STUDIES IN PHYSIOLOGY OF SEED GERMINATION

II. Seedling Growth, Carbohydrate, Protein Metabolism and Amylase Activity Under Different Light Conditions

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INTRODUCTION

RECENTLY a few workers have reported metabolic changes during germination (Ingle *et al.*, 1964; Beevers and Guernsey, 1966; Rai and Laloraya, 1965, 1967 and Banerjee and Laloraya, 1967). The present study was undertaken to elucidate the effect of different light treatments and exogenous application of ascorbic acid and sucrose upon seedling growth, carbohydrate and protein metabolism as well as amylase activity.

MATERIAL AND METHODS

Seeds of *Triticum aestivum* L. Var. N.P. 718 were incubated in sterilized Petri dishes, lined with Whatman No. 1 filter-paper moistened with distilled water, ascorbic acid (25 ppm) and sucrose (1%) separately under similar conditions as reported earlier (Abraham *et al.* (1970).

Samples were taken at different stages of germination separately for embryo axis and endosperm and the estimations in triplicate were carried out for total sugars, starch, protein content, seedling dry weight and amylase activity. Sugar, starch and protein contents are expressed as per cent dry weight: seedling growth in terms of mean dry weight (mg/100 seedlings) and amylase activity as mg. starch digested per hr. per gm. fr. wt. Sugars were determined according to the method of Weinmann (1947) and the method of Chinoy (1939) was employed for strach content and amylase activity. Seedling

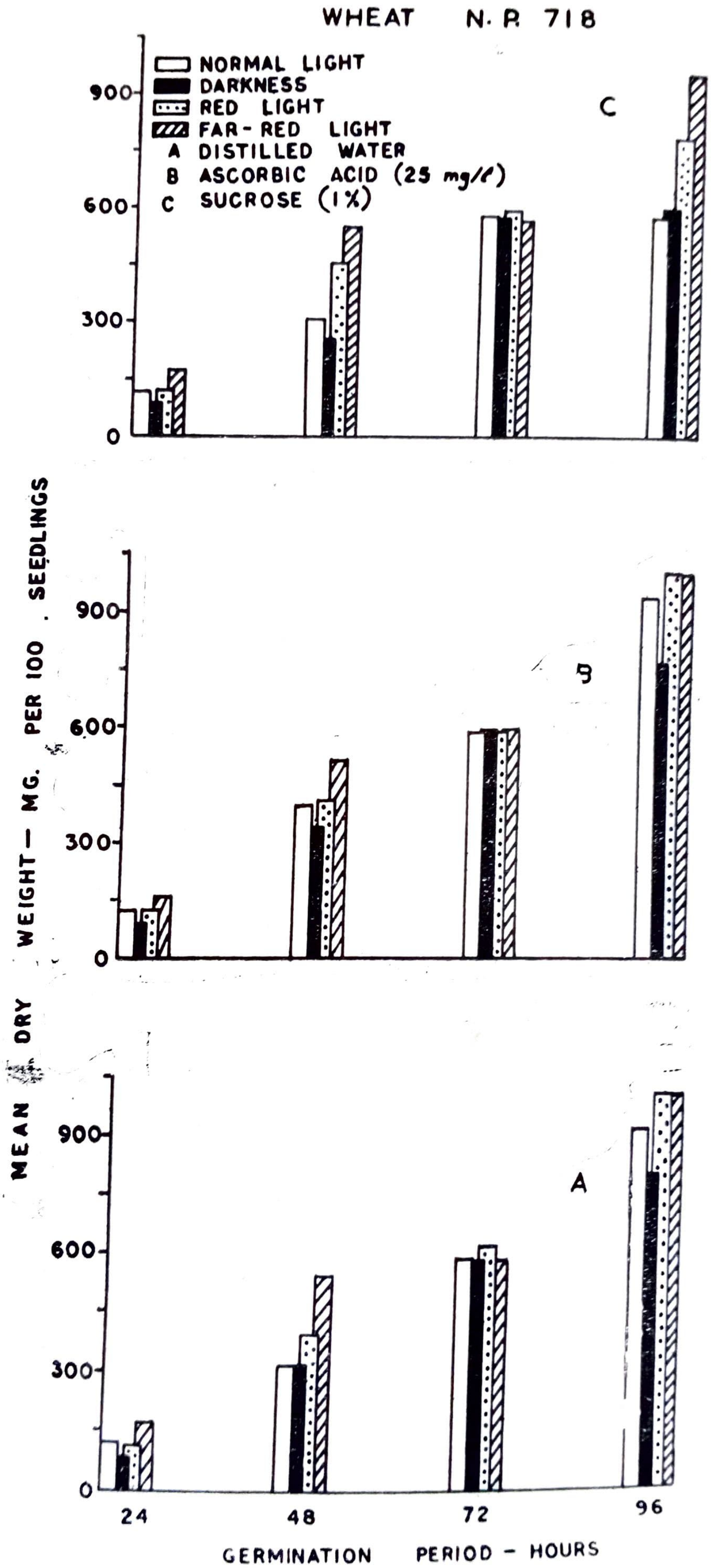

FIG. 1 Seedling growth (mean dry wt. mg/100 seedlings) under different lights and substrates.

growth was determined according to the method of Nanda *et al.* (1959). Protein content was calculated by multiplying the nitrogen content obtained by using the microkjeldahl method (Doneen, 1932) by a factor 5·7 (A.O.A.C. methods, 1955). The data were analysed statistically using Fisher's method of analysis of variance (1954).

OBSERVATIONS

Seedling growth.—There is progressive increase in dry weight with the advance of germination (Fig. 1). The enhancing effect of red (R) and far-red (FR) was more pronounced than that of dark (D) after 48 and 96 hours with all the substrates. Mean dry weight is the highest with ascorbic acid (AA) and the lowest with sucrose. However, the interaction of sucrose with R and FR lights shows better seedling growth as compared to that in normal day light (ND) and dark.

Total sugar and starch in the embryo.—There is no significant difference in total sugar content under different treatments at Stage I. Seedling grown under ND in AA show highest sugar content. The content in sucrose grown seedlings is slightly lower under R and FR than in dark and normal light. The sugar content increases as germination advances. R and FR increase sugar content of seedlings grown in all the three substrates upto Stages II and III as compared to ND and D. During the subsequent stages the content in dark and normal light are higher in all the three substrates. The highest values are obtained in sucrose grown seedling under FR light (Fig. 2).

Light treatments are significant at 5% level. The differences between mean sugar content of seedlings grown under R and FR as well as D, are significant. F values for growth stages and substrates are highly significant. There is significant increase in the mean content of total sugar at Stages III, IV and V as compared to Stage I. The mean values recorded for AA and sucrose substrates are higher than those of DW. However, the difference in case of AA substrate is not significant.

Starch was absent in case of embryo throughout the experimental period.

Protein content of the embryo.—Protein content of the embryo axis is at a higher level at Stages I and II followed by a gradual decrease (Fig. 4). At Stage I, highest value is recorded under R with AA and sucrose media, followed by FR and ND respectively. At the Stage II, a similar trend is obtained in sets of all the three substrates excepting that lowest values are recorded under ND with AA and sucrose as media. At the Stage III and onwards the values for light treatments or substrates do not differ much excepting that a slightly higher value is recorded for R set with sucrose as the substrate at Stage IV. Values for the D are relatively lower in comparison to those in

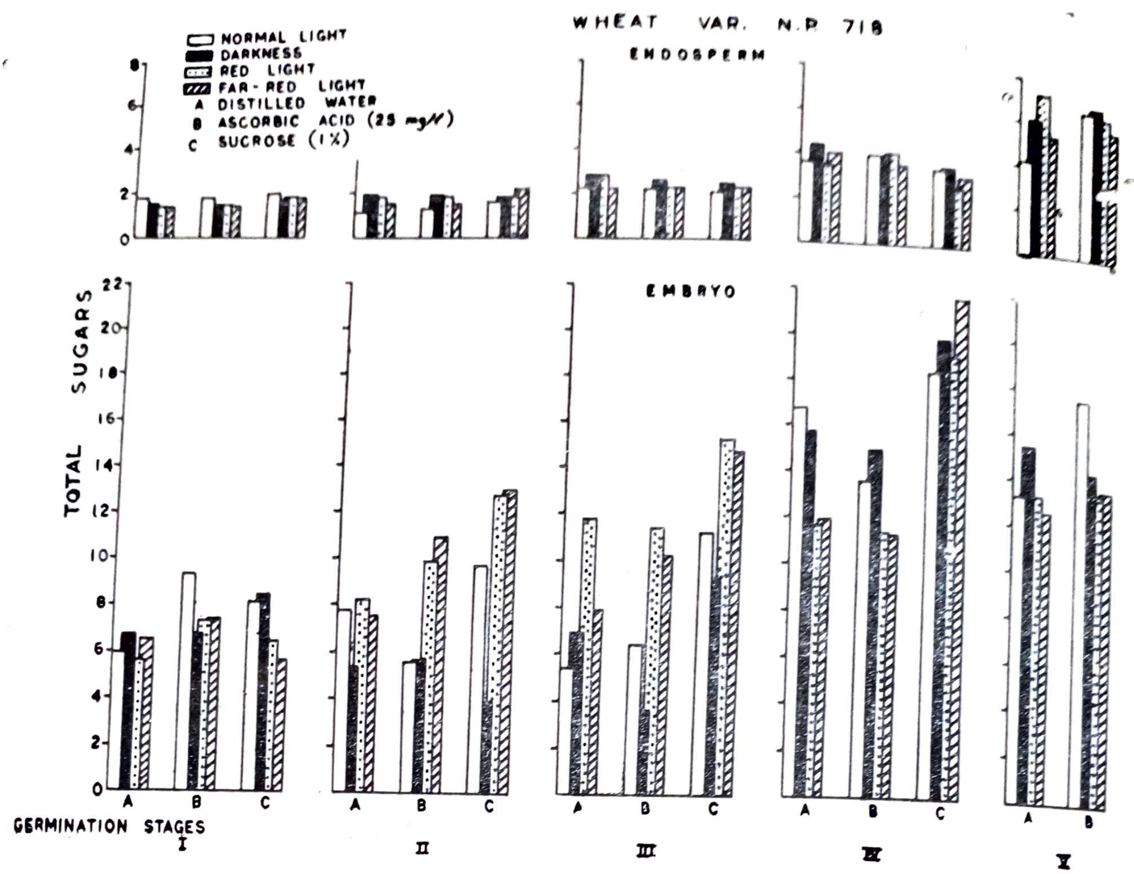

FIG. 2. Effect of different light treatments, substrates and growth stages on total sugar content in embryo axis and endosperm.

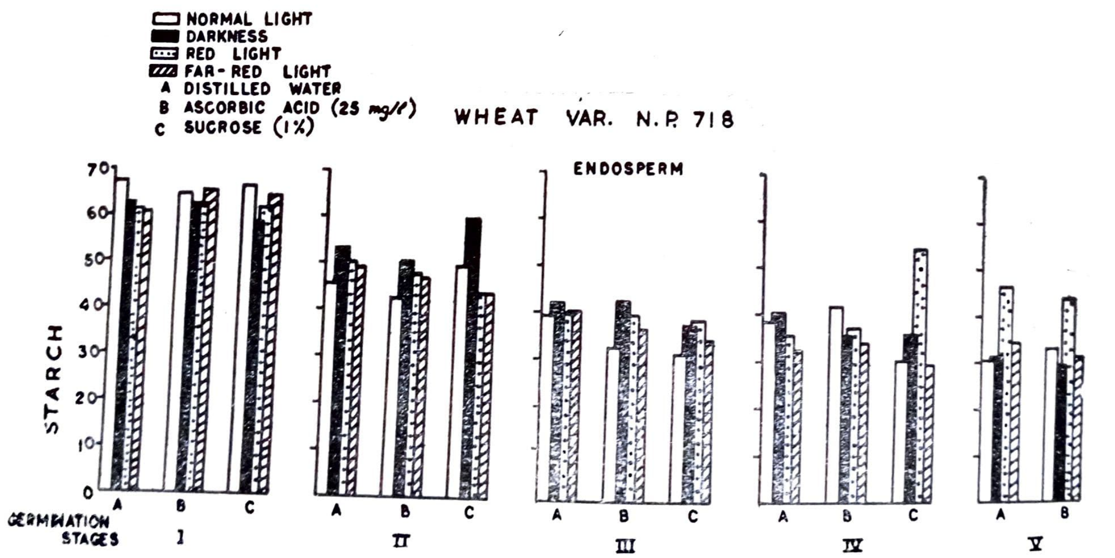

FIG. 3 Effect of different light treatments, substrates and growth stages on starch content in endosperm.

other light treatments for all the stages of germination as well as all the substrates. There is, however, an exception in seedlings of Stage II grown in D with AA, sucrose as substrates are better than the corresponding ones under ND.

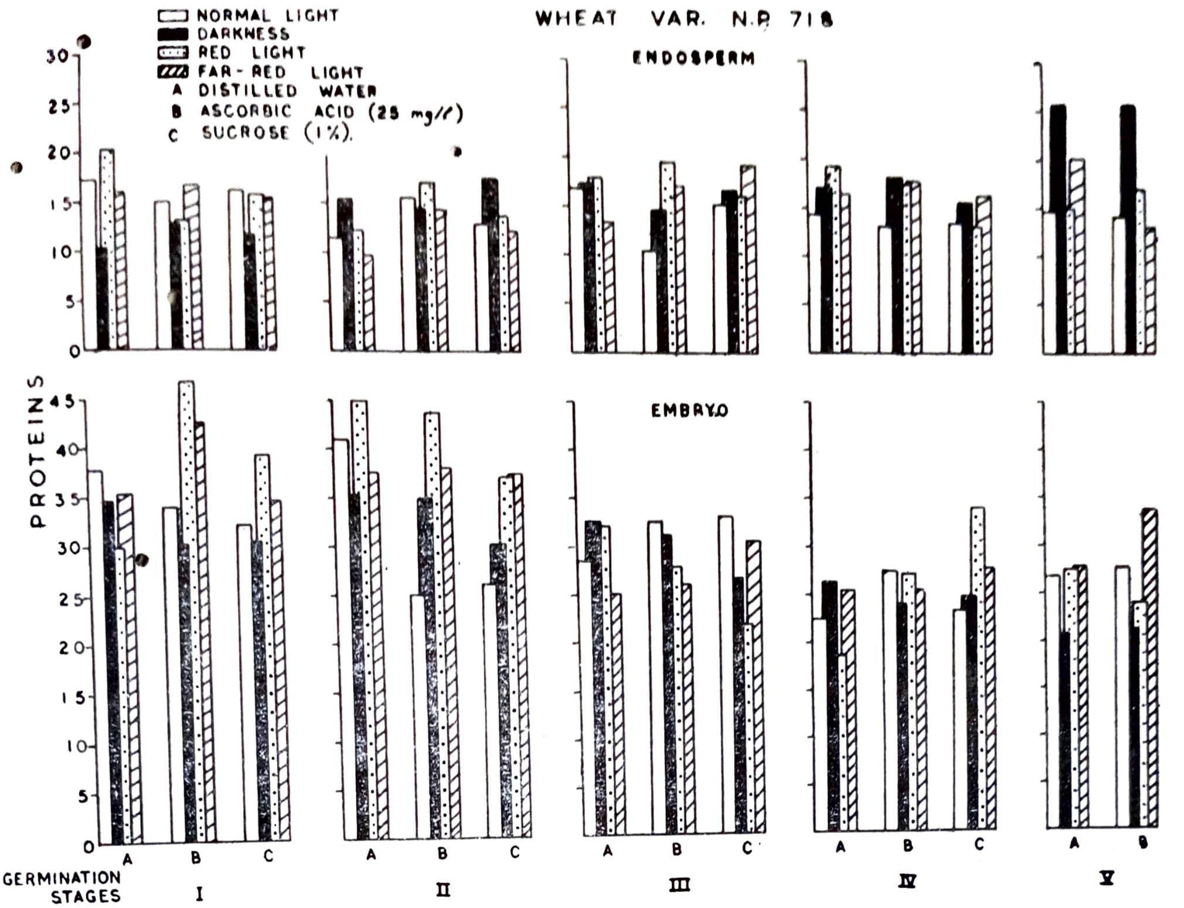

FIG. 4. Effect of different light treatments, substrates and stages on protein in embryo axis and endosperm.

Amylase activity.—There is higher amylase activity in the embryo axis at Stage I in D and AA, sucrose substrates. No marked effect is seen due to R and FR at Stages I and II. Stage II onwards activity increases until Stage IV, followed by a fall at Stage V. R and FR seem to be inhibitory at the Stages III and IV in AA growth medium as compared with ND and D. Reverse trend is found in the Stage V (Fig. 5).

Statistical analysis reveals the effect of growth stages only to be highly significant and the values are markedly lower at Stages II and III as compared to Stage I. The highest activity is obtained at Stage IV, but the difference is not significant.

Total sugars and starch in the endosperm.—Starch content decreases with growth accompanied by a concomitant increase in the sugar content (Figs. 2, 3). Maximum starch and minimum sugar content is found at Stage I under all the three substrates and light conditions. Decrease in starch and simultaneous increase in sugar during subsequent growth stages indicate a higher hydrolytic activity. Starch content is higher in D compared to other light treatment at Stages II and III. This suggests that D has an inhibitory effect upon breakdown of starch. The lowest starch content is found in ND at Stages II and III suggesting a promoting effect upon hydrolysis of starch.

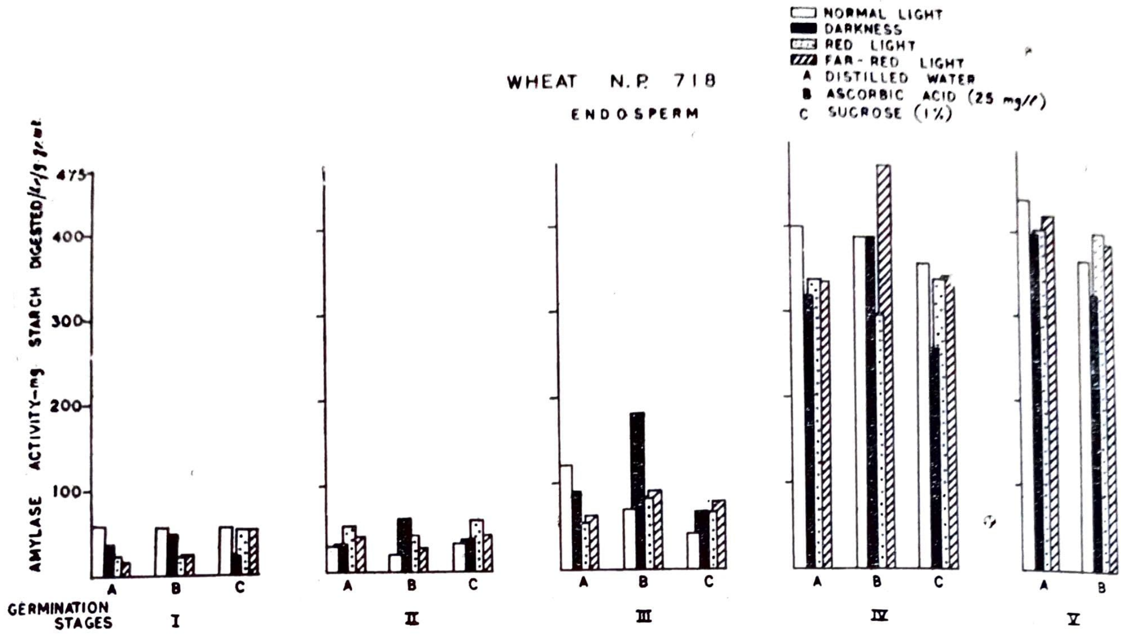

FIG. 5. Effect of different light treatments, substrates and growth stages on amylase activity in embryo axis.

Effect of growth stages is only significant in case of sugar. Mean values of sugar at Stages II to V are significantly higher in comparison to mean value of the Stage I. Reverse trend is found in case of the starch content.

Protein content in endosperm.—There is almost no significant change in protein content of the endosperm throughout the experimental period, in any of the treatments and also there is no significant effect of light treatments or substrates (Fig. 4). A higher value is, however, recorded in D in comparison to other light treatments at Stage I.

Statistical analysis shows that only the effect due to growth stages is significant. However, a comparison of mean values does not show any significant critical difference, except at the Stage V where the mean value is higher.

Amylase activity in the endosperm is at a considerably lower level during the Stages I to III (Fig. 6). and a significant upsurge in its activity is recorded thereafter. Only minor variations in amylase activity due to light treatments and substrates are observed during the first two stages of germination. Similar trend is observed in Stage III also. Higher activity is seen in D with AA as medium. At Stage IV the activity is the highest in FR and the lowest under R in AA substrates. In sucrose the activity is similar under the influence of ND, R and FR and lowest in dark. There is no significant difference in the values for light treatment and substrates at Stage V. The effect of growth stages is highly significant. The mean value of amylase activity in the endosperm increases progressively with the advancement of germination and it is significantly higher than the mean value at Stage I. The effect of light treatments and substrates are not significant.

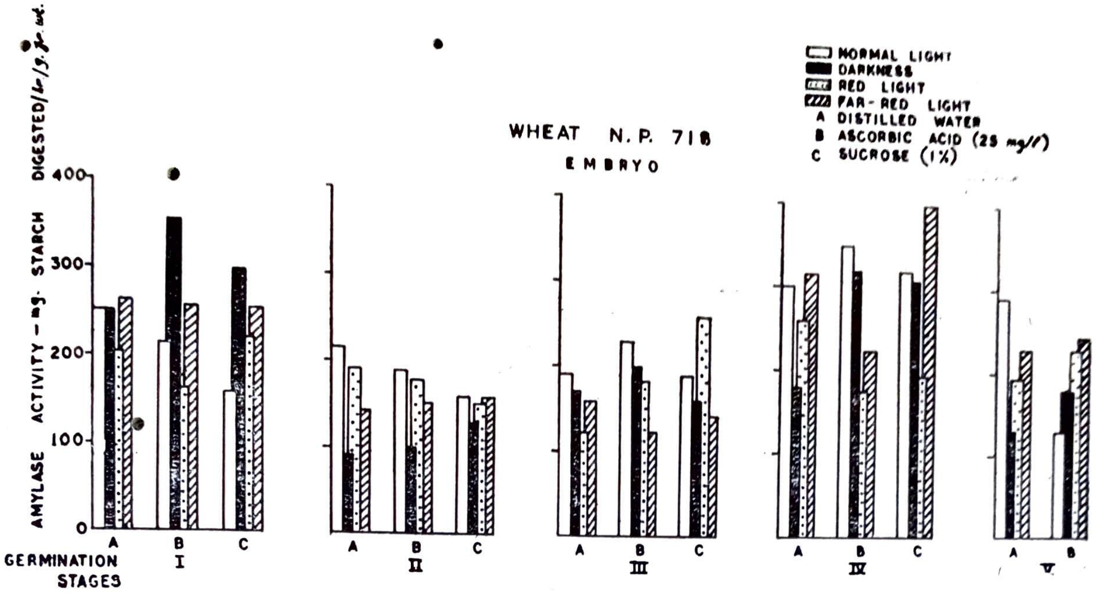

FIG. 6. Effect of different light treatments, substrates and growth stages on amylase activity in endosperm.

DISCUSSION

Germination process begin with imbition of water by the seed. Hydrolysis of the starch in endosperm increases with the advance of germination. This finding is corroborated by the increased content of total sugars as well as a progressive increase in the amylase activity in the endosperm. The total sugars increase also in the embryo axis with advance of germination. The other noteworthy feature is the higher amylase activity in the embryo axis in spite of the fact that starch is absent. R and FR effect sugar contents of embryo axis favourably as compared to ND and D. Instead of inhibiting seedling growth as well as a number of metabolic processes as is seen in positive photoblastic seed, the FR significantly enhances them over those obtained under ND.

The protein content of the endosperm is maintained at a steady level, whereas there is a progressive decrease in its concentration in the growing embryo axis. This finding is supported by an earlier work showing that RNA content (Res. Prog. Report No. VI) of the embryo axis exhibits a downward trend during the course) of early germination. This is most probably due to the fact that RNA is actively associated with protein synthesis. Protein content is independent of the influence of any of the light treatments. It, therefore, appears that judging either by the rate of germination, rate of seedling growth or by metabolic studies on carbohydrates, protein etc., the seeds of the wheat variety under study do not show the operation of R-FR system like majority of seeds as reported by Giese (1964). Growth was restricted after IV Stage of germination in the presence of sucrose probably due to the adverse osmotic relations in operation.

It is clear that ascorbic acid and sucrose substrates bring about changes in amylase activity and seedling growth in comparison to the effect of DW alone. This suggests an inductive effect of ascorbic acid as shown earlier (Chinoy, 1962; Abraham *et al.*, 1970; Patel, 1967 and Chinoy *et al.*, 1967). Presence of ascorbic acid in the substrate in combination with red as well as far-red lights enhances the seedling growth and metabolic processes.

Summary

The effect of R, FR, ND and D were studied on the mobilization patterns of carbohydrates, protein as well as seedling growth during germination of wheat cv. N.P. 718 incubated in DW, AA (25 ppm), sucrose (1%). It appears that R–FR system does not operate like majority of cases as germination percentage is not affected under any light treatment. AA activates amylase activity indicating faster mobilization of starch in endosperm. This is further confirmed by higher sugar content in embryo. Protein content is unaffected by light treatments.

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References

Abraham, P. G., I. C. Dave, O. P. Saxena, and R. B. Pandya. 1970. Studies in the physiology of seed germination. I. Ascorbic acid metabolism in germination of wheat under different light conditions. *J. Indian bot. Soc.* **49**: 41–49.

A.O.A.C. 1955. *Methods of Analysis*. (8th edition.) William Horwitz *et al.* (Eds). A. O. A. C. Washington.

Banerjee, D., and M. M. Laloraya. 1967. Correlative studies on plant growth and metabolism. III. Metabolic changes accompanying inhibition of the longitudinal growth of stem and root by kinetin. *Pl. Physiol.* **42**: 623–627.

Beevers, L., and F. S. Guernsey. 1966. Changes in some nitrogenous compounds during the germination of pea seeds. *Ibid.* **41**: 1455–1458.

Chinoy, J. J. 1939. A new colorimetric method for the determination of starch applied tos oluble starch, natural starches and flour. *Mikrochemie microchem. Acta* **26**: 132–142.

———. 1962. Formation and utilization of ascorbic acid in shoot apex of wheat as factors of growth and development. *Indian J. Pl. Physiol.* **5**: 172–201.

CHINORY, J. J. 1967. Role of ascorbic acid in crop production. *Poona Agric. Coll. Mag.* **57**: 1–6.

——, C. K. SHAH, H. T. PATEL, AND H. K. SUTHAR. 1967. Role of auxin and gibberellin in the synthesis of ascorbic acid and growth of tissue explants. *Biologia Pl.* **9**: 181–194.

DONEEN, L. D. 1932. A micromethod for nitrogen in plant material. *Pl. Physiol.* **1**: 717–720.

FISHER, R. A. 1954. *Statistical Methods for Research Workers*. (12th ed.) Oliver and Boyd, London.

GIESE, A. C. 1964. *Photophysiology, General Properties, Action of Light on Plants.* Academic Press, New York.

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

NANDA, K. K., J. J. CHINOY, AND S. M. GUPTA. 1959. A method for the determination of the rate of seedling growth and its application to the study of the effect of presowing hardening treatment on wheat grain. *Phyton.* **12**: 153–161.

PATEL, H. D. 1967. *Inductive Effects of Seed hormonization as well as Vernalization Upon Ascorbic Acid Metabolism Growth and Development of Some Crop Plants.* Ph.D. Thesis, Gujarat Univ.

RAI, V. K., AND M. M. LALORAYA. 1965. Correlation studies on plant growth and metabolism. I. Changes in protein and soluble nitrogen accompanying gibberellin-induced growth in lettuce seedlings. *Pl. Physiol.* **40**: 437–447.

——, AND ——. 1967. Correlative studies on plant growth and metabolism. II. Effect of light and gibberellic acid changes in protein and soluble nitrogen in lettuce seedlings. *Ibid.* **42**: 440–444.

Research Progress Report No. VI (April 1966–March 1967) P.L. 480 Research Project No. FG-In-182 (A7–CR–87) entitled: Physiology of seed germination in relation to the early production of ascorbic acid and ascorbic acid oxidase in wheat (*Triticum*) and peanut (Arachis). Botany Department, Gujarat University, Ahmedabad–9.

WEINMANN, H. 1947. Determination of total available carbohydrates in plants. *Pl. Physiol.* **22**: 279–290.