fraction was evident, but this did not reach the significant level (Tables IV, VIII). On the other hand,

TABLE IV

Nitrogen fractions, as percentage of dry weight, in the root of IAA treated rice plant

Treatment	Age of the plant in days	Total N	Total soluble N	Protein N	Amide N	α -amino N
Control	28	3.077	1.081	1.996	0.297	0.352
	60	2.151	0.995	1.156	0.280	0.338
	96	1.652	0.777	0.875	0.225	0.274
10^{-1} mg/l	28	2.610	1.123	1.486	0.402	0.280
	60	2.554	0.891	1.663	0.268	0.249
	96	2.439	1.381	1.058	0.405	0.431
10^{-2} mg/l	28	3.062	0.945	2.117	0.292	0.284
	60	2.758	1.801	0.957	0.465	0.457
	96	2.446	1.271	1.175	0.397	0.397
10^{-3} mg/l	28	3.075	0.859	2.216	0.274	0.228
	60	2.675	1.640	1.035	0.392	0.445
	96	2.425	1.158	1.267	0.324	0.340
10^{-4} mg/l	28	3.103	0.920	2.182	0.282	0.282
	60	2.735	1.738	0.997	0.472	0.444
	96	2.608	1.404	1.204	0.405	0.433
C.D. for inter-treatment \times stage—						
at 5% P		0.404	0.464	0.437	NS	NS
at 1% P		—	—	0.606	—	—

NS—Not significant.

in the shoot no significant difference in the nitrogenous constituents and the level of acid hydrolysable polysaccharides was apparent, but an increase was noted in the components of the total sugars, viz. sucrose and reducing sugar (Tables VI, VIII). The accumulation of soluble carbohydrate fractions in the shoot and their decrease in the root presumably indicate slowing down of translocation of these fractions from the shoot to the root when considered along with the reduction in growth and protein synthesis in the root, for which availability of soluble carbohydrates and amino acids would be an essential feature. The restricted availability of amino acids appears to have been caused by the synthesis of amide nitrogen in the root (Table IV). It is

evident from these metabolic changes that the reduction in protein synthesis is related directly with the decrease in root elongation. The net total and protein nitrogen contents of the root at this concentration of IAA were lower than the control, indicating inhibition of nitrogen uptake by high concentration of auxin (Table X). Smith (1951) cited examples of inhibition of nitrate uptake by roots treated with auxins as also inhibition of root growth. Small concentrations of 2, 4-D have been found by Nance (1949) to drastically inhibit nitrate uptake by wheat roots.

TABLE V
Nitrogen fractions, as percentage of dry weight, in the root of MH treated rice plant

Treatment	Age of the plant in days	Total N	Total soluble N	Protein N	Amide N	α -amino N
Control	28	2.496	1.108	1.389	0.297	0.329
	60	3.358	0.872	2.486	0.316	0.206
	96	2.364	1.181	1.183	0.382	0.374
10^{-1} mg/l	28	2.084	0.796	1.288	0.251	0.286
	60	3.404	0.490	2.914	0.181	0.162
	96	2.302	1.003	1.299	0.276	0.338
10^{-2} mg/l	28	1.810	0.746	1.063	0.239	0.263
	60	3.190	0.418	2.771	0.143	0.152
	96	2.322	0.848	1.474	0.258	0.232
10^{-3} mg/l	28	1.911	0.785	1.125	0.241	0.262
	60	3.083	0.384	2.699	0.161	0.112
	96	2.262	0.741	1.521	0.226	0.219
10^{-4} mg/l	28	1.874	0.748	1.127	0.236	0.260
	60	3.067	0.377	2.689	0.150	0.120
	96	2.221	0.508	1.713	0.175	0.182
C.D. for inter-treatment \times stage—						
at 5% P		—	0.147	0.205	0.053	—
at 1% P		—	—	0.284	—	—

At the age of 28 days a decrease was seen in the total and protein nitrogen contents of the roots treated with 10^{-1} mg/l of MH, though this missed the level of significance (Table V), while in the shoot these fractions were very little affected (Table VII). Along with the lower uptake of nitrogen, soluble nitrogen content decreased significantly in the root (Table V). But amide nitrogen was accumulated in the shoot (Table VII) indicating that protein synthesis is presumably checked at the amide level. The downward trend observed in carbohydrate fractions (Table IX) also gave evidence to the fact that the main metabolic activities are slowed down by the treatments.

These relate favourably to the inhibition of root growth coupled to reduction in protein synthesis.

After 60 and 96 days' growth, there was an increase in the total and protein nitrogen contents of the roots treated with 10^{-1} mg/l IAA and also accelerated root growth (Tables I, IV). Hydrolysis of polysaccharides and translocation of the products from the shoot to the root was indicated by the presence of higher contents of soluble carbohydrates in both root and shoot of the treated plants at the age of 60 days, the amount of total sugars in the

TABLE VI

Nitrogen fractions, as percentage of dry weight, in the shoot of IAA treated rice plant

Treatment	Age of the plant in days	Total N	Total soluble N	Protein N	Amide N	α -amino N
Control	28	5.174	1.430	3.744	0.377	0.447
	60	3.409	1.333	2.075	0.397	0.404
	96	3.136	1.137	1.999	0.330	0.341
10^{-1} mg/l	28	4.413	1.349	3.064	0.337	0.429
	60	4.206	1.594	2.609	0.448	0.517
	96	3.575	1.293	2.282	0.386	0.406
10^{-2} mg/l	28	4.701	1.255	3.446	0.317	0.411
	60	3.812	1.456	2.356	0.383	0.524
	96	3.603	1.543	2.060	0.433	0.507
10^{-3} mg/l	28	4.764	1.280	3.484	0.308	0.409
	60	4.020	1.287	2.733	0.393	0.425
	96	3.954	1.514	2.440	0.451	0.501
10^{-4} mg/l	28	5.122	1.416	3.706	0.366	0.430
	60	4.033	1.241	2.792	0.346	0.440
	96	3.846	1.532	2.314	0.398	0.478
C.D. for inter-treatment \times stage— at 5% P		NS	0.270	NS	NS	NS

NS—Not significant.

roots being evidently in excess after utilization in protein synthesis. After 96 days more or less similar results were obtained. Even though at this stage there was no excess of soluble carbohydrates in the root as observed in the previous stage, this is obviously due to their conversion to acid hydrolysable polysaccharides (Table VIII).

From Table V it will be seen that in the plants treated with 10^{-1} mg/l MH, total nitrogen of the root did not vary much from the control at the tillering and pre-flowering stages. This is in contrast to the effect of IAA

where marked increase was recorded under identical conditions (Table IV). Similar to the condition found at the early stage, there was decrease of soluble nitrogen in the root at these stages, but protein nitrogen increased significantly. At the same time, higher value of total nitrogen in the shoot was reflected in increased amide nitrogen without any appreciable rise in protein nitrogen (Table VII). Pronounced increases in carbohydrate fractions were generally observed in the plant at the tillering stage. At the later stage this trend was more or less reversed in that the total sugar, reducing sugar and acid

TABLE VII

Nitrogen fractions, as percentage of dry weight, in the shoot of MH treated rice plant

Treatment	Age of the plant in days	Total N	Total soluble N	Protein N	Amide N	α -amino N
Control	28	4.381	2.046	2.335	0.425	0.620
	60	3.623	0.833	2.791	0.244	0.359
	96	3.297	0.492	2.805	0.165	0.151
10 ⁻¹ mg/l	28	4.258	1.935	2.323	0.494	0.588
	60	3.932	0.867	3.065	0.306	0.304
	96	3.426	0.661	2.765	0.226	0.237
10 ⁻² mg/l	28	4.142	1.833	2.308	0.497	0.582
	60	3.954	0.858	3.095	0.331	0.296
	96	4.023	1.274	2.749	0.356	0.347
10 ⁻³ mg/l	28	4.055	1.854	2.201	0.485	0.585
	60	3.984	0.870	3.115	0.296	0.322
	96	4.024	1.223	2.801	0.315	0.367
10 ⁻⁴ mg/l	28	3.925	1.829	2.096	0.499	0.586
	60	4.061	0.880	3.181	0.320	0.324
	96	4.007	1.209	2.798	0.320	0.329
C.D. for inter-treatment \times stage—						
at 5% P		—	0.189	—	—	0.064
at 1% P		—	0.263	—	—	0.089

hydrolysable polysaccharides of the shoot showed no marked change from the control. In the root, while total sugar was slightly reduced, reducing sugar increased with corresponding decline in non-reducing sugar. Likewise the acid hydrolysable polysaccharides were not appreciably altered (Table IX). These, considered along with the increase in protein nitrogen of the root, are parallel events in the overall effect of the growth substance on the morphogenic changes of the plant.

With lower concentrations of IAA, viz. 10⁻² to 10⁻⁴ mg/l, the total and protein nitrogen per root increased (Table X). This is suggestive of increased

nitrogen metabolism due to IAA treatment. The increase in the total sugar of the shoot may be caused by greater carbohydrate assimilation. The overall reduction in the total sugar content of the roots is presumably due to the depletion of sugar because of the rapid growth of roots (Table VIII). Similar observations were made in IAA treated etiolated pea stem sections by Christiansen and Thimann (1950a) and in excised wheat leaves by Austin (1961). The intense metabolic activities occurring in the root after the auxin treatment are also evident from increase in free α -amino N and amide N in the roots of 60 and 96 days old plants (Table IV).

The data obtained by treating the plants with 10^{-2} to 10^{-4} mg/l MH at the three stages of growth indicate a general increase in the acid hydrolysable polysaccharides of the plant. Total soluble carbohydrates decreased significantly at the young stage in both the root and shoot of the plant, while

TABLE VIII
Carbohydrate fractions, as percentage of dry weight, in the root and shoot of IAA treated rice plant

Treatment	Age of the plant in days	Root				Shoot			
		Total sugar	Reducing sugar	Non-reducing sugar	Acid hydrolysable polysaccharides	Total sugar	Reducing sugar	Non-reducing sugar	Acid hydrolysable polysaccharides
Control	28	0.313	0.028	0.286	19.92	1.103	0.167	0.936	11.04
	60	0.255	0.025	0.230	20.74	1.428	0.193	1.236	17.12
	96	0.369	0.018	0.351	18.46	0.764	0.524	0.240	19.23
10^{-1} mg/l	28	0.161	0.016	0.145	16.76	1.885	0.667	1.219	12.05
	60	0.337	0.064	0.272	20.04	1.707	0.401	1.306	14.24
	96	0.183	0.034	0.150	22.05	1.127	0.909	0.219	20.07
10^{-2} mg/l	28	0.148	0.059	0.090	13.33	1.440	0.501	0.939	15.32
	60	0.254	0.045	0.210	21.37	1.894	0.318	1.576	16.71
	96	0.158	0.035	0.123	22.73	1.714	0.957	0.758	18.14
10^{-3} mg/l	28	0.158	0.058	0.100	15.75	1.332	0.533	0.799	15.10
	60	0.251	0.065	0.186	20.68	1.860	0.303	1.556	17.27
	96	0.154	0.031	0.122	22.87	1.619	0.919	0.700	18.54
10^{-4} mg/l	28	0.181	0.074	0.108	15.92	1.417	0.458	0.959	15.18
	60	0.236	0.064	0.172	20.86	1.689	0.275	1.414	17.12
	96	0.160	0.023	0.137	23.29	1.516	0.854	0.662	18.04
C.D. for T \times S									
at 5% P		0.077	0.022	0.071	2.00	0.186	0.109	0.153	1.48
at 1% P		0.108	0.030	0.099	2.78	0.258	0.152	0.213	2.06

higher values were noted in the shoot at the tillering and pre-flowering stages. Reducing sugar decreased in the treated roots at the tillering stage, but increased significantly at the pre-flowering. In the shoot, however, this fraction was less than the control at the young stage, but higher at the later two stages. Non-reducing sugar of the treated plants was significantly lower than the control at the young and pre-flowering stages, but it was high at the tillering

TABLE IX
Carbohydrate fractions, as percentage of dry weight, in the root and shoot of MH treated rice plant

Treatment	Age of the plant in days	Root				Shoot			
		Total sugar	Reducing sugar	Non-reducing sugar	Acid hydrolysable polysaccharides	Total sugar	Reducing sugar	Non-reducing sugar	Acid hydrolysable polysaccharides
Control	28	0.588	0.091	0.497	20.50	1.441	0.201	1.239	14.21
	60	0.592	0.365	0.227	15.79	0.853	0.482	0.371	16.57
	96	0.998	0.275	0.724	21.53	1.923	0.916	1.007	23.19
10 ⁻¹ mg/l	28	0.306	0.080	0.226	18.34	1.187	0.255	0.932	15.99
	60	1.089	0.550	0.539	16.58	1.646	0.793	0.853	17.89
	96	0.772	0.499	0.273	22.60	1.812	1.042	0.770	22.53
10 ⁻² mg/l	28	0.215	0.065	0.150	16.88	0.754	0.096	0.658	16.34
	60	0.484	0.209	0.275	19.87	1.427	0.600	0.826	21.88
	96	0.824	0.491	0.333	23.86	2.226	1.390	0.836	23.04
10 ⁻³ mg/l	28	0.268	0.065	0.203	22.86	0.791	0.108	0.683	15.08
	60	0.504	0.248	0.255	18.91	1.456	0.611	0.844	18.42
	96	0.951	0.557	0.394	24.03	2.160	1.450	0.711	23.62
10 ⁻⁴ mg/l	28	0.238	0.050	0.188	22.45	0.702	0.100	0.602	14.35
	60	0.554	0.185	0.369	20.00	1.371	0.546	0.826	13.27
	96	1.022	0.554	0.468	23.43	2.367	1.676	0.691	23.42
C.D. for T × S									
at 5% P		0.091	0.105	0.109	2.03	0.254	0.181	0.207	—
at 1% P		0.126	0.146	0.152	2.81	0.352	0.251	0.287	—

stage (Table IX). Tables V and VII reveal that the overall treatment effect on total nitrogen was generally not significant in the root and shoot. But when the nitrogen fractions were considered separately, variations were apparent. Soluble nitrogen showed a decrease in the treated roots at all stages and in the treated shoot at the age of 28 days. At the tillering stage, however, this fraction did not differ much from the control in the treated

shoot, but accumulated by 96 days. The amide nitrogen fraction showed reduction in the root with corresponding increase in the shoot. The values of α -amino nitrogen decreased in the treated roots at the three stages of growth. At the pre-flowering stage, this fraction was in excess of the control in the shoot of treated plants.

These results show that early stimulation of root growth by MH is not accompanied by increased protein synthesis, while at the later stages protein synthesis follows parallel to increased growth rate.

TABLE X

Amount of total and protein nitrogen in the roots of an IAA treated rice plant, in mg

Treatment	Age of the plant in days	Total N	Protein N
Control	28	0.102	0.073
	60	0.556	0.296
	96	1.617	0.670
10 ⁻¹ mg/l	28	0.068	0.044
	60	0.913	0.633
	96	3.500	1.094
10 ⁻² mg/l	28	0.121	0.089
	60	0.992	0.309
	96	3.574	1.417
10 ⁻³ mg/l	28	0.148	0.114
	60	1.119	0.391
	96	3.847	1.795
10 ⁻⁴ mg/l	28	0.175	0.135
	60	1.174	0.395
	96	4.473	1.769

Mention may be made here of the results obtained by earlier workers on the relationship between the growth substances IAA and MH and metabolism in different plants. Increase in sugars observed in excised tobacco stem sections after IAA treatment has been observed to be not due to starch hydrolysis, but presumably caused by the presence of the auxin in culture medium (Skoog and Robinson 1950). While Burström (1951) reported that auxins do not alter the rate of protein synthesis nor influence the turnover of protein, Christiansen and Thimann (1950b) found a slight enhancement of protein synthesis in pea stem sections by these substances. Osborne (1958) suggested a dual role for IAA in controlling growth. In addition to function as a growth

promoter it may stimulate the formation of a growth inhibitor, which would be a safety mechanism to maintain the indigenous level of auxin. Andreeva and Morozova (1959) reported an increase in growth of shoots and roots, volume of roots, uptake of nutrients, photosynthesis and respiration in tomato seedlings after treatment with 20 mg/l of the potassium salt of IAA. Increase in water-soluble carbohydrates in different plants due to treatment with IAA has been shown by Beljdenkova and Konovalov (1959) and in the rooting of *Justicia* cuttings by Sen and Basu (1960). The results obtained by Booth *et al.* (1962) in young plants of *Solanum andigena* indicated that the external application of IAA was not primarily to create a metabolic 'sink' by stimulating synthesis, but rather to enhance active uptake and accumulation of the metabolites by the tissues to which it is applied. Increased sucrose and starch have been noted in MH treated cotton plants by McIlrath (1950) and in barley by Currier *et al.* (1951). In tobacco leaves treated with one per cent solution of MH, total nitrogen content showed considerable reduction and ammonium nitrogen, an increase (Shimizu and Iwazaki 1956). Tibbitts and Wedin (1957), however, noted that total nitrogen in tobacco leaves increased by MH treatment. Various MH treatments of the flue-cured tobacco at topping time have been shown to increase sugar contents of the plant (Gaines 1959; Birch and Vickery 1961; Harris and Miles 1961). Increase in total soluble matter, sucrose, starch and free amino compounds, particularly glutamine and glutamate, was recorded by Samborski and Shaw (1957) in the first leaf of *Khapli* wheat.

In conclusion it may be stated that in the rice plant, during germination the growth substances IAA and MH slow down translocation of soluble nitrogen from the endosperm to the embryo by primarily retarding the enzyme activity responsible for the hydrolysis of protein in the endosperm (Sircar and Datta Ray 1962), but at the growth phases of the plant up to pre-flowering, the overall effect of the growth substances shows a direct relation of stimulation of root growth to protein synthesis. The increase in protein synthesis associated with stimulation of root growth is presumably involved in two ways, firstly, increased protein demand for growth in length and number and secondly, protein is utilized in binding with added IAA. However, in the case of MH, a slightly different picture is presented by concentrations lower than 10^{-1} mg/l, where stimulation of root growth is not accompanied by increased protein synthesis at the early stage, while at the later stages stimulation of root growth is directly related to increased protein synthesis.

ACKNOWLEDGEMENT

Thanks are due to the Council of Scientific and Industrial Research for awarding a Research Fellowship to one (S. G.) of the authors for conducting these studies.

REFERENCES

- Andreae, W. A., and Good, N. E. (1957). Studies on 3-indoleacetic acid metabolism. IV. Conjugation with aspartic acid and ammonia as processes in the metabolism of carboxylic acids. *Pl. Physiol.*, **32**, 566-72.
- Andreae, W. A., and Van Yesselstein, M. W. H. (1960a). Studies on 3-indoleacetic acid metabolism. V. Effect of calcium ions on 3-indoleacetic acid uptake and metabolism by pea roots. *Pl. Physiol.*, **35**, 220-24.
- (1960b). Studies on 3-indoleacetic acid metabolism. VI. 3-indoleacetic acid uptake and metabolism by pea roots and epicotyls. *Pl. Physiol.*, **35**, 225-32.
- Andreeva, R. A., and Morozova, I. B. (1959). The influence of IAA treatment of the root system upon the growth and metabolism of tomato seedlings. *Dokl. Akad. Nauk SSSR (Bot. Sci. Sect.)*, **125**, 90-92.
- Audus, L. J., and Das, N. (1955). The interaction of auxins and antiauxins in the stimulation of root growth. *J. exp. Bot.*, **6**, 328-47.
- Audus, L. J., and Thresh, R. (1956). The effects of synthetic growth regulator treatments on the levels of free endogenous growth substances in plants. *Ann. Bot.*, **20**, 439-59.
- Austin, A. (1961). Effect of indole-3-acetic acid on the nitrogen and carbohydrate metabolism of excised wheat leaves. *Curr. Sci.*, **30**, 24-25.
- Beljdenkova, A. F., and Konovalov, I. N. (1959). An investigation of the physiological effect of growth substances on plants. *Congr. Int. Bot.*, **9th**, **2**, 27.
- Birch, E. C., and Vickery, L. S. (1961). The effect of maleic hydrazide on certain chemical constituents of flue-cured tobacco. *Canad. J. Pl. Sci.*, **41**, 170-75.
- Booth, A., Moorby, J., Davies, C. R., Jones, H., and Wareing, P. F. (1962). Effects of indole-3-acetic acid on the movement of nutrients within plants. *Nature, Lond.*, **194**, 204-05.
- Bose, P. C., and Hamner, C. L. (1960). Growth, development and mineral uptake in tomato plants as affected by MH. *Indian J. agric. Sci.*, **30**, 170-76.
- Buis, R. (1957). Action de l'hydrazide maleique sur le développement du Mais, en rapport avec la concentration et avec l'âge des plantules. *Bull. Soc. bot. Fr.*, **104**, 33-37.
- Burström, H. (1951). Studies on growth and metabolism of roots. V. Cell elongation and dry matter content. *Physiologia Pl.*, **4**, 199-208.
- (1957). Auxin and the mechanism of root growth. *Symp. Soc. exp. Biol.*, **11**, 44-62.
- Christiansen, G. S., and Thimann, K. V. (1950a). The metabolism of stem tissue during growth and its inhibition. II. Respiration and other soluble material. *Arch. Biochem.*, **26**, 248-59.
- (1950b). The metabolism of stem tissue during growth and its inhibition. III. Nitrogen metabolism. *Arch. Biochem.*, **28**, 117-29.
- Currier, H. B., Day, B. E., and Crafts, A. S. (1951). Some effects of maleic hydrazide on plants. *Bot. Gaz.*, **112**, 272-80.
- Fransson, P. (1958). Studies on the interaction of anti-auxin and native auxin in wheat roots, *Physiologia Pl.*, **11**, 644-54.
- Gaines, J. G. (1959). Influence of maleic hydrazide on flue-cured tobacco quality. *Tobacco, Lond.*, **148**, 20-23.
- Galston, A. W. (1956). Some metabolic consequences of the administration of indole-3-acetic acid in plant cells. In *The Chemistry and mode of action of Plant Growth Substances*. Ed. Wain, R. L., and Wightman, F. Butterworth Scientific Publications, London, 1956.
- Harris, J. B., and Miles, J. D. (1961). Some effects of urea and maleic hydrazide on the flue-cured tobacco. *Tobacco, N.Y.*, **153**, 19-23.
- Hawk, P. B., Oser, B. L., and Summerson, W. H. (1947). *Practical Physiological Chemistry*, 12th ed., Blakiston, New York.
- Kozlova, N. A., Ermolaeva, E. Y., and Batska, P. (1960). Effect of maleic hydrazide on flowering of *Perilla ocymoides* in a long-day environment. *Dokl. Akad. Nauk SSSR. (Bot. Sci. Sect.)*, **130**, 22-24.
- Manos, G. E. (1961). The effects of growth substances on attached and detached root tips of *Pisum sativum* L. *Physiologia Pl.*, **14**, 697-711.

- McIlrath, W. J. (1950). Response of the cotton plant to maleic hydrazide. *Am. J. Bot.*, **37**, 816-19.
- Mitchell, W. D., and Wittwer, S. H. (1962). Response of plants to root absorbed chemical growth substances applied in culture solutions. *Nature, Lond.*, **195**, 725-26.
- Nance, J. F. (1949). The inhibition of salt accumulation in excised wheat roots by 2-, 4-dichlorophenoxy-acetic acid. *Science*, **109**, 174-76.
- Oland, K. (1959). Nitrogenous reserves of apple trees. *Physiologia Pl.*, **12**, 594-648.
- Osborne, D. J. (1958). Growth of etiolated sections of pea internode following exposures to indole-3-acetic acid, 2-, 4-dichlorophenoxy-acetic acid and 2-, 5-dichlorobenzoic acid. *Pl. Physiol.*, **33**, 46-57.
- Pilet, P. E. (1959). Un cas d'adaptation auxines-oxydasique (racine). *Revue gén. Bot.*, **66**, 450-60.
- Samborski, D. J., and Shaw, M. (1957). The physiology of host-parasite relations. IV A. The effect of maleic hydrazide on the carbohydrate, nitrogen and free amino acid content of the first leaf of *Khapli* wheat. *Canad. J. Bot.*, **35**, 457-61.
- Sen, P. K., and Basu, R. N. (1960). Effect of growth substances on root formation in cuttings of *Justicia gendarussa* L. as influenced by varying levels of nitrogen nutrition of stock plants. *Indian J. Pl. Physiol.*, **3**, 72-83.
- Sen, P. K., and Bose, T. C. (1959). Effect of growth-regulating substances on rice. *Indian Agric.*, **3**, 13-16.
- Shimizu, Y., and Iwazaki, H. (1956). Influence of maleic hydrazide on ripening of tobacco leaves. *Proc. Crop Sci. Soc. Japan*, **25**, 96-98.
- Sircar, S. M. (1958). Auxin relations of rice plant. In *Modern Developments in Plant Physiology: A seminar*, University of Delhi, 76-80.
- (1963). Physiology of the rice plant. Presidential address. *Proc. Indian Sci. Congr.*, 1-20.
- (1965). Hormonal control of root growth. *Bull. bot. Soc. Beng.*, **19**, 63-66.
- Sircar, S. M., and Das, T. M. (1954). Studies on the physiology of rice. IX. Auxin content of the vernalized seed. *Proc. natn. Inst. Sci. India*, **20**, 673-92.
- Sircar, S. M., and Datta, S. C. (1957). Studies on the physiology of rice. X. Effects of potassium deficiency on growth and nitrogen metabolism. *Indian J. agric. Sci.*, **27**, 1-24.
- Sircar, S. M., and Datta Ray, P. (1962). Studies on the physiology of rice. XV. Changes in the metabolism of the seed during germination and their relation to the application of growth regulators. *J. exp. Bot.*, **13**, 61-74.
- Sircar, S. M., and Kundu, M. (1960). Studies on the physiology of rice. XVI. Root and shoot growth in relation to the application of growth regulators and changes in the endogenous free auxin contents. *Proc. natn. Inst. Sci. India*, **26 B** (Suppl.), 165-89.
- Sircar, S. M., and Parija, P. (1949). Studies on the physiology of rice. V. Photoperiodic response in five varieties of rice. *Proc. natn. Inst. Sci. India*, **15**, 93-107.
- Skoog, F., and Robinson, B. J. (1950). A direct relationship between indoleacetic acid effects on growth and reducing sugar in tobacco tissue. *Proc. Soc. exp. Biol.*, **74**, 565-68.
- Smith, F. G. (1951). Respiratory changes in relation to toxicity. In *Plant Growth Substances*. Ed. Skoog, F. Univ. of Wisconsin Press, Madison, p. 476.
- Thurman, D. A., and Street, H. E. (1962). Metabolism of some indole auxins in excised tomato roots. *J. exp. Bot.*, **13**, 369-77.
- Tibbitts, T. W., and Wedin, W. (1957). Quality of tobacco harvested from plants treated with maleic hydrazide. *Tobacco, N.Y.*, **145**, 22-24.
- Van der Plank, J. E. (1936). The estimation of sugars in the leaf of the mangold (*Beta vulgaris*). *Biochem. J.*, **30**, 457-83.
- Whelan, W. J. (1955). Starch, Glycogen, Fructosans and similar Polysaccharides. In *Modern methods of Plant Analysis*, Vol. II, Ed. Paech, K. and Tracey, M. V., p. 626.