STUDIES ON THE PHYSIOLOGY OF RICE

XII. Culture of excised embryos in relation to endosperm auxin and other growth factors

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(*Received January 4; approved for reading on May 3, 1957*)

Introduction

In a previous paper of this series (Sircar and Das, 1954) evidence of the presence of a large amount of auxin in the endosperm of rice and its gradual disappearance with the germination of the embryo was presented. Hatcher (1945) observed that during the development of the rye grain auxin of the endosperm mainly accumulates in aleurone cells. With ripening, however, the total auxin of the seed decreases (Avery *et al.*, 1942 ; Popoff, 1941 and Hatcher, 1945). Hatcher has further shown that this disappearance at maturity is due to the desiccation of the grain during ripening and not due to translocation from the grain nor any growth activity. During the early stages of germination the bound auxin is set free and the liberated auxin presumably indolyl acetic acid disappears within a few days and appears to be inactivated (Avery *et al.*, 1940 and Guttenberg *et al.*, 1947). Funke and Soding (1948) suggested that this inactivation takes place in the first internode and moves upward in the inactive form. When it reaches the coleoptile tip it becomes activated once again and moves downward in this form. According to this hypothesis indolyl acetic acid plays an important rôle in the dynamics of the early germination of embryo. Das (1954) has further shown that the embryo of the *petkus* winter rye gradually accumulates auxin with age. A similar result has been obtained by the present authors with winter rice var. *Bhasamanik*. In order to throw further light on the auxin relation of rice endosperm comprehensive experiments on the germination of rice embryo have been carried out by eliminating portions of endosperm and substituting endosperm extract and indolyl acetic acid. A brief summary of the results showing the presence of inhibitory factors in the early growth of embryo has already been published (Sircar, Das and Lahiri, 1955). The part played by the endosperm extract and added indolyl acetic acid on germination was studied by growing embryos in nutrient media. The scope of the work was extended further to analyse the effect of different growth factors on the germination of excised rice embryo.

Experimental Methods

Fractioning of endosperm and germination in indolyl acetic acid and endosperm extract.—Rice grains var. *Bhasamanik* received from the State Rice Research Station, Chinsurah, West Bengal, were carefully husked so that the embryos were not damaged during husking. A mm. graph was fixed on a slide and the grain was held on it. Different fractions of endosperm were then carefully removed with the help of a sterile scalpel. The embryos with different fractions of endosperm were subsequently soaked in sterile distilled water for 24 hours in darkness at 25 ± 10°C. They were then transferred to 1·5% sterile agar slants inside a closed culture room. No seed treatment was done to avoid the effects of other factors that might influence the growth rate of embryo. Agar tubes showing fungus contamination were immediately rejected.

In other experiments embryos with different fractions were grown in sterile agar media containing a range of conc. of indolyl acetic acid (I.A.A.) from 10 mg./L to 0·001 mg./L and endosperm extract. Endosperm extracts (0·212% and 0·0212%) were prepared according to the method described by Sircar and Das (1954). In all cases control sets with full endosperm were maintained. The seedlings in agar tubes were kept in a temperature controlled chamber (25 ± 1°C.) and exposed for five minutes to diffuse sunlight every day. The root and the shoot growth was measured every day by a mm. graph paper held on the tube.

Culture of excised embryo in nutrient media.—Rice embryo is usually very small (approx. 1 to 0·5 mm. in length). It was noticed that during excision any injury to the embryo or the scutellum brought about a failure of germination in most cases even when the excised embryo was placed in media containing minerals, carbohydrate, auxin and vitamins. This is presumably due to the inevitable damage to the epithelial layer during excision resulting in the disruption of translocation of nutrients to the embryo. In order to avoid any injury and damage to the epithelial layer the following procedure was adopted for these experiments. Dehusked grains were soaked in sterile distilled water for 24 hours in darkness at 25 ± 1°C. and the excision of the embryos was made under aspectic conditions in a closed culture room. The incision was given behind the epithelial layer within 1 mm. on the endosperm tissue by a sharp arrow-headed needle under the high power of a Zeiss stereo-binocular microscope. To exhaust the food stored in the residual endosperm, embryos were germinated on sterile agar plates without nutrients. In course of 48 hours the germinating embryo used up the food of the endosperm, it grew no further and subsequently died. The 48 hours old embryos were then transferred to sterilized agar slants, one embryo per tube, containing nutrients. The excised embryos in the tubes were kept in a temperature controlled chamber at 25 ± 1°C. in darkness. The growth readings were taken under the illumination of a red lamp fitted inside the chamber. Any tube showing slightest fungus contamination was rejected. The combination of mineral nutrients adopted by Prof. Gregory and Dr. Purvis at the Imperial College of Science, London (Das, 1954), was used in the culture of rice embryos. In addition the effects of the following growth factors were studied: Sucrose, thiamine (Vitamin B_1), pyridoxine (Vitamin B_6), nicotinic acid, indolyl acetic acid, Dl-tryptophane. Media were prepared in all cases with 1·5% agar containing different growth factors. It may be noted here that experimental limitations did not permit the authors to try sub-culture for rice embryos or to continue the culture in agar tubes for longer than 144 hours on account of the large size of the seedlings.

Results

Fractioning of endosperm and culture of embryo in endosperm extract and indolyl acetic acid media.—The results of embryo growth with different fractions of endosperm have been presented in Table I. Significance of the treatments was assessed by the application of *T*-test. It is apparent that the growth of root is increased when the embryo is growing with three-fourth endosperm. As regards the coleoptile growth significant difference has not been noted in all cases but root growth with three-fourth endosperm is significantly greater in all the periods.

In the medium containing endosperm extract the embryos with different fractions of endosperm show pronounced suppression of root length. In some cases embryos with three-fourth endosperm when grown in the medium containing the extract root length becomes almost equal to that of the control, i.e. embryo with full endosperm growing in the medium without added extract (Table II). This behaviour of the growth of root is clearly manifested in all the periods. No specific effect is indicated in the coleoptile growth by the addition of endosperm extract. As such the percentage of leaf break and the length of the first leaf is not much affected by the addition of endosperm extract, on the contrary embryo with one-eighth

TABLE I

Growth of rice embryos with different fractions of endosperm. Temperature 25 ± 1°C.

Treatment	24 hours				48 hours			
	Shoot length in mm.	S.E.	Root length in mm.	S.E.	Shoot length in mm.	S.E.	Root length in mm.	S.E.
1. Embryos with full endosperm	2·961	0·2628	6·769	0·6918	10·417	2·220	34·562	2·375
2. Embryos with ¾ endosperm	3·333	0·1597	8·267	0·6935	14·000	0·651	39·961	1·338
3. Embryos with ½ endosperm	3·300	0·2056	6·533	0·2698	12·906	0·750	31·471	1·855
4. Embryos with ¼ endosperm	2·900	0·1114	6·300	0·6572	11·533	0·895	25·961	2·117
5. Embryos with ⅛ endosperm	2·500	0·2621	3·727	0·7650	4·700	0·786	7·285	2·526
6. Embryos with wounded endosperm	2·469	0·1852	9·033	0·7778	8·607	1·062	22·231	3·471

TABLE I—*contd.*

Growth of rice embryos with different fractions of endosperm. Temperature 25 ± 1°C.

Treatment	72 hours				96 hours			
	Shoot length in mm.	S.E.	Root length in mm.	S.E.	Shoot length in mm.	S.E.	Root length in mm.	S.E.
1. Embryos with full endosperm	24·214	1·525	47·808	1·754	33·143	3·272	54·692	6·290
2. Embryos with ¾ endosperm	26·291	2·746	53·769	1·634	35·692	3·573	64·615	4·699
3. Embryos with ½ endosperm	22·133	1·421	49·300	4·495	29·751	3·797	50·625	2·845
4. Embryos with ¼ endosperm	16·462	2·150	38·091	3·212	22·357	0·888	44·800	3·828
5. Embryos with ⅛ endosperm	7·233	1·133	18·045	3·045	14·636	1·703	28·272	5·312
6. Embryos with wounded endosperm	15·143	1·529	42·393	3·682	23·250	3·617	44·000	..

endosperm shows very poor growth and coleoptile breaking does not occur up to 96 hours of culture. When the embryos with different fractions of endosperm are grown in the medium containing I.A.A. the acceleration of root growth due to elimination of endosperm fraction as noted previously is retarded and in some cases it becomes almost equal to the root length of embryos with full endosperm growing without I.A.A. (Fig. 1). A general survey from the figure will show that in all cases I.A.A. exerts a retarding effect on root growth in a definite sequence with increasing concentration. In the control set (embryos with different fractions of endosperm growing in the media without I.A.A.) the embryos with half endosperm

TABLE II

Growth of rice embryos (attached to endosperm) in different concentrations of endosperm extract. Temperature 27 ± 1°C.

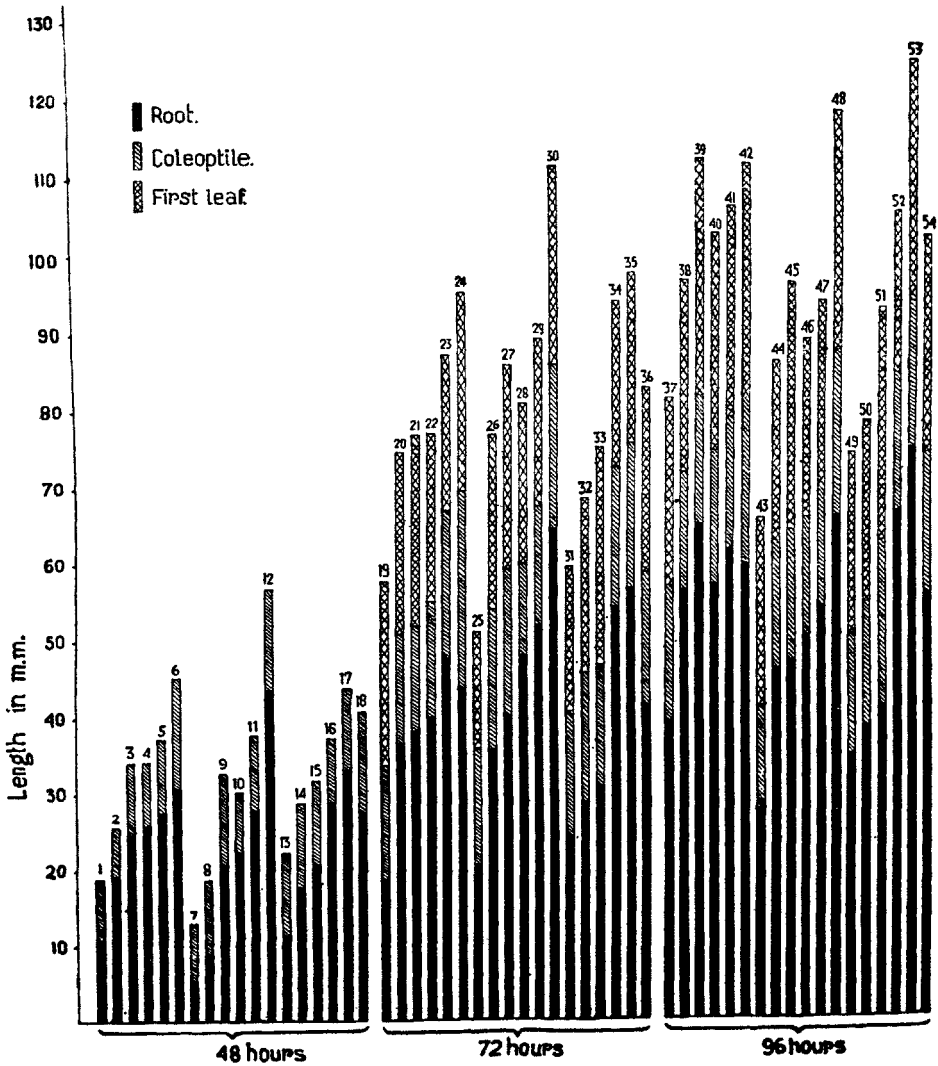
Treatment	24 hours			48 hours	
	Conc. of endosperm extract	Length of coleoptile in mm.	Length of root in mm.	Length of coleoptile in mm.	Length of root in mm.
1. Embryos with full endosperm	0·212%	1·97	4·53	5·93	22·65
	0·0212%	1·73	4·20	5·33	16·95
	Control	2·97	6·71	7·63	23·71
2. Embryos with ½ endosperm	0·212%	1·73	4·70	4·53	18·93
	0·0212%	1·36	3·20	3·53	18·33
	Control	2·20	6·53	5·93	22·00
3. Embryos with ¾ endosperm	0·212%	1·83	3·72	6·20	19·96
	0·0212%	1·67	5·50	5·06	23·00
	Control	2·43	8·27	6·73	28·15
4. Embryos with ¼ endosperm	0·212%	1·60	2·50	3·88	6·25
	0·0212%	1·57	2·20	3·56	8·00
	Control	2·50	3·73	4·25	6·42

TABLE II—*contd.*

Growth of rice embryos (attached to endosperm) in different concentrations of endosperm extract. Temperature 27 ± 1°C.

Treatment	72 hours				96 hours			
	Length of coleoptile in mm.	Length of first leaf in mm.	% of leaf break	Length of root in mm.	Length of coleoptile in mm.	Length of first leaf in mm.	% of leaf break	Length of root in mm.
1. Embryos with full endosperm	12·91	22·0	8·0	36·50	16·83	28·38	66·7	53·83
	11·71	..	..	34·62	15·79	27·27	58·3	49·91
	13·77	19·50	18·20	45·45	20·73	27·70	90·9	66·00
2. Embryos with ½ endosperm	12·25	..	..	34·04	16·58	22·40	41·60	44·54
	11·33	..	..	39·44	13·36	20·57	46·40	50·54
	10·67	17·00	27·20	38·50	15·10	23·37	80·00	56·20
3. Embryos with ¾ endosperm	13·75	15·00	8·00	38·36	16·41	24·70	83·30	68·50
	16·11	22·00	9·00	47·64	18·33	29·42	100·00	65·33
	16·30	14·75	16·70	49·58	16·34	25·15	100·00	72·08
4. Embryos with ¼ endosperm	4·90	..	..	6·75	5·20	..	..	7·88
	5·14	..	..	8·29	6·59	..	..	8·35
	5·79	..	..	6·79	6·21	..	..	6·83

show greater root length over all other treatments. Growth of root in this particular treatment is significantly greater in all the periods. The shoot growth, however, is not much affected by the presence of I.A.A. in the culture media.



Growth of rice embryo with fractions of endosperm in media containing different concentrations of I.A.A.

FIG. 1. 48 hours.—1–5: embryos with full endosperm in 10 mg./L, 1 mg./L, 0·1 mg./L, 0·01 mg./L, 0·001 mg./L respectively of I.A.A. media and 6 is control; 7–11: embryos with $\frac{1}{2}$ endosperm in the same concentrations of I.A.A. and 12 is control; 13–17: embryos with $\frac{1}{4}$ endosperm in I.A.A. media and 18 is control.
72 hours.—19–23: embryos with full endosperm in I.A.A. media and 24 is control; 25–29: embryos with $\frac{1}{2}$ endosperm in I.A.A. media and 30 is control; 31–35: embryos with $\frac{1}{4}$ endosperm in I.A.A. media and 36 is control.
96 hours.—37–41: embryos with full endosperm in I.A.A. media and 42 is control; 43–47: embryos with $\frac{1}{2}$ endosperm in I.A.A. media and 48 is control; 49–53: embryos with $\frac{1}{4}$ endosperm in I.A.A. media and 54 is control.
The same concentration is used for different durations.

TABLE III

Growth of excised rice embryo (*var.* Bhasamanik) *at different concentrations of sucrose with standard mineral nutrients. Temperature* $25 \pm 1°C$.

Treatments	48 hours				72 hours				96 hours			
	Length of coleoptile in mm.	% of control	Length of root in mm.	% of control	Length of coleoptile in mm.	% of control	Length of root in mm.	% of control	Length of coleoptile in mm.	% of control	Length of root in mm.	% of control
1. Control (with only mineral nutrients)	4·27	..	2·7	..	5·0	..	2·7	..	5·6	..	3·5	..
2. 10% sucrose + mineral nutrients	2·54	59·48	2·0	74·07	5·6	112·00	5·1	196·15	6·2	110·72	7·3	208·57
3. 2% sucrose + mineral nutrients	5·54	129·74	4·45	164·81	7·0	140·00	5·7	219·23	10·00	178·57	10·00	285·71
4. 0·4% sucrose + mineral nutrients	4·45	104·21	2·7	100·00	7·4	148·00	4·2	169·53	7·7	119·64	4·7	134·28
5. 0·08% sucrose + mineral nutrients	2·6	60·88	1·7	62·96	4·1	82·00	3·2	84·61	6·6	117·85	4·4	176·00

The effect of sucrose and other growth factors on excised embryos.—In order to observe the effect of sugar excised embryos are cultured in the media containing different concentrations of sucrose. In all cases mineral nutrients are added. In control no sucrose is given, only minerals are present. Without sucrose the embryos show very limited growth; when both sucrose and minerals are present maximum growth of embryo is observed at two per cent sugar (Table III).

Normally the embryos attached with the endosperm show breaking of coleoptile and initiation of first leaf on agar slants within 72 hours after sprouting and the coleoptile length usually is 1·4 to 1·5 mm. at this stage. In the culture of embryos in different concentrations of sugar no coleoptile breaking is observed even after 96 hours. In general the seedlings are very small in comparison to those remaining attached with the endosperm. When only minerals or sucrose is present in the medium the embryo growth is limited. The essential rôle of thiamine (Vitamin B_1) in plant tissue culture has been emphasized by various workers. Its presence in the pericarp of rice and the disease caused by its absence in polished rice is an established fact. Bonner (Schopfer, 1943) has shown its action is quantitative. Within certain limits any increased dosage caused an increase in growth but beyond the optimum level no effect is produced. In the present study thiamine in various concentrations is supplied with two per cent sucrose-mineral nutrients and growth rate of excised embryos are studied. It is evident from Fig. 2 that in the first 24 hours' supply

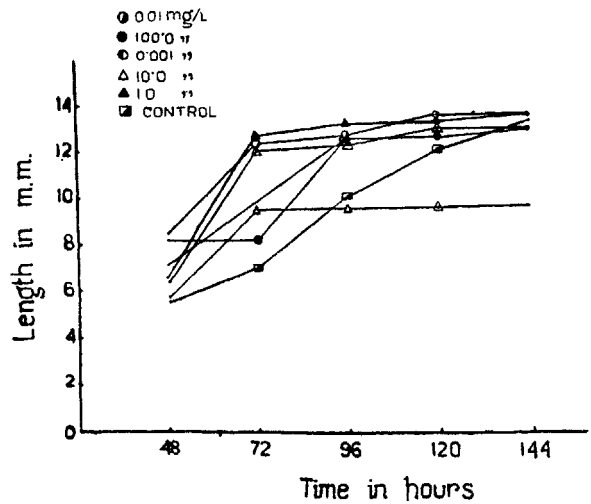


FIG. 2. Effects of different concentrations of thiamine on the coleoptile growth of excised embryos in the media containing sucrose and minerals.

of thiamine there is an increase in the growth rate up to the length of the coleoptile where it usually breaks. In the control the growth of the coleoptile is slow and it takes about 144 hours to reach this length. Coleoptile breaking and elongation of first leaf, however, did not occur up to 144 hours' growth in any of the treatments which would indicate that possibly some other factor responsible for elongation of leaf is lacking in the media. Thiamine seems to play an important rôle in the root growth of excised embryos. In its presence increase in root length takes place in almost all the concentrations. Best root growth is obtained in 0 01 mg./L concentration (Fig. 3) which appears to be the optimum for the culture of excised rice embryo.

It has been reported that rice grains contain large amount of pyridoxine (Schopfer, 1943). Its presence in the grain suggests that it may have some rôle in

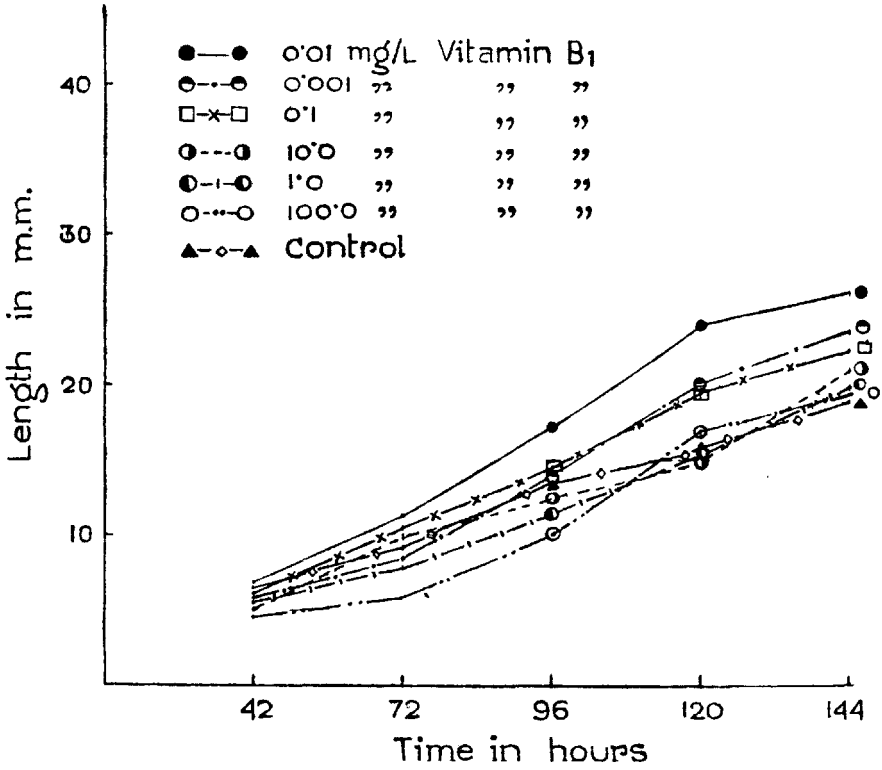


FIG. 3. Effects of different concentrations of thiamine on the root growth of excised embryos in the media of sucrose and minerals.

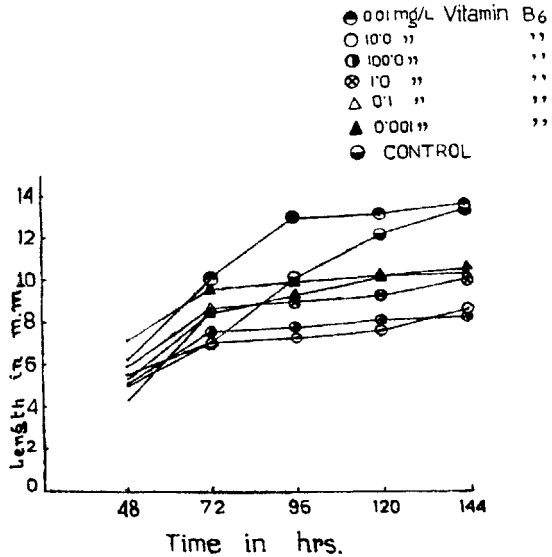


FIG. 4. Effects of different concentrations of pyridoxine on the coleoptile growth of excised embryos in the media of sucrose and minerals.

the metabolism of the growing embryo. In order to study its effect on the growth of excised rice embryo pyridoxine in combination with two per cent sucrose and minerals is used in the medium. Control set is prepared with two per cent sucrose and minerals only. Figs. 4 and 5 will show that 0 01 mg./L conc. of pyridoxine (Vitamin B_6)

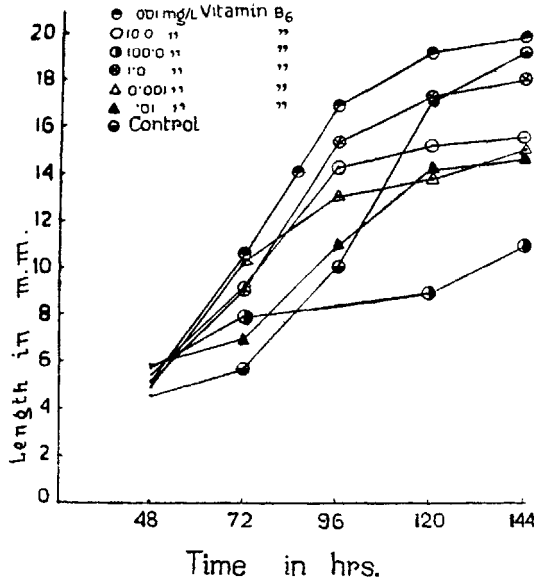


FIG. 5. Effect of pyridoxine on the root growth of excised embryos in the media containing sucrose and minerals.

increases the growth rate of root and of coleoptile at the initial stage while other concentrations do not seem to have any effect on shoot growth but root growth is slightly increased. It appears that concentrations of 10 mg./L or above have a depressing effect on root and shoot growth. In no case, however, coleoptile breaking or leaf elongation has been observed up to 144 hours.

In the next series of experiments the following combinations of growth factors have been used to study their effects on the excised embryos :—

1. Mineral nutrients + I.A.A. (0·01 mg./L) and two per cent sucrose
2. ,, ,, + I.A.A. (0·001 mg./L)
3. ,, ,, + thiamine (0·01 mg./L)
4. ,, ,, + I.A.A. (0·001 mg./L) + thiamine (0 01 mg./L) + pyridoxine (0·01 mg./L)
5. ,, ,, + I.A.A. (0 001 mg./L) + pyridoxine (0·01 mg./L) + nicotinic acid (0 01 mg./L)
6. ,, ,, + I.A.A. (0·001 mg./L) + pyridoxine (0·01 mg./L) + nicotinic acid (0·01 mg./L) + thiamine (0·01 mg./L)
7. ,, ,, + Dl-tryptophane (0 01 mg./L) + thiamine (0·01 mg./L) + pyridoxine (0 01 mg./L)
8. ,, ,, + Dl-tryptophane (0·01 mg./L) + thiamine (0·01 mg./L) + nicotinic acid (0·01 mg./L) + pyridoxine (0·01 mg./L)

A remarkable shoot growth has been observed when I.A.A. is present in the medium in combination with sucrose and minerals (treatments 1 and 2 in Fig. 6).

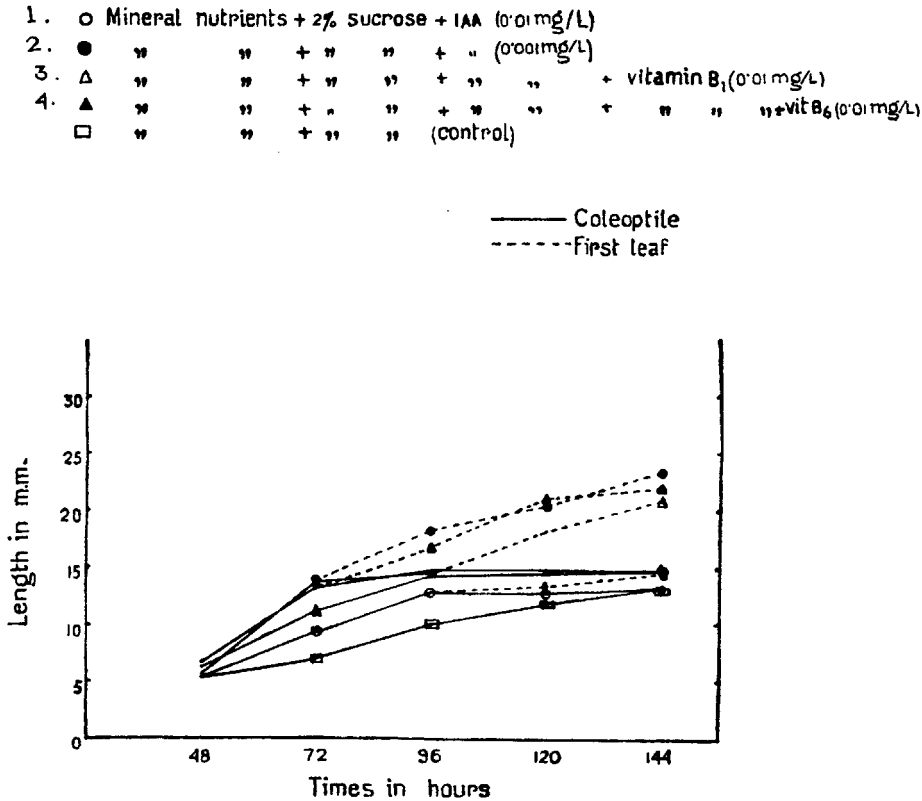


FIG. 6. Shoot growth of excised embryos in complex nutrient media.

The root growth is also accelerated on addition of I.A.A. at low concentrations (0·01 mg./L and 0·001 mg./L), growth with 0·001 mg./L being greater. Coleoptile breaking and elongation of first leaf are observed within 120 hours in treatment 2. When thiamine, pyridoxine and I.A.A. are present in the medium the embryo growth is very much favoured (treatment 4 in Figs. 6 and 7). Here root length is much greater in comparison to previous treatments. The presence of thiamine and pyridoxine together greatly favours root growth than when they are present alone. The coleoptile breaking and the elongation of first leaf is observed within 96 hours. Nicotinic acid when added with I.A.A. and pyridoxine does not show any marked improvement in growth although root and shoot development is satisfactory. Addition of thiamine in this combination does not accelerate embryo growth (treatments 5 and 6 in Figs. 8 and 9). When Dl-tryptophane in combination with thiamine, pyridoxine, sucrose and minerals is used as a substitute for I.A.A. growth of both root and shoot development is accelerated (treatment 7 in Figs. 8 and 9 and Fig. 10). In addition of nicotinic acid to these factors the roots show somewhat reduced growth while the shoot development is not affected (treatment 8 in Figs. 8 and 9). It thus appears that whenever I.A.A. or Dl-tryptophane is present in the medium the shoot development, i.e. coleoptile breaking and appearance of first leaf, is accelerated otherwise the coleoptile shows very limited growth.

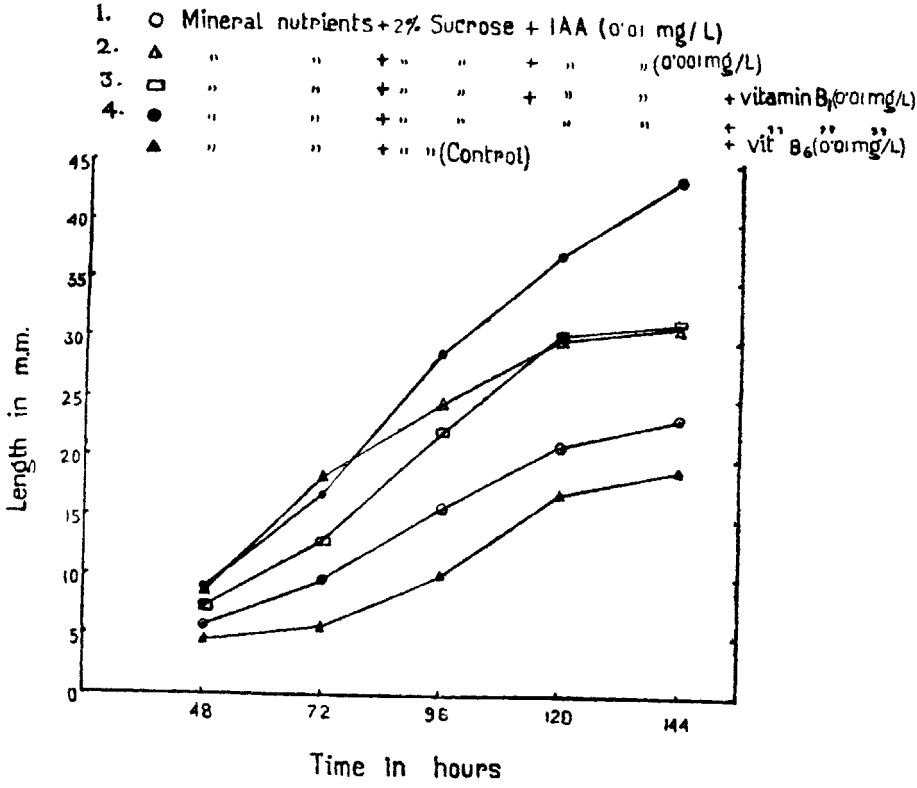


FIG. 7. Root growth of excised embryos in complex nutrient media.

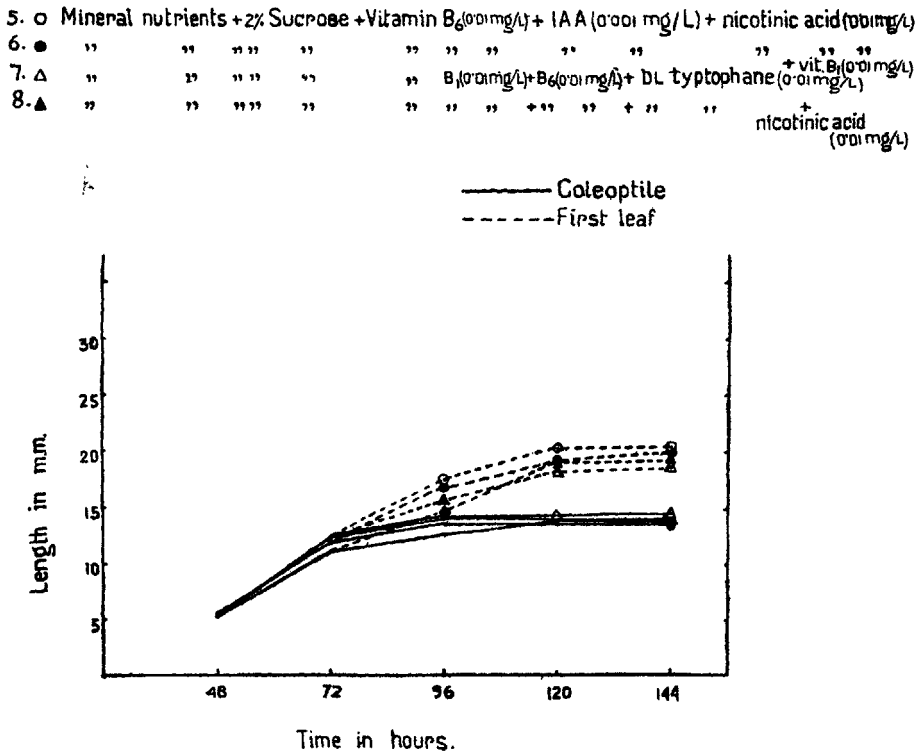


FIG. 8. Shoot growth of excised embryos in complex nutrient media.

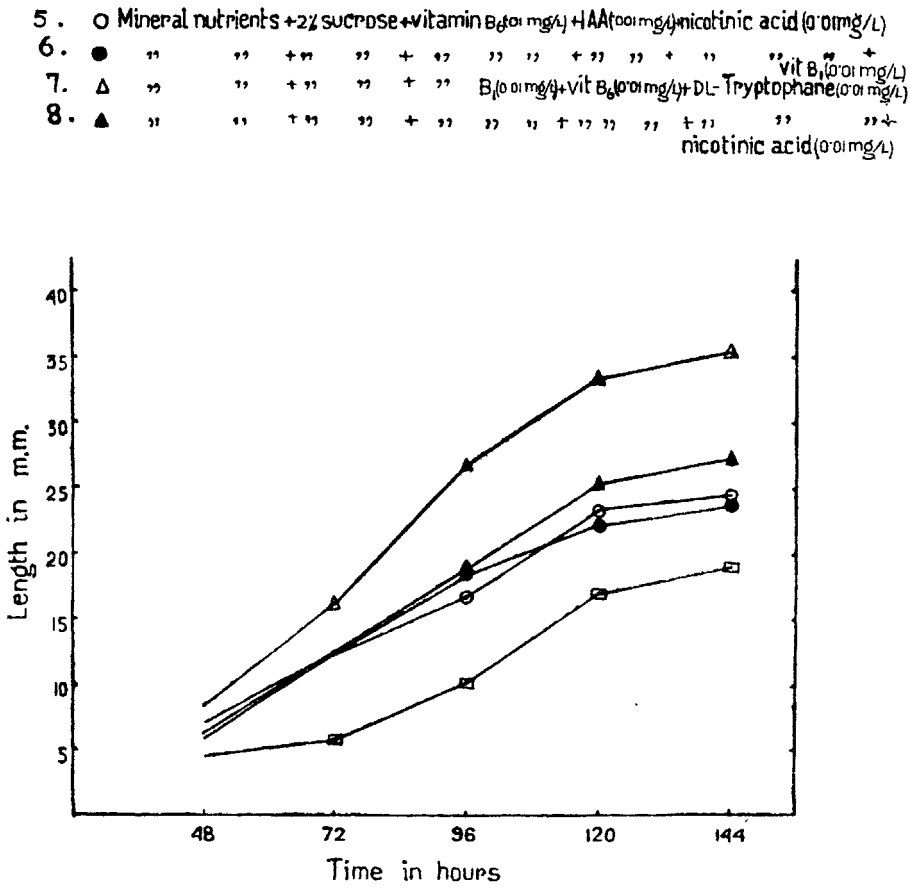


FIG. 9. Root growth of excised embryos in complex nutrient media.

DISCUSSION

This work shows acceleration of root growth of embryos when a portion of endosperm is eliminated. When more than half is removed such an effect is not found there. That this increased growth due to fractioning of endosperm is not caused by wound is clear from Table I. When very little endosperm adheres to the embryo (i.e. one-eighth endosperm) there is a fall in growth rate due to the lack of nutritive substances. Hypothesis advanced to account for this is the presence of supra-optimal auxin or some other unknown factor present in the endosperm. Hence, elimination of a portion of endosperm makes the level of auxin or the unknown factor optimal for growth until such elimination reaches a limiting value (Sircar, Das and Lahiri, 1955). In order to study the nature of inhibitory factor of endosperm rice embryos with different fractions of endosperm were grown on sterile agar media containing endosperm extracts. In all cases addition of endosperm extracts results in diminished root growth in comparison with control without added extract. This accelerated root growth due to the elimination of endosperm fraction is suppressed when endosperm extract is added to the media. Thus the root length with three-fourth fractions of endosperm and added extract becomes almost equal to that of the full endosperm growing in the medium without the extract.

It may be observed, again, that increased root growth by the fractioning of endosperm (i.e. half endosperm) is retarded when it is grown in the medium containing I.A.A. and it becomes almost equal to the embryo growing in agar medium (Fig. 1). The inhibition of root length caused by the presence of I.A.A. in the medium may be compared with the inhibitory factor of the endosperm which suppresses the root growth in the early stages of germination. On the basis of these findings attempts have been made to investigate the essential growth factors necessary for the embryo in the initial stages of germination. For this purpose excised embryo culture is adopted. It has been noted before that excised embryos in general show less growth in comparison with those attached to seeds. The diminished growth might be due to the following reasons:—

(*a*) Mechanical injury during excision.
(*b*) Limited auxin supply.
(*c*) Lack of growth factors other than auxins, possibly of vitamin nature.

The effects of injury are of primary consideration in the work of embryo culture. The rice embryos fail to germinate when they are completely excised from endosperm and are placed in the media containing sugar, vitamins, and I.A.A. or endosperm extract. This is presumably due to the damage of the epithelial layer. In the present study the effect of injury has been eliminated by the adoption of a special technique of excision. By this method the epithelial layer is not injured, hence translocation of food substances from the media to the embryo is maintained. Sucrose has been proved to be the best form of carbohydrate for culturing tissues and embryos of several plants. In the post-germinal embryo culture of rice it has also been noted that the embryo growth corresponds directly with the increasing concentrations of sucrose up to two per cent level. With minerals only the embryos show very limited growth but with the addition of sucrose in the medium better results are obtained. The plants, however, are small in comparison to those that had intact endosperm. In the excised embryo culture of rice thiamine and pyridoxine have been found to be helpful for the root and shoot growth. They are known to be essential for the growth of roots. Excised roots of the majority of plants investigated are unable to synthesise them. Bonner and Bonner (1948) have shown that green leaves can synthesise thiamine and pyridoxine and these are translocated to roots. It is not known whether coleoptile tip can synthesise thiamine and pyridoxine. In order to prove conclusively that a substance is an essential factor it should be shown by experiment that not only growth is stimulated by the substance but no growth takes place in its absence. Since growth in the excised embryo of rice did not stop in absence of added thiamine or pyridoxine, rather these embryos were found to show increasing growth during the period of experimentation and there was stimulation after addition of these factors, it leads one to consider the importance of thiamine and pyridoxine for the growth of rice embryo. There may be two possibilities, either the coleoptile and root tips might have carried them from the endosperm during the period of residual germination or the tips are capable of synthesising them from sucrose and nutrients.

Presence of I.A.A. facilitates the shoot development to a great extent while root growth is found to be more dependent on growth factors of vitamin nature. When I.A.A. is added to the medium in combination with thiamine, pyridoxine, sucrose and minerals the optimal growth of embryo results. Tryptophane facilitates the embryo growth. The capacity of plant tissue to form auxin from tryptophane has been demonstrated by a number of workers. Such conversion probably takes place in the rice embryos in the media containing tryptophane.

It should be noted, however, that in all the different media that have been used in the present study the excised embryos show less growth in comparison to that obtained with the intact endosperm. Presumably some other unknown growth factor or factors are present which have not been included in the present investigation.

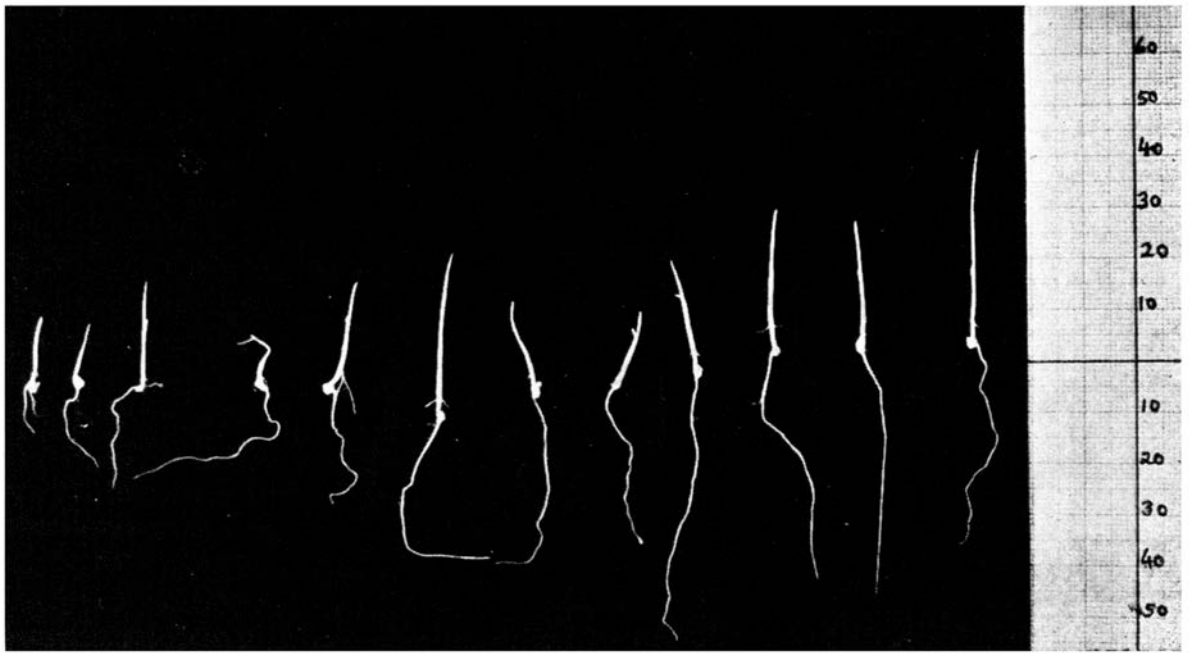


FIG. 10. Photograph showing the growth and coleoptile breaking of excised embryos at different stages in the media containing 0·01 mg./L thiamine and 0·01 mg./L pyridoxine and DI-tryptophane media. In all cases thin root system, absence of visible root hairs and delicate growth of embryos are remarkable.

Summary

Experiments on the germination of rice embryo have been carried out by eliminating fractions of endosperm and substituting endosperm extract and indolyl acetic acid.

Some factor or factors are present in the endosperm in supra-optimal concentration which exerts a retarding effect on the embryo growth in the initial stages of germination. Elimination of a fraction of endosperm makes the level of the factor optimal for embryo growth until such elimination reaches a limiting value. That this unknown factor is of auxin in nature has been shown from substitution of endosperm extract and I.A.A. in the culture media.

Attempts to culture rice embryo have shown that it fails to germinate when completely excised from the endosperm and placed in nutrient media. This was due to the damage of the epithelial layer during excision and could be eliminated by adopting a special technique.

Culture of excised embryo has shown its growth is dependent on endosperm food factors. Some of these factors are sucrose, salts, I.A.A. and vitamins B_1 and B_6. Dl-tryptophane acts as a substitute for I.A.A. for the growth of embryo showing its conversion to I.A.A. in the embryo cells.

Acknowledgements

The authors express their thanks to Dr. I. Banerjee, F.N.I., Head of the Department of Botany, for facilities offered in this investigation and to Dr. T. M. Das for many valuable suggestions and unreserved help.

References

Avery, G. S. Jr., Creighton, H. B., and Shalucha, B. (1940) Extraction methods in relation to hormone content of maize endosperms. *Amer. Jour. Bot.*, **27**, 289–300.

Avery, G. S. Jr., Berger, J., and Shalucha, B. (1942). Auxin content of maize kernels during ontogeny from plants of varying heterotic vigor. *Amer. Jour. Bot.*, **29**, 765–772.

Bonner, J., and Bonner, H. (1948). The B vitamins as plant hormones; vitamins and hormones. Academic Proc. Inc. Publishers, New York, U.S.A., **5**, 225–275.

Das, T. M. (1954). The relation of auxin to the extension growth of the stem in cereals. Ph.D. Thesis, University of London.

Funke, H., and Soding, H. (1948). *Planta*, **35**, 341–370. Cited by P. Larsen in *Ann. Rev. of Plant Physiology*, **2**, 169–198, 1951.

Guttenberg, H. V., and Lehle-Joesges, E. (1947). *Planta*, **35**, 281–296. Cited by P. Larsen in *Ann. Rev. of Plant Physiology*, **2**, 169–198, 1951.

Hatcher, E. S. J. (1945). Studies in the vernalisation of cereals. IX. Auxin production during development and ripening of the anther and carpel of spring and winter rye. *Ann. Bot. N.S.*, **9**, 235–266.

Larsen, P. (1951). Formation, occurrence and inactivation of growth substances. *Ann. Rev. Plant Physiology*. Ann. Rev. Inc. Stanford, California, U.S.A., **2**, 169–198.

Popoff, A. (1941). *Augew Botan.*, **23**, 254–285. Cited by P. Larsen in *Ann. Rev. of Plant Physiology*, **2**, 169–198, 1951.

Schopfer, W. H. (1943). Plants and Vitamins. Chronica Botanica Co., Waltham., Mass., U.S.A.

Sircar, S. M., and Das, T. M. (1954). Studies on the physiology of rice. IX. Auxin content of the vernalized seed. *Proc. Nat. Int. Sci. Ind.*, **20**, 673–682.

Sircar, S. M., Das, T. M., and Lahiri, A. N. (1955). Germination of rice embryo under water and its relation of growth to endosperm fractions. *Nature*, **175**, 1045–1047.

Issued July 10, 1957.