CHAPTER X

CONCLUSIONS
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From the results and findings of the three stress treatments on Wheat and Blackgram seedlings, during germination, the following conclusions are drawn:

1. The embryo axis is the chief centre of the enzymic activity and not the endosperm or cotyledon. This corroborates Brown's (1965) findings.

2. Water deficits in seedlings were simulated by germinating the seedlings under various osmotic and by subjecting the germinated seedlings to desiccation. The behaviour and responses of Blackgram Seedlings were different under these two water stresses. But the behaviour and responses of Wheat Seedlings were almost same under these two water stresses.

3. Various metabolites and enzymic activities in Wheat as well as Blackgram Seedlings were affected differently under different osmotic except under 5 PEG. Under 5 PEG, almost all the metabolites and enzymic activities of Blackgram Seedlings were affected in the same way, i.e. they were substantially lowered.

4. Osmotica affected the moisture content adversely in Wheat Seedlings whereas it had little effect on the moisture
content in Blackgram Seedlings. This is probably due to the high concentration of "Sugar" in Blackgram Seedlings.

5. Seedling growth of both Wheat and Blackgram was adversely affected under 5 osmotic.

6. Desiccated Weights of both Wheat and Blackgram Seedlings decreased with increase in period of desiccation treatment. Desiccated Weights were more than the Dry Weights of the Controlled (Undesiccated) Seedlings. Moisture loss increased with the duration of desiccation treatment.

7. Activities of Oxidizing enzymes (viz. catalase and Peroxidase) were lowered under osmotic and by desiccation in both Wheat and Blackgram seedlings. Lowered activities of the oxidizing enzymes under water stress observed here, corroborates with Todd's findings (1972) that water stress causes an overall decrease in enzyme level. It is shown by Altman et al (1966) that lowering of respiration is concomitant with the lowering of the activities of oxidizing enzymes. This lowering of respiration causes seedlings to grow with poor
differentiation and ultimately leads to its arrested growth. Siegel and Galston (1967) have also correlated seedling growth and its differentiation with Peroxidase activity.

8. It is observed that there is a greater depletion of Starch Content of endosperm of Wheat Seedlings under Stress (Osmotica, Desiccation and Low Temperature) than in the endosperm of non-stressed seedlings. This perhaps indicates that under stress there is a greater mobilization of energy sources (Starch) from "Storage organ" (endosperm) to the "Growing Organ" (embryo axis). Under stress the amylase activity of Wheat Seedlings was also lowered. This indicates a possibility of new enzymes like Phosphorylase being synthesized to hydrolyze starch, for it is well known that under stress, there takes place a "denovo" synthesis of new enzymes. (Kessler, 1961 = "denovo" synthesis of new enzymes under stress; Stumpf, 1952 = Phosphorylase might have hydrolyzed starch; Takoki (1968) = 100% increase of Phosphorylase in Cowpea under water stress).
9. Reducing Sugar Content under osmotic and by desiccation increased in both Wheat and Blackgram Seedlings with a concomitant increase in Invertase Activity and a decrease in Non-Reducing Sugar Content. The rise in reducing sugar content in embryo axis could be a result of a biomechanism to survive stress conditions. As postulated by Maranville and Paulsen (1970), Parker (1968) and Klotz (1958) increase in concentration of low molecular weight carbohydrates (reducing sugars) helps the seedlings under stress in retaining turgidity or in stabilizing proteins. In embryo axis and in cotyledons of Blackgram Seedlings under osmotic, moisture content was little affected and the seedling growth was arrested, despite the fact that there was an increase in reducing sugar content in embryo axis. This indicates that this sugar is not being converted to polysaccharides for cell wall formation. Instead, it is used for protection, i.e. for retaining turgidity, for stabilizing proteins, etc.

10. Protease Activity in embryo axis of seedlings germinated for a long time (96 hours) under osmotica increased in the case of Blackgram and was lowered in the case of Wheat. Protease Activity in cotyledons of Blackgram Seedlings
was affected under osmotica, the effects under various osmotica were different. Protease activity in endosperm of Wheat Seedlings was lowered under osmotica. Protein Content of embryo axis of Blackgram Seedlings was lowered under osmotica. Protein Content of cotyledon of Blackgram Seedlings increased under osmotica during prolonged germination. The effects of various osmotica on histone content of embryo axis and of cotyledons of Blackgram Seedlings were different. Protein Content and Histone content of embryo axis and of endosperm of Wheat Seedlings were lowered under osmotica. Thus, it can be concluded that effects of osmotica on Wheat and Blackgram Seedlings were different.

11. Protease Activity, Protein Content and Histone Content of embryo axis of Wheat and Blackgram Seedlings was lowered by desiccation. Protease Activity in endosperm of Wheat Seedlings and in cotyledon of Blackgram Seedlings increased during prolonged desiccation treatment whereas Protein Content and Histone Content was lowered by desiccation treatments. Thus it can be concluded that effects of desiccation on both Wheat and Blackgram Seedlings were same.
12. **RNAse Activity, RNA Content and DNA Content** of both Wheat and Blackgram Seedlings were adversely affected under osmotic and by desiccation. Thus both osmotic and desiccation adversely affected the various metabolisms of Wheat and Blackgram Seedlings. Low Protein content indicates either a lowering in the rate of protein synthesis or a breakdown of proteins. Since protease activity was also low, a lowering in the rate of protein synthesis is more likely. This corroborates with the findings of Ben-zioni et al (1967) and Greenway et al (1972). Low rate of protein in seedlings under stress could be a result of non-availability of energy due to lowered respiratory activity because of lowered activities of oxidizing enzymes.

13. Another interesting observation was that shorter periods of desiccation had a more adverse effect on macromolecules (Protein, Histone, etc.) and enzymic activities than either longer periods of desiccation or osmotic stress.

14. **Low Temperature treatments** created a severe effect in both Wheat and Blackgram Seedlings, i.e. all metabolites and enzymic activities were substantially lowered.
15. Shorter periods of low temperature treatment lowered the sugar content whereas longer periods of treatment increased this content despite being more prolonged stress in both Wheat and Blackgram Seedlings. It is a well known fact that sugar depresses the freezing point of water and retards ice formation. Thus, the rise in sugar content on prolonged low temperature treatment could be a result of biomechanism for overcoming the low temperature stress effect.

16. Both the oxidizing enzymes (viz. Catalase and Peroxidase) were substantially lowered by low temperature treatments in both Wheat and Blackgram Seedlings indicating a lower status of respiration, thus not releasing enough energy for the metabolic activities.

Thus in conclusion, it can be stated "that it is the reaction phase - oxidation, hydrolysis and destruction" which dominates in stressed Wheat and Blackgram Seedlings. This corroborates with Stocker's (1960) theory. But the restitution phase also occurred as evidenced by increase in concentration of sugars. This is specially true of desiccated seedlings for whereas osmotica causes only a restriction in the supply
of water, desiccation causes a slow dehydration and in fact a complete removal of water. This perhaps also explains the differences in behaviours of these two seedlings under these two water stresses.

A number of questions arise as a consequence of this study. An explanation has to be found for the suppression of amylase activity under stress. Why is there a "de novo synthesis" of new enzymes like phosphorylase in seedlings under stress? The differences in behaviour of Wheat and Blackgram Seedlings under osmotic stress remain to be explained. Can the differences be ascribed to the difference in the type of food stored in these two seedlings, Wheat being "Starchy" and Blackgram being "Proteinaceous"? What is the real reason for the lowering of protein content in stressed seedlings? Is it due to a lowering in the rate of protein synthesis? Or is it due to the degradation of proteins to amino acids? It has been mentioned that sugars are utilized for overcoming the effect of stress. However, no explanation is available for a reduction in utilization of sugars for seedling growth.

Still much is unknown about the physiology of seeds in the state of suspended growth except that the seeds become
partly dehydrated (Abrol and McIlrath, 1966). Similarly, literature is scanty on the role of proline under water stress during germination. Similarly, not much is known about the intricate mechanisms and physiological processes involved in cold hardiness of plants. Although biologically important reactions are limited to a small temperature range, it is still a mystery, how plants display remarkable thermal resilience, by occupying sites as diverse as Antarctica at one extreme and Death Valley, California at the other. Thus much remains to be studied on how plants adapt themselves to different stresses like Water Stress, Temperature Stress, Chemical Stress, Light Stress, etc.

As said in the introduction, "Germination" is the most essential and primary part of a plant’s growth, development and differentiation. Hence, a lot remains to be known on how the plant’s physiological systems adjust themselves to adverse environmental conditions especially during germination. This knowledge could help us to manipulate the growth of crop plants, fruit trees and all other economically important plants especially under adverse conditions, thus once for all overcoming the great "food problem" which the whole world is facing.
This work could be considered as just a "beginning" for solving the bigger problem before us.