BIOLOGIA PLANTARUM (PRAHA)

22 (6) : 414—419, 1980

Proteolytic Activity and Nitrogen Transfer in Maize Seeds during Imbibition

B. BOSE and H. S. SRIVASTAVA**

Department of Botany, University of Gorakhpur*

Abstract. Ethanol-soluble and insoluble nitrogen and protease activity in maize seeds during imbibition period of 6 to 50 h at 30 ± 2 °C were determined both in light and in the dark. In light, soluble and insoluble nitrogen in the embryo were similar to that in the dark. But the increase in soluble nitrogen in the endosperm up to 38 h was higher in light than in the dark. Decrease in insoluble nitrogen was correlated with increase in soluble nitrogen, the level always being higher in the dark. Light increased protease activity also in the endosperm. Among various light qualities, red light was most effective in inducing proteolysis, and loss of nitrogen from the endosperm. Further, the growth and organic nitrogen of primary leaves from seedlings raised from light pretreated seeds were better than those from dark pretreated ones.

The effect of light on seed germination is well documented, varying from promotory to inhibitory or to no effect in some cases (TOOLE *et al.* 1956, MAYER and POLJAKOFF-MAYBER 1963, KOLLER *et al.* 1962). During germination, the storage products of the cotyledons or endosperm are hydrolyzed and then transferred to the embryonic axis involving activation and synthesis of various molecules (BEEVERS and GUERSNEY 1966, BEEVERS and SPLITTSTOESSER 1968, ABDUL-BAKI 1969, CHING 1972). Light as an effector of seed germination may modify one or more biochemical reactions during germination. In fact KOLLER *et al.* (1962) have reported that red light effective in lettuce seed causes an increase in the soluble nitrogen of the cotyledons. Increase in the soluble nitrogen and protease activity by light during germination, has been reported in *Cucurbita maxima* (WILLEY and ASHTON 1967) and in rice grain (PALMIANO and JULIANO 1972).

For germination of mature seeds light does not seem to be necessary. On the other hand, there is slight promotion of the rate of germination in the dark (SRIVASTAVA 1976a). In order to study the mechanism of the effect of light on seed germination, the hydrolysis of endosperm proteins and the transfer of hydrolysate to the embryo were examined in light and in the dark.

Received October 18, 1979; accepted January 15, 1980

* *Address:* Gorakhpur 273001, India.

** *Present address:* Department of Life Sciences, Vigyan Bhawan, Khandwa Road, Indore, 452001, India.

MATERIAL AND METHODS

Seed and Seed Germination

Seeds of *Zea mays* L. cv. Ganga-2 used in the present investigation were purchased from a local dealer. After sterilizing them with a dilute solution of bleaching powder for a brief period, they were washed thoroughly and allowed to imbibe distilled water for 6 h either in light or in the dark. They

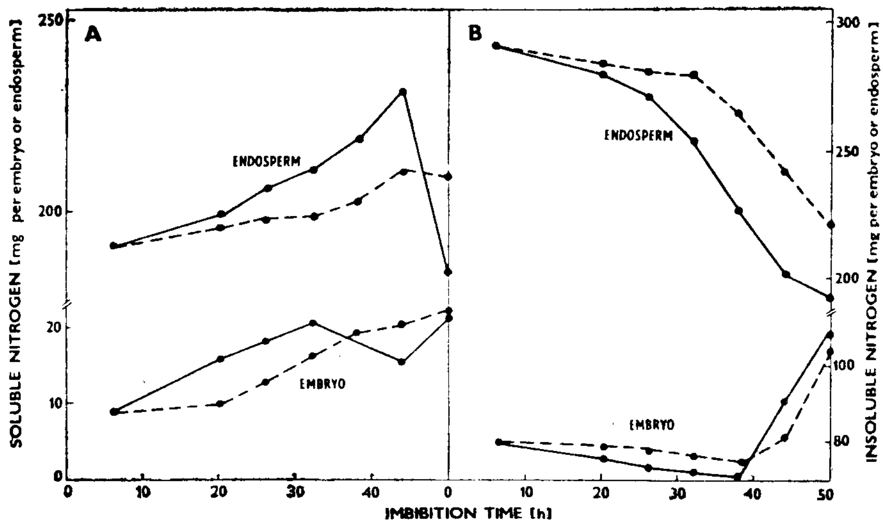

Fig. 1. Changes in ethanol-soluble (A) and ethanol-insoluble (B) nitrogen of the maize seeds during imbibition. Continuous lines — light; dashed lines — dark.

were then planted on filter paper circles (moistened with 10 mM $CaCl_2$, 2 H_2O) contained in large Petri dishes and incubated either in light (2 klx) or in the dark at 28 to 32 °C for the desired period. Light of desired quality was obtained through the use of coloured cellophane. Endosperm and embryo were dissected out aseptically for analytical analysis. Scutellum was included in the embryo portion.

Measurement of Growth

Dry weight of leaves was taken as an index of growth. Leaves from uniformly grown seedlings were collected and dried in an oven at 70 °C for 24 h.

Nitrogen Determination

Ethanol-soluble and insoluble nitrogen in oven dried samples of endosperm and embryo were measured according to a modified micro-Kjeldahl method (Lang 1958).

Nitrate Reductase Activity

In vivo activity of nitrate reductase in the leaves was assayed by a procedure described earlier (Srivastava 1974).

TABLE 1

Effect of different light qualities on ethanol-soluble and insoluble nitrogen in embryo and endosperm and protease activity in the endosperm of maize seeds

Light condition	Nitrogen [mg/embryo]		Nitrogen [mg/endosperm]		Protease activity
	Soluble N	Insoluble N	Soluble N	Insoluble N	mg protein released h^{-1} per endosperm
Dark	0.22	1.03	2.09	2.19	2.42
White light	0.21	1.07	1.83	1.91	3.96
Red-light	0.30	1.10	2.16	1.64	4.96
Yellow-light	0.29	1.09	2.11	1.73	4.34
Green-light	0.24	1.05	2.10	1.97	3.35
Blue-light	0.23	1.03	2.10	2.10	2.73

Nitrogen in the endosperm and embryo were measured after 50 h of imbibition under various light conditions while protease activity was measured after 75 h.

Protease Activity

Protease activity in the endosperm was assayed and extracted according to a slight modification of the method of HARVEY and OAKS (1974a, b) using bovine serum albumin (Sigma chemical Co., U.S.A.) as substrate. Residual protein after the activity of protease was precipitated out with 20% TCA. The washed precipitate was digested with conc. H_2SO_4 and protein nitrogen was determined by Nesslerisation (LANG 1958). Protein was calculated by multiplying the nitrogen value with a factor of 6.25. The data expressed in this paper are average values from three repeated experiments.

RESULTS

Effect of Light on Transfer of Nitrogen from Endosperm to the Embryo

Ethanol-soluble nitrogen of the embryo increased gradually both in light and in the dark at almost similar rates (Fig. 1A). Ethanol-soluble nitrogen of the endosperm also increased gradually both in light and in the dark. But in light it was appreciably higher between 26 and 44 h. Trends in changes in ethanol insoluble nitrogen of endosperm and embryo were almost opposite to the soluble nitrogen (Fig. 1B). There was a gradual decline in endosperm nitrogen, again the rate being higher in light than in the dark. Ethanol insoluble nitrogen in the embryo declined slowly up to 38 h and then it increased. Statistically, the differences between dark and light treated nitrogen of endosperm (both soluble and insoluble) were significant at $P = 0.05$.

Effect of Light Quality on Nitrogenous Components and Protease Activity

As shown in Table 1 significant increase (over dark) in soluble nitrogen was observed in red and yellow light. Correspondingly, protein nitrogen of the endosperm was higher in red and yellow light. On the basis of nitrogen solubilisation and its loss from the endosperm, the effect of different light

TABLE 2
Growth, nitrate reductase activity and nitrogen content of primary leaves from 8-day maize plants as affected by a pretreatment of seeds with light or dark

Parameters	Pretreatment condition	
	Light	Dark
Fresh weight [mg/leaf]	141.75	129.09
Dry weight [mg/leaf]	16.40	15.22
Nitrate reductase activity [nmol NO_2 h^{-1} g^{-1} f.w.]	948.00	660.00
Ethanol-soluble N [mg g^{-1} dry weight]	11.26	10.27
Ethanol insoluble N [mg g^{-1} dry weight]	19.95	18.81

Seeds were imbibed in distilled water either in light or in dark. Then they were planted in pots and seedlings were raised for 8 days in the garden at an average day and night temperature of 32 °C and 28 °C, respectively. Photoperiod was about 12 h.

qualities can be expressed in the order: Red > yellow > white > green > blue > dark. A similar effect was observed on protease activity, too. It was highest in the endosperm of the seeds germinated in red light and lowest in those from dark germinated seeds.

Growth and Nitrogen Content of Primary Leaves as Affected by Pretreatment of Seeds with Light or Dark

It was observed that in the leaves of the plants from light pretreated seeds all the parameters studied *i.e.* fresh weight, dry weight, nitrate reductase activity, soluble nitrogen and insoluble nitrogen were higher than in the leaves of dark pretreated seeds (Table 2).

DISCUSSION

The experiments described here demonstrate that light is a promotive factor for the internal changes involved in the solubilisation and transfer of nitrogen from endosperm to embryo during imbibition. Changes in nitrogenous components during the imbibition period, which are indicative of changes in other molecules also, have been reported by several other authors (DURE 1960, INGLE *et al.* 1964, SIMON and MEANY 1965, LAWRENCE and

TABLE 3
Effect of light on transfer of nitrogen from endosperm to the embryo between 6 and 50 h of imbibition

	Total nitrogen [mg/part]	
	Dark	Light
Total loss from endosperm	0.533	1.073
Total gain in embryo	0.362	0.392
Leaching in the medium	0.171	0.681

JANET 1969, SRIVASTAVA 1976b). From these studies and also from our own studies with nitrogen changes in maize seedlings (SRIVASTAVA 1976b, SRIVASTAVA *et al.* 1976), three phases of nitrogen changes during seed germination may be recognized: (i) Internal redistribution of nitrogen in the embryo; (ii) hydrolysis of protein in the endosperm; (iii) transfer of soluble nitrogen from endosperm to embryo. In the present investigation, germination of seeds after 50 h of imbibition was about 10 to 15% lower in light than that in the dark. Further, the effect of light on nitrogenous components of the embryo was less pronounced than that on endosperm (Fig. 1A, B). Since germination of maize seeds appears to be independent of the changes in the endosperm (SRIVASTAVA *et al.* 1976), any effect of light on the phases (ii) and (iii) of germination may not be correlated to its effects on seed germination. The observation that light induces solubilisation and transfer of nitrogen from endosperm (Fig. 1A, B; Table 3) but inhibits seed germination is consistent which this statement. It is also apparent that most of the light accelerated transfer of nitrogen from endosperm is not gained in the embryo (Table 3).

Light increased the activity of protease also. The increase in protease by light has been observed earlier in *Cucurbita maxima* (WILLEY and ASHTON 1967) and rice seedlings (PALMIANO and JULIANO 1972). The increase in protease may be directly responsible for the increase in soluble nitrogen in the endosperm.

Red-light appeared to be more effective in increasing soluble nitrogen and protease activity. Effectiveness of red-light in promoting seed germination of many photoblastic seeds has been demonstrated (DOWNS 1955, JONES and BAILEY 1956, WASSINK and STOLWIJK 1956, CUNNING 1963). These effects of red-light may be mediated *via* phytochrome as reported by MANCINELLI *et al.* (1967), MANCINELLI and TOLKOVSKÝ (1968), and HOLDSWORTH (1972). The inducing effect of light on endosperm proteolysis and transfer of nitrogen appears to be carried over to the further growth of the seedling. Hence leaves obtained from the seedlings raised from light pretreated seeds had better growth, nitrate reductase activity and organic nitrogen than those from dark pretreated ones.

Acknowledgement

One of us (BB) is grateful to the C.S.I.R., New Delhi for financial assistance. The authors wish to thank Prof. S. N. Mathur for laboratory facilities.

REFERENCES

ABDUL-BAKI, A. A.: Metabolism of barley seeds during early hour of germination. — Plant Physiol. 44 : 733—738, 1969.

BEEVERS, L., GUERSNEY, F. S.: Changes in some nitrogenous compounds during the germination of pea seeds. — Plant Physiol. 41 : 1455—1458, 1966.

BEEVERS, L., SPLITTSTOESSER, E. W.: Protein and nucleic acid metabolism in germinating peas. — J. exp. Bot. 19 : 698—711, 1968.

CHING, T. M.: Metabolism of germinating seeds. — In: KOZLOWSKI, T. T. (ed.): Seed Biology. Pp. 156—180. Academic Press, New York 1972.

CUNNING, B. G.: The dependence of germination on photoperiod, light quality and temperature in *Chenopodium* spp. — Can. J. Bot. 41 : 1211—1218, 1963.

DOWNS, R. J.: Photoreversibility of leaf and hypocotyl elongation of dark grown red kidney bean seedlings. — Plant Physiol. 30 : 468—473, 1955.

DUBE, L. S.: Gross nutritional contribution of maize endosperm and scutellum to germination growth of maize axis. — Plant Physiol. 35 : 919—925, 1960.

HARVEY, B. M. R., OAKS, A.: Characteristics of an acid protease from maize endosperm. — Plant Physiol. **53** : 449—452, 1974a.

HARVEY, B. M. R., OAKS, A.: The hydrolysis of endosperm protein in *Zea mays* L. — Plant Physiol. **53** : 453—457, 1974b.

HOLDSWORTH, M.: Phytochrome and seed germination. — New Phytol. **71** : 105—118, 1972.

INGLE, J., BEEVERS, L., HAGEMAN, R. H.: Metabolic changes associated with the germination of corn I. Changes in weight and metabolites and their redistribution in the embryo axis, scutellum and endosperm. — Plant Physiol. **39** : 735—740, 1964.

JONES, M. B., BAILEY, F. L.: Light effects on the germination of seeds of henbit. — Plant Physiol. **31** : 347—349, 1956.

KOLLER, D., MAYER, A. M., POLJAKOFF-MAYBER, A., KLEIN, S.: Seed germination. — Annu. Rev. Plant Physiol. **13** : 437—465, 1962.

LANG, C. A.: Simple micro-determination of Kjeldahl nitrogen in biological materials. — Anal. Chem. **30** : 1692—1694, 1958.

LAWRENCE, K. M., JANET, E. S.: Nitrogen mobilisation in pea seedlings. — Plant Physiol. **34** : 668—674, 1969.

MANCINELLI, A. L., TOLKOWSKY, A.: Phytochrome and seed germination V. Changes of phytochrome content during the germination of cucumber seeds. — Plant Physiol. **43** : 489—494, 1968.

MANCINELLI, A. L., YANIV, Z., SMITH, P.: Phytochrome and seed germination I. Temperature dependence and relative P_{FR} levels in the germination of dark - germinating tomato seeds. — Plant Physiol. **42** : 333—337, 1967.

MAYER, A. M., POLJAKOFF-MAYBER, A.: The Germination of Seeds. — Pergamon Press, Oxford 1963.

PALMIANO, E. P., JULIANO, B. O.: Biochemical changes in the rice grain during germination. — Plant Physiol. **49** : 751—756, 1972.

SIMON, E. W., MEANY, A.: Utilisation of reserves in germinating *Phaseolus* seeds. — Plant Physiol. **40** : 1136—1139, 1965.

SRIVASTAVA, H. S.: *In vivo* activity of nitrate reductase in maize seedlings. — Indian J. Biochem. Biophys. **11** : 230—232, 1974.

SRIVASTAVA, H. S.: Inhibition of maize seedling growth by chloramphenicol. — Indian J. Plant Physiol. **19** : 53—59, 1976a.

SRIVASTAVA, H. S.: Some aspects of nitrate assimilation in normal and opaque-2 mutant of maize. — J. exp. Bot. **27** : 373—380, 1976b.

SRIVASTAVA, H. S., OAKS, A., BAKYTA, I. L.: The effect of nitrate on early seedling growth in *Zea mays* L. — Can. J. Bot. **54** : 923—929, 1976.

TOOLE, E. H., HENDRICKS, S. B., BORTHWICK, H. A., TOOLE, V. K.: Physiology of seed germination. — Annu. Rev. Plant Physiol. **7** : 299—325, 1956.

WASSINK, E. C., STOLWIJK, J. A. J.: Effect of light quality on plant growth. — Annu. Rev. Plant Physiol. **7** : 373—400, 1956.

WILLEY, L., ASHTON, F. M.: Influence of embryonic axis on protein hydrolysis in cotyledons of *Cucurbita maxima*. — Physiol. Plant. **20** : 688—696, 1967.