STUDIES IN THE PHYSIOLOGY OF SEED GERMINATION

I. Ascorbic Acid Metabolism in Germination of Wheat Under Different Light Conditions

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INTRODUCTION

THE germination of photosensitive seeds is promoted by red and inhibited by far-red light (Evenari, 1965). Nakata and Lockhart (1966)

reported that stem elongation, cell division and cell elongation were promoted by far-red light. There was virtually no difference either in the rate of leaf initiation or duration of growth of internodes under far-red. An explanation for the promotive effects of high energy levels of far-red has been advanced by Mohr (1962).

The role of ascorbic acid in growth and metabolism has been studied by a number of workers (Reid, 1937, 1938, 1941 *a*, *b*, *c*, 1942; Aberg, 1958; Mapson, 1958; Arnon, 1961; Chinoy, 1962, 1967; Chinoy *et al.*, 1967). This paper deals with ascorbic acid metabolism of germinating wheat under varying conditions of light when treated with exogenous ascorbic acid and sucrose.

Material and Methods

Seeds of wheat (*Triticum aestivum* L. var. N.P. 718) were incubated in sterilized Petri dishes, lined with Whatman No. 1 filter-papers moistened with distilled water, ascorbic acid (25 ppm) and sucrose (1%) separately and were exposed in each case to normal day (ND), continuous darkness (D), red and far-red light.

Red light (R) was obtained through a fluorescent light source with an interposed filter of 2 sheets of red cellophane (6 f.c.) and far-red (FR) with 2 layers each of red and blue cellophane papers (2 f.c.) as suggested by Hillman (1962).

The substrates were changed twice a day. Samples were taken at different stages of germination (Fig. 1) separately for embryo axis and

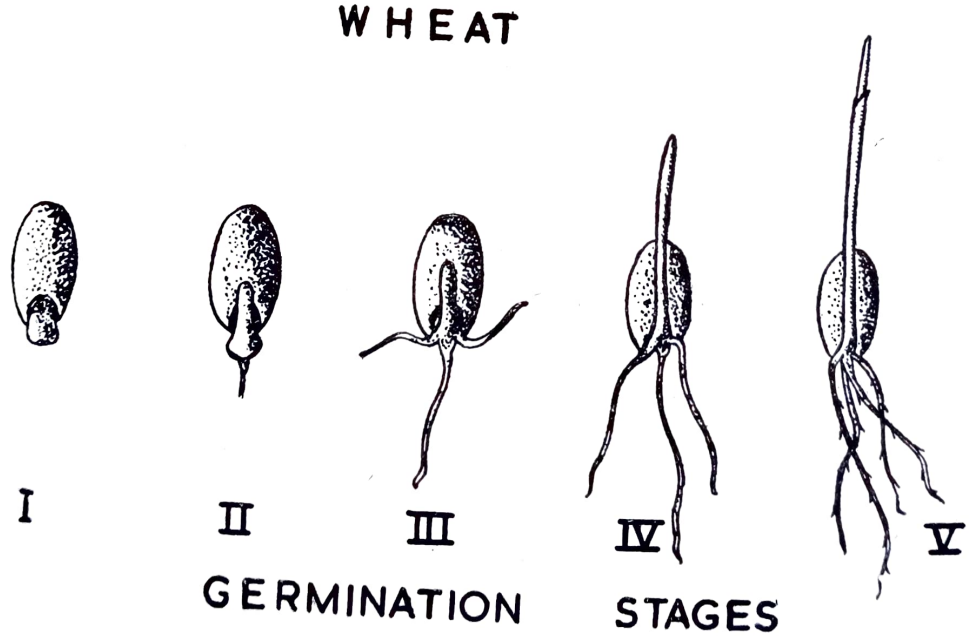

FIG. 1. The germination stages of wheat. *I stage*: Coleorhiza just emerging. *II stage*: Plumule just emerging. *III. Stage*: Two lateral roots emerging and coleoptile still colourless. *IV stage*: Coleoptile becoming green and less than 4 cm. *V stage*: First leaf just emerged and about 1 cm long.

endosperm and estimations in triplicate were carried out for ascorbic acid (AA), ascorbigen (ASG) and total ascorbic acid (TAA). These were expressed as mg/g fr. wt. as well as per cent ascorbic acid utilization (AAU) as described previously (Chinoy, 1962; Chinoy *et al.*, 1967). The data were analysed statistically using Fisher's method of analysis of variance (1954).

OBSERVATIONS

1. Free ascorbic acid (AA), ascorbigen (ASG) and total ascorbic acid (TAA) = (AA + ASG) contents as well as per cent ascorbic acid utilization (AAU) in seedlings are presented in Figs. 2 and 3.

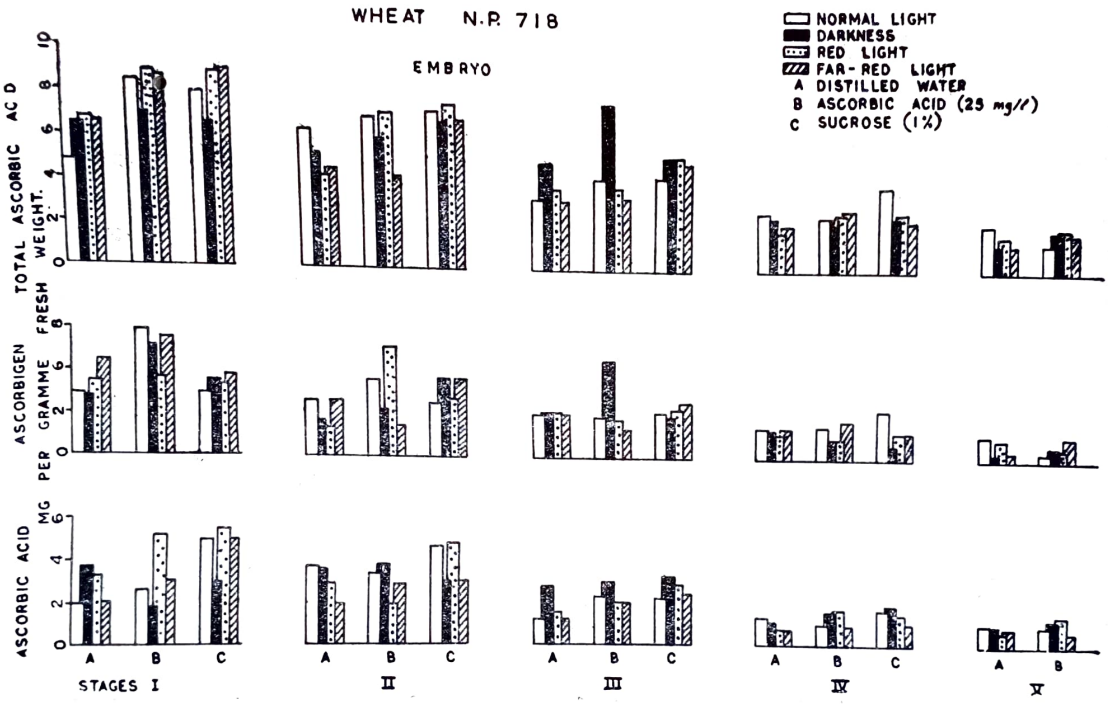

FIG. 2. Effect of different light treatments, substrates and growth stages on ascorbic acid, ascorbigen and total ascorbic acid contents in embryo axis.

At stage I, AA content in sucrose as well as AA substrates is higher under R, FR and ND than in dark. But in water AA content is higher in dark and red light than in ND and FR light. The content decreases gradually with the advancement of growth and no effect of light treatments is descernible from stage III onwards. At stage III the content is the highest in dark in all the 3 substrates.

Statistical analysis for AA content shows the effect of growth stages and substrate to be highly significant, while the same for the light treatment is not significant. There is a significant decrease in AA content with advance of germination. Mean AA content in seedlings grown in sucrose is significantly higher than the same for distilled water. Also mean AA content in seedlings grown in AA as substrate is higher than in case of distilled water but the difference is not significant. More or less similar trend is obtained in the

case of ASG, except that a higher ASG content is generally accompanied by a lower AA content and *vice versa*. The effect of light treatment and substrate on AA content is not significant, while the effect of growth stages is highly significant on the content. A significant fall in the mean ASG content is obtained from stage I onwards.

TAA content also exhibits a trend similar to that of AA.

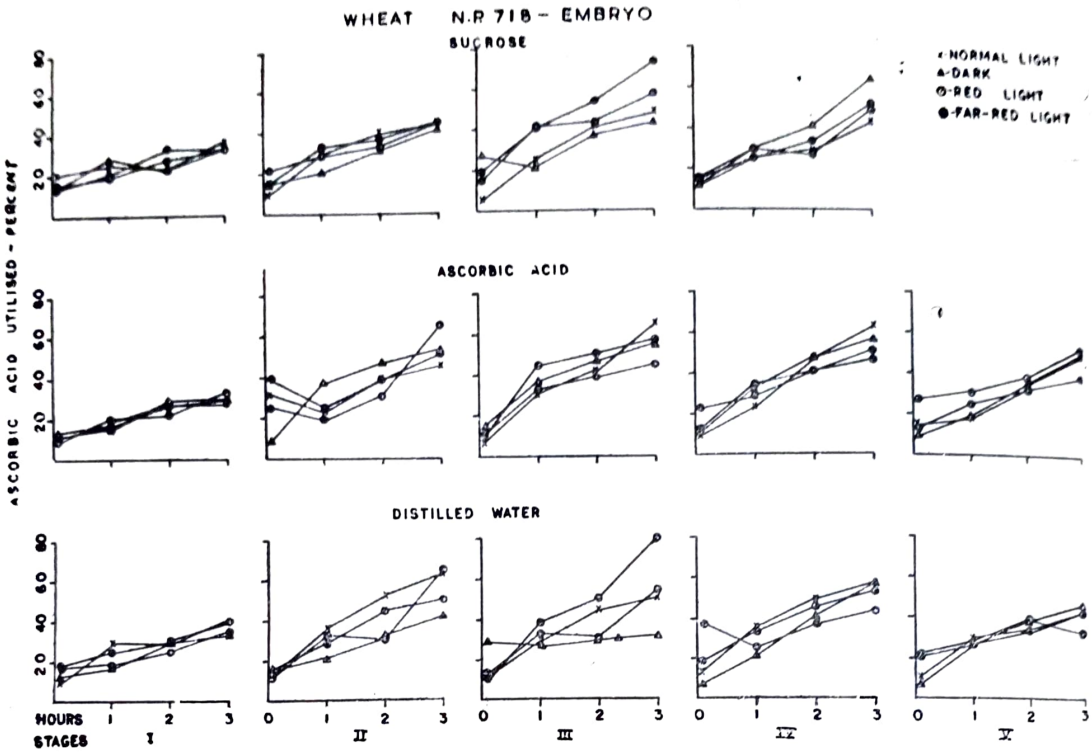

FIG. 3. Effect of different light treatments, substrates and growth stages on ascorbic acid utilization in embryo axis.

Results of AAU are presented in Fig. 3. There is no difference in AAU due either to light treatments or substrates at stage I. AAU increases in red light at stage III in all the substrates. Maximum AAU in red light is recorded after incubation for 3 hrs. in case of distilled water and sucrose substrates, followed by far-red, ND and dark sets respectively. This value for all the light treatments is nearly the same in AA substrate. From this stage onwards, the activity is maintained at that level until stage V.

While the effect of light treatment is significant at 5% level, that of growth stages and incubation period is highly significant. The values are significantly higher in red, ND and far-red lights compared to dark. The highest significant difference is under red light set. Alongwith advance of growth, mean AAU also increases significantly throughout the duration of experiment as can be seen from the mean values and their significant differences for growth stage. Mean AAU exhibits a significant multifold increase with the lengthening of the incubation period.

2. Data of free AA, ASG, total AA contents as well as per cent AAU in endosperm are presented in Figs. 4 and 5.

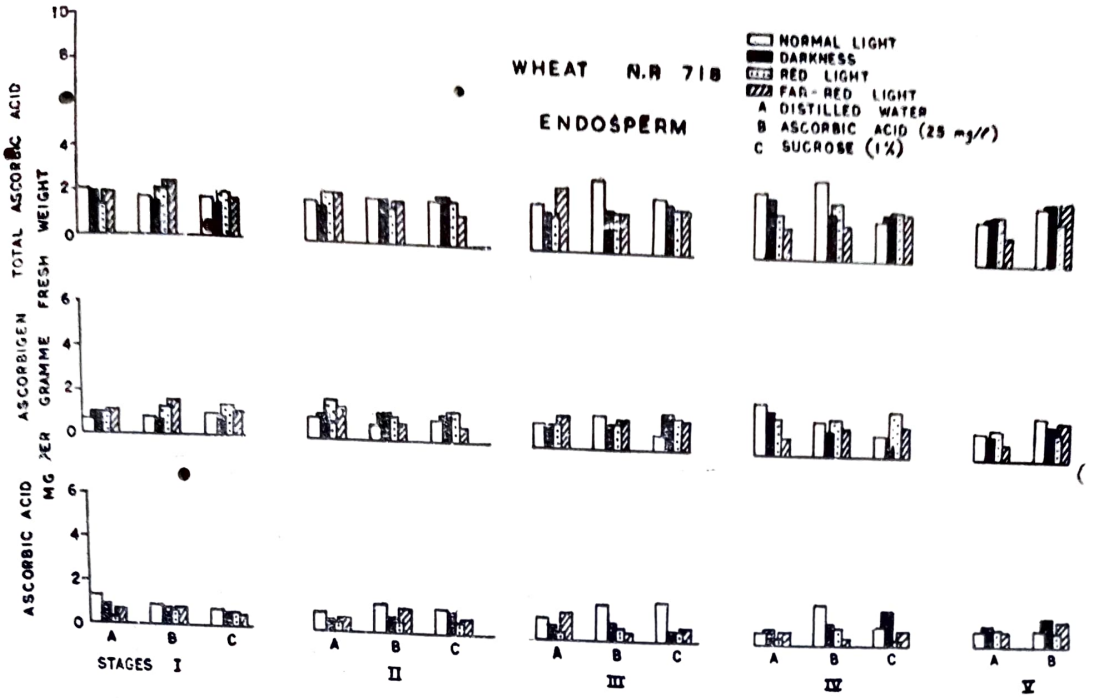

FIG. 4. Effect of different light treatments, substrates and growth stages on ascorbic acid, ascorbigen and total ascorbic acid contents in endosperm.

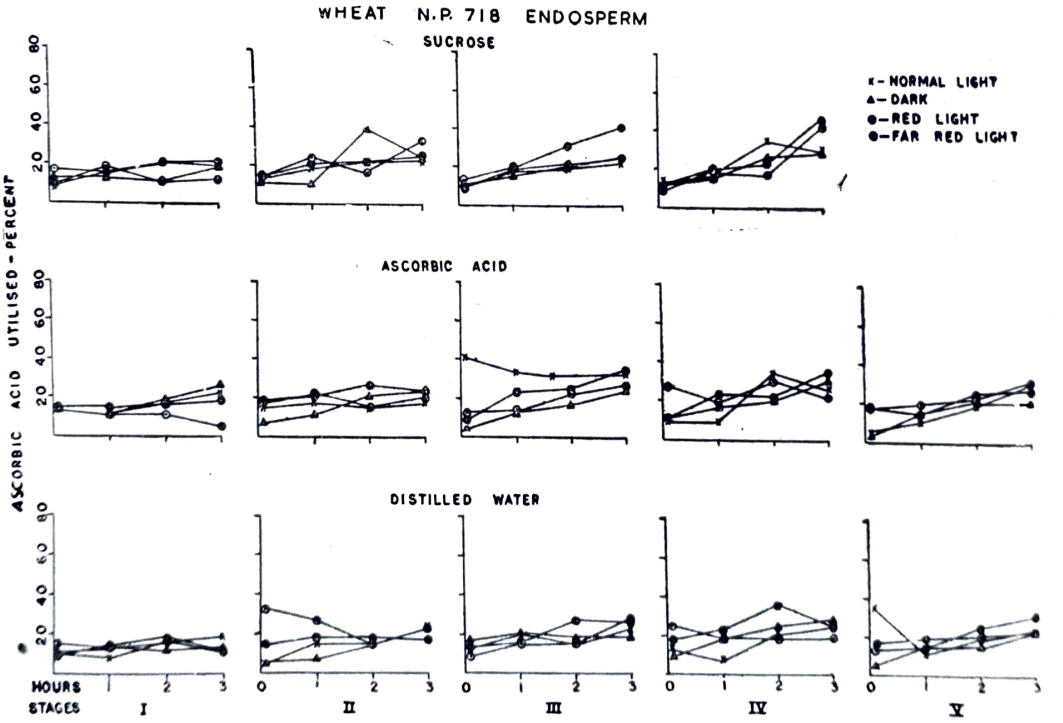

FIG. 5. Effect of different light treatments, substrates and growth stages on ascorbic acid utilization in endosperm.

There is no significant difference in AA content of endosperm under different light treatments or substrates throughout the experimental period except at stage III where ND (especially with AA and

sucrose substrates) favours a higher biosynthesis of AA as compared to other light treatments. Total AA remains almost at a similar level at all growth stages. However, values of AA, ASG and TAA upto stage III in endosperm are consistent with the corresponding higher values in the seedlings, indicating a coptinuous biosynthesis of AA and its mobilization from the endosperm to the seedlings. These findings are corroborated by the comparatively lower AAU in the endosperm than in the seedling (Fig. 5). At stages I and II, AAU does not differ from different reaction periods and substrates. Red light tends to lower the activity with the prolongation of incubation period in sucrose and AA substrates during stage I. A similar trend is observed in all the three substrates upto the second hour of incubation, followed by an upsurge in the AAU activity at stage II. From stage II onwards the activity increases markedly in all cases and the enhancing effect of FR especially in sucrose and both R and FR lights in all the three substrates for stage III is significant. The differences in the values under different light treatments for stage IV under all the substrates are also significant. Interaction of sucrose substrate with R and FR lights brings about a significant upsurge in AAU after three hours of incubation. No marked difference in AAU is noticeable at stage V except that under R and FR, AAU is maintained at a slightly higher level.

Statistical analysis shows that the effect due to light treatments and substrates is significant at 1% level. The effects of growth stages and incubation period are also highly significant. Amongst light treatments, the positive difference in the mean values between FR light and darkness is significant. From stage I onwards, AAU increases significantly. There is a significant enchancement in the AAU under the influence of AA and sucrose. An increase in the incubation period also significantly enhances AAU.

Discussion

Germination process begins with the imbibition of water by the seed. AA as well as ASG contents are maintained at a more or less similar level in the endosperm suggesting that a certain minimum level of AA is necessary in the endosperm for helping in the mobilization and transport of carbohydrates to the embryo. It is probable that this AA is being synthesized locally in the endosperm. There is a progressive decrease in the AA as well as ASG contents in the embryo axis with a concomitant increase in AAU. This denotes that AA is synthesized continuously in the embryo axis from carbohydrates, translocated to it and is being actively metabolized which supports earlier findings of Chinoy *et al.* (1958, 1961). A part of AA is stored in bound from (ASG) and the rest is utilized in the growth of the embryo axis.

The anabolic processes associated with synthesis and growth are localized in the embryo axis while the catabolic processes concerned

with nutrition are localized in the endosperm. This is further corroborated with the earlier findings (*Res. Prog. Rep. No. VI*) that there is a progressive decrease in catalase activity of the embryo axis which indicates that oxidative reactions decrease in the embryo axis during the early period of germination whereas a progressive increase in endospermic catalase activity indicates an increase in its oxidative reactions.

None of the four light treatments had any effect on AA and ASG contents. Likewise a comparatively lower catalase activity (*Res. Prog. Rep. No. VI*) under R and FR light treatments suggests that oxidative activity proceeds at a lower rate under these light treatments compared to ND and dark. Higher AAU under R and FR lights suggests that more of the AA is being metabolised to maintain the redox balance and as a result the activities of the enzymes containing -SH groups is maintained at a higher level (*loc. cit.*).

The present investigation shows that the germination percentage of wheat is not affected materially by different light treatments. This is not in conformity with the reports of Evenari (1965) and Mancinelli and Tolkowsky (1967) for photoblastic seeds. From this it is concluded that the classical photomorphogenic pigment (phytochrome) is not operative in wheat like majority of cases of seeds (Giese, 1964).

The present investigations confirm the enhanced biosynthesis of ascorbic acid under the influence of exogenous application of AA and sucrose. This suggests an inductive effects of AA shown earlier (Chinoy, 1962, 1967; Patel, 1967; Chinoy *et al.*, 1967).

Summary

Changes in the ascorbic acid metabolism during various stages of germination of wheat seed have been studied under red, far-red and normal lights as well as under darkness. Ascorbic acid and sucrose were supplied exogenously and their effects compared with distilled water as control. It is observed that the reversible R–FR system is not operative in wheat because per cent germination and seedling growth as well as the metabolic changes studied show no reversible, inhibitory or stimulatory effect. Ascorbic acid and sucrose as substrates effect favourably the various metabolic changes studied. Ascorbic acid associated with R and FR light accelerates the seedling growth as well as the metabolic processes investigated.

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REFERENCES

ABERG, B. 1958. Ascorbic acid (formation, storage, mobilization and transformation of carbohydrates). In *Encyclopaedia Plant Physiology*. W. Ruhland (Ed.) Springe-Verlag, Berl n **6**: 479–499.

ARNON, D. I. 1961. Photosynthetic phosphorylation and the energy conversion process in photosynthesis. In *Biological Structure and Function*. T. W. Goodwin, and O. Lindberg. (Eds.), Vol. II: Academic Press, London. 339-400.

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

———. 1967. Role of ascorbic acid in crop production. *Poona Agric. Coll-Mag.* **57**: 1–6.

——, R. GROVER, AND K. K. NANDA. 1958. Sucrose as precursor for the regulatory substances in *Avena* coleoptile. In *Proc. Seminar on Modern Developments in Plant Physiology*. University of Delhi. Aug. 26–30, 1957: 12–14.

——— 1961. Role of sucrose in growth reaction of *Avena* coleoptile sections. *Flora* **150**: 1–10.

——, R. B. PANDYA, O. P. SAXENA, I. C. DAVE, AND P. G. ABRAHAM. 1967. Pattern of ascorbic acid metabolism during vernalization of wheat. *J. Indian bot. Soc.* **46**: 344–350.

EVENARI, M. 1965. Light and seed dormancy. In *Encyclopaedia Plant Physiology*. W. Ruhland, (Ed). Springer-Verlag Berlin, **15**: 804–847.

FISCHER, R. A. 1954. *Statistical Methods for Research Workers*. 12th Ed., Oliver and Boyd, London.

GIESE, A. C. 1964. *Photophysiology, General Principles, Action of Light on Plants*. Academic Press, New York. **1 A**: 15–16.

HILLMAN, W. S. 1962. *The Physiology of Flowering*. Holt, Reinhart and Winston, New York.

MANCINELLI, A. L., AND A. TOLKOWSKY. 1967. Changes in the level of phytochrome during the germination of cucumber seeds. *Pl. Physiol.* (Suppl) **42**: 8–9.

MAPSON, L. W. 1958. Metabolism of ascorbic acid in plants. Part I. Function. *Ann. Rev. Pl. Physiol.* **9**: 119–150.

MOHR, H. 1962. Primary effects of light on growth. *Ibid.* **13**: 465–488.

NAKATA, S., AND J. A. LOCKHART. 1966. Effects of red and far-red radiation on cell division and elongation in the stem of pinto been seedlings. *Am. J. Bot.* **53**: 12–20.

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

REID, M. E. 1937. Localization of ascorbic acid in the cowpea plant at different periods of development. *Am. J. Bot.* **24**: 445–447.

———. 1938. The effect of light on the accumulation of ascorbic acid in young cowpea plants. *Ibid.* **25**: 701–711.

———. 1941 *a*. Relation of vitamin C to cell size in the growing region of the primary root of cowpea seedlings. *Ibid.* **28**: 410–415.

———. 1941 *b*. Metabolism of ascorbic acid in cowpea plants. *Bull. Torrey bot. Club* **68**: 359–371.

REID, M.E. 1941 *c*. Relation of temperature to the ascorbic acid content of cowpea. plants. *Ibid.* **68**: 519–530.

———. 1943. Interrelations of calcium and ascorbic acid to cell surfaces and intercellular substances and to physiological action. *Physiol. Rev.* **23**: 76–99

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