EXPERIMENTAL FINDINGS

PART I

Effect of different periods of desiccation on the enzymatic activities and ascorbic acid metabolism of untreated and pretreated seeds of Wheat N.P. 710, Ragi Co. 5 and Sesamum N.P. 6 during germination.

The data of the following activities (for Wheat, Ragi and Sesamum) have been presented here:

a. Catalase activity
b. Amylase activity
c. Sulphydryl content (-SH)
d. Ascorbic acid content (AA)
e. Ascorbic acid utilization (AAU)
f. Ascorbigen content (ASG)

a. Catalase activity:

Data of catalase activity for the three crop plants, Wheat, Ragi and Sesamum are presented as histograms in Figures 2, 3 and 4 respectively.

In order to bring out clearly the effects of single factors as well as their interactions the data has been grouped in different ways to obtain mean values for each factor as well as its interactions with another factor. Thus, for example, to obtain mean values for different stages of germination in Wheat (I to VIII) all the
determinations for the five desiccation treatments, for the three pretreatments and for all the three replicates were added up for each stages of germination and divided by the total number of determinations which were \((5 \times 3 \times 3) = 45\) for each germination stage.

Therefore, each of the eight histograms for the germination stages of Wheat shown in the upper left hand corner of Figure 2 is a mean of 45 determinations. Similarly for the five desiccation treatments values for the eight seedling stages, for the three pretreatments and three replicates, i.e. \((8 \times 3 \times 3) = 72\) separate determinations were added and the figure divided by 72 to obtain mean values for each of the five desiccation treatments.

In the same manner histogram for each of the three pretreatments is a mean of 120 individual determinations. Each histogram for a combination of five desiccation treatments and three pretreatments (central set of histograms) is a mean of 24 determinations; and each reading for a combination of eight stages of germination and five desiccation treatments (lower set of histograms) is a mean of nine determinations.

In this manner the data has been processed for each crop plant as well as for each biochemical determination or growth character. In Ragi and Sesamum also there were in all 360 treatment combinations in each case and these were grouped appropriately to
obtain the means for each treatment or its combination with another factor by adding up the values and dividing by the appropriate number of determinations.

Taking the data in Figures 2, 3 and 4 into consideration the following points emerge:

(i) There is a progressive increase in the catalase activity with the advance in stages of seedlings growth in the embryo axis of Wheat and Ragi and in the whole seed of Sesamum. In the case of endosperm there is a continuous fall in catalase activity after the initial rise in the second stage.

(ii) There is a sharp fall in catalase activity of embryo axis of Wheat and Ragi and seed of Sesamum during both the 5-day as well as 10-day desiccation (For Wheat and Ragi) and 2-day and 4-day desiccation (For Sesamum). In the endosperm on the other hand there is a tendency for enhancement in catalase activity in Wheat.

(iii) Catalase activity of the embryo axis of ascorbic acid pretreated seed is significantly higher compared to the catalase activity of untreated as well as distilled water treated embryo axis of both Wheat and Ragi. In the case of Sesamum also there is a significant enhancing effect of AA-pretreatment on the
catalase activity. AA-pretreatment also accelerates the catalase activity of endosperms of Wheat and Ragi.

(iv) The highly significant enhancing effect of AA-pretreatment over the other two pretreatments on catalase activity is again clearly brought out when these data are considered separately for the five desiccation treatments (central set of histograms). This enhancement is highly significant both for the embryo axis as well as for the endosperm in Wheat and Ragi and also in Sesamum seedling.

(v) Studying the results of catalase activity for the five desiccation treatments separately for the eight stages of germination (lower set of histograms) it becomes clear that there is a rising trend in the enzymic activity of embryo axis with advance in germination and fall in the activity of the endosperm of Wheat and Ragi. Seed of Sesamum also shows a rising trend in catalase activity with the march of germination. Once again in almost all cases there is a sharp decline in the catalase activity of desiccated embryo axis, which attain almost original levels and sometimes surpass them during the period of
revival. Endosperms of both Wheat and Ragi also show similar decline in catalase activity during desiccation with subsequent upsurge in the enzymic activity after revival.

**Statistical significance of the data**

The data of catalase activity in Wheat, Ragi and Sesamum was subjected to analysis of variance using Fisher's method (1954). The analysis is presented in Tables 1, 2 and 3 of Appendix I.

Effects of desiccation, pretreatment and germination stages were highly significant ($P > 0.01$) in all the three crop plants.
FIGURE 2. Catalase activity in Wheat N.P. 710.
WHEAT N.P. 710

EMBRYO

GERMINATION STAGES

ENDOSPERM

GERMINATION STAGES
FIGURE 3. Catalase activity in Ragi Co. 5.
RAGI CO. 5

EMBRYO

ENDOSPERM

STAGES

DESiCCATION

PRETREATMENT

STAGES

DESiCCATION

PRETREATMENT

CATALASE ACTIVITY

GERMINATION STAGES
CATALASE ACTIVITY
SESAMUM N.P. 6

STAGES DESICCATION PRETREATMENT

GERMINATION STAGES
b. **Amylase activity:**

Amylase activity in Wheat, Ragi and Sesamum was studied at 30 minutes and 60 minutes intervals respectively. Histograms in Figures 5, 6 and 7 represent the data for amylase activity at 60 minutes interval in the three crop plants. The data for amylase activity studied after 30 minutes are not given here for the sake of brevity and avoiding repetition.

The effects of single factors and their interactions are brought out clearly by grouping the data in the same manner as that described for catalase activity.

The data brings out some very interesting facts as follows:

(i) From histograms shown in the upper left hand corner of the three Figures, amylase activity in the embryo axis of Wheat and Ragi as well as in the seed of Sesamum appears to be maximum in the 4th and 5th stage of germination. In the later stages it decreases in the case of Wheat and Ragi embryo axis while in Sesamum seedling it remains more or less at the same level. In the endosperm of Wheat and Ragi the enzymic activity is low in the beginning, increasing during the 4th
stage and afterwards it remains more or less at the same level.

(ii) In the case of Wheat and Ragi amylase activity is very much enhanced during 5-day and 10-day desiccation treatments and their respective revivals as well. This is observed in both embryo and endosperm. The same fact is true in case of Sesamum seedling also where 2 and 4-day desiccation treatments as well as their revivals are quite beneficial for the amylase activity.

(iii) Amylase activity in the ascorbic acid pretreated seed is significantly higher compared to the amylase activity of untreated and distilled water treated embryo axis of both Wheat and Ragi. AA-pretreatment also accelerates amylase activity of Ragi endosperm. In the case of Sesamum pretreatments do not appear to be of much advantage over the untreated series.

(iv) The highly significant enhancing effect of AA-pretreatment over the other two series is again clearly brought out when these data are considered separately for the five desiccation treatments (central set of histograms). This enhancement is quite
obvious in the embryo axis of both Wheat and Ragi as well as in Ragi endosperm. In the case of Sesamum seedling there is not much difference in the amylase activity between the pretreated and untreated series.

(v) Considering the results of amylase activity for the five desiccation treatments separately for the eight stages of germination (lower set of histograms) it is once again clear that amylase activity is higher in the beginning compared to the amylase activity in the later stages of growth. Again, in all the three crop plants, the two desiccation treatments as well as their revivals are highly advantageous for amylase activity.

Statistical significance of the data

The data of amylase activity in Wheat, Ragi and Sesamum was subjected to analysis of variance using the same method as used for catalase activity. The analysis is presented in Tables 1, 2 and 3 of Appendix II.

Effects of pretreatment, desiccation treatments and stages were highly significant in all the three crop plants.
FIGURE 5. Amylase activity in Wheat N.P. 710.
WHEAT N.R. 710

ENDOSPERM
60 MINUTES

EMBRYO
60 MINUTES

DESISSCATION
PRETREATMENT

STAGES

GERMINATION STAGES

AMYLAΣE ACTIVITY

CONTROL
DIST. WATER
ASCORBIC ACID

F 5D 5R 10D 10R

STAGES
I-VIII

F 5D 5R 10D 10R

1 2 3 4 5 6

FIGURE 6. Amylase activity in Ragi Co. 5.
Sesamum N.P. 6

60 Minutes

Stages Desiccation Pretreatment

- I
- II
- III
- IV
- V
- VI
- VII
- VIII

Amylase Activity

- Control
- Dist. Water
- Ascorbic Acid

Germination Stages

- Undesiccated
- Desiccated (5 Days)
- Revived (After 5 Days)
- Desiccated (10 Days)
- Revived (After 10 Days)
c. Sulfhydryl content (-SH):

Data of sulfhydryl content in the three crop plants is presented in Figures 8, 9 and 10 respectively.

In order to bring out clearly the effects of single factors as well as their interactions the data has been grouped in different ways to obtain mean values for each factor as well as its interaction with another factor. For this purpose the same method as described previously was used.

Taking the data in Figures 8, 9 and 10 into consideration, the following points emerge:

(i) There is a progressive increase in the -SH content with the advance in stages of seedling growth in the embryo axis of Wheat and Ragi and in the whole seed of Sesamum. In the case of endosperm the -SH content decreases after the initial rise in the third stage.

(ii) 5-day and 10-day desiccation treatments for embryo and endosperm of Wheat and Ragi, as well as 2-day and 4-day desiccation treatments for seed of Sesamum cause a considerable depression in the -SH content. However, revival after these desiccation treatments increases the -SH content to a considerable extent in all the three crop plants almost
attaining the level of normally watered series.

(iii) Pretreatments with ascorbic acid is of great advantage to -SH content of all the three crop plants. In case of Wheat, pretreatment with distilled water is also beneficial to a slight extent.

(iv) The highly significant enhancing effect of AA-pretreatment over the other two pretreatments on -SH content is again clearly brought out when these data are considered separately for the five desiccation treatments (central set of histograms). This enhancement is highly significant both for the embryo axis as well as the endosperm of Wheat and Ragi and also for seed of Sesamum.

(v) Studying the results of -SH content for the five desiccation treatments separately for the eight stages of germination (lower set of histograms), it becomes clear that there is a rising trend in the -SH content of embryo axis with the advance in germination and a steady state in the -SH content of endosperm of Wheat and Ragi after an initial rise upto the IIInd stage of germination. Seed of Sesamum also shows a rising trend in -SH content with the march of germination. Once
again in almost all cases there is a sharp decline in the -SH content of desiccated embryo axis as well as endosperm of Wheat and Ragi and Sesamum seedling, while during the period of revival, there is a considerable increase in the -SH content of all the three crop plants.

**Statistical significance of the data**

The data of -SH content of Wheat, Ragi and Sesamum were subjected to analysis of variance using the same method as described previously. The analysis is presented in Tables 1, 2 and 3 of Appendix III.

Effects of pretreatment, desiccation and germination stages were highly significant ($P > 0.01$) in all the three crop plants.
FIGURE 8. Sulfhydryl content of Wheat N.P. 710.
WHEAT N.R. 710

GERMINATION STAGES

EMBRYO

STAGES 1-13

DESICCATION

PRETREATMENT

ENDOSPERM

STAGES 1-13

DESICCATION

PRETREATMENT

S.H. CONTENT #/100 SEEDLINGS

F  SD  SR  10D  10R

CONTROL

DIST. WATER

ASCORBIC ACID

GERMINATION STAGES

undecked

desiccated (5 days)

revived (after 5 days)

desiccated (10 days)

revived (after 10 days)
FIGURE 9. Sulfhydryl content of Ragi Co. 5.
RAGI CO. 5

GERMINATION STAGES

EMBRYO

ENDOSPERM

SH CONTENT mg/g. D. Wt.

STAGES DESICCATION PRETREATMENT

STAGES DESICCATION PRETREATMENT

CONTROL
DIST. WATER
ASCORBIC ACID

UNDESICCATED
DESICCATED (5 DAYS)
REVIVED (AFTER 5 DAYS)
DESICCATED (10 DAYS)
REVIVED (AFTER 10 DAYS)
FIGURE 10. Sulphydryl content of Sesamum N.P. 6.
d. Ascorbic acid content (AA):

Data of ascorbic acid content of Wheat, Ragi and Sesamum are presented as histograms in Figures 11, 12 and 13 respectively.

Effects of single factors and also their interactions are brought out clearly by grouping the data in different ways as described previously. The results lead to the following facts of interest:

(i) Histograms for the germination stages shown in the upper left hand corner of all the three Figures indicate that there is a progressive increase in the ascorbic acid content with the advance in stages of seedling growth in the embryo axis of Wheat and in the whole seed of Sesamum. The same fact is observed in Wheat and Ragi endosperms. In the embryo axis of Ragi, however, ascorbic acid content is maintained at more or less the same higher level in the later stages of germination.

(ii) Ascorbic acid concentration increases appreciably in all the three crop plants during the two desiccation periods as well as after revival in the embryo axis and endosperm of Wheat and Ragi as well as in the whole seedling of Sesamum.
(iii) Ascorbic acid pretreatment is evidently advantageous for ascorbic acid content of embryo axis of Wheat and Ragi as well as of whole seedling of Sesamum. AA-pretreatment also seems to increase AA content of Wheat and Ragi endosperms. In Ragi distilled water pretreatment is also beneficial to some extent.

(iv) The highly significant enhancing effect of AA-pretreatment over the other two pretreatments on ascorbic acid content is again clearly brought out when these data are considered separately for the five desiccation treatments (central set of histograms). This enhancement is quite significant in all the three crop plants.

(v) Studying the results of ascorbic acid content for the five desiccation treatments separately for the eight stages of germination (lower set of histograms) it becomes clear that there is a gradual rise in ascorbic acid content of embryo axis as well as endosperms of Wheat and Ragi and seedling of Sesamum. This holds good for desiccated and revived material. In undesiccated material ascorbic acid content decreases considerably in the later stages of germination. Once again in almost all cases 5-day and 10-day desiccation
treatments for Wheat and Ragi as well as 2-day and 4-day desiccation treatments for Sesamum, increase the AA-content to a considerable extent. During the period of revival also AA-content is significantly higher compared to the undesiccated material of all the three crop plants.

Statistical significance of the data

The data of ascorbic acid content of Wheat, Ragi and Sesamum was subjected to analysis of variance using the same method as described previously. The analysis is presented in Tables 1, 2 and 3 of Appendix IV.

Effects of pretreatment, desiccation and germination stages are highly significant (P > 0.01) in all the three crop plants.
FIGURE 11. Ascorbic acid content of Wheat N.P. 710.
WHEAT N.P. 710

EMBRYO

STAGES I-VIII

DESI CCATION PRETREATMENT

ASCORBIC ACID mg/g

D. Wt.

F 5D 5R 10D 10R

GERMINATION STAGES

ENDOSPERM

STAGES I-VIII

DESI CCATION PRETREATMENT

CONTROL DIST. WATER ASCORBIC ACID

G 5D 5R 10D 10R

10 R

REVIVED (AFTER 5 DAYS)

REVIVED (AFTER 10 DAYS)
FIGURE 12. Ascorbic acid content of Ragi Co. 5.
PRETREATMENT

ENDOSPERM

GERMINATION STAGES
FIGURE 13. Ascorbic acid content of Sesamum N.P. 6
SESAMUM N.P. 6

ASCORBIC ACID
SESAMUM N.P. 6
GERMINATION STAGES

STAGES
I-VIII

DESICTION
PRETREATMENT

ASCORBIC ACID
mg/g. D. Wt.

CONTROL
DIST. WATER
ASCORBIC ACID

UNDESICCATED
DESICCATED (5 DAYS)
REVIVED (AFTER 5 DAYS)
DESICCATED (10 DAYS)
REVIVED (AFTER 10 DAYS)

GERMINATION STAGES
Histograms in Figures 14, 15 and 16 represent the effects of single factors and their interactions on ascorbic acid utilization in Wheat, Ragi and Sesamum respectively. These effects were obtained by grouping the data in the same manner as described earlier.

Considering the Figures 14, 15 and 16, the following points emerge:

(i) There is a progressive increase in utilization of ascorbic acid with the advance in stages of seedling growth in the embryo axis as well as in the endosperms of Wheat and Ragi. In whole seedling of Sesamum, however, AAU increases up to the fifth stage of germination after which there is a slight decline.

(ii) In the embryo axis and the endosperm of Wheat and Ragi 5-day and 10-day desiccation treatments depress the utilization of ascorbic acid only to a slight extent. After revival, however, the fall in AA utilization is significant. In the case of Sesamum also, in 2-day and 4-day desiccation treatments AA utilization is almost the same as in the fully watered series and it is only
after revival that there is a fall in AA utilization.

(iii) Seeds pretreated with ascorbic acid utilize more ascorbic acid in Wheat and Sesamum. In case of Ragi, pretreatments with ascorbic acid as well as distilled water are quite beneficial to ascorbic acid utilization.

(iv) The highly significant enhancing effect of AA-pretreatment over the other two series on ascorbic acid utilization is again clearly brought out when the data are considered separately for the five desiccation treatments (Central set of histograms). This enhancement is highly significant both for the embryo axis and the endosperm of Wheat and also for the whole seed of Sesamum. In the case of Ragi embryo axis and endosperm, pretreatments with ascorbic acid as well as distilled water are advantageous for utilization of ascorbic acid.

(v) Studying the results of AAU for the five desiccation treatments separately for the eight stages of germination (lower set of histograms), it becomes clear that there is a rising trend in the utilization of ascorbic acid in embryo axis as well as endosperms of
Wheat and Ragi with the advance in germination. Seed of Sesamum shows a rising trend up to the fifth stage of growth after which stage AAU begins to decline. Once again in almost all cases there is a considerable increase in AAU in desiccated embryo axis as well as endosperms of Wheat and Ragi and in Sesamum seedling.

**Statistical significance of the data**

The data of ascorbic acid utilization in Wheat, Ragi and Sesamum was subjected to analysis of variance using the same method as mentioned before. The analysis is presented in Tables 4, 5 and 6 of the Appendix IV.

Effects of pretreatment, desiccation and germination stages were highly significant ($P < 0.01$) in all the three crop plants.
FIGURE 15. Ascorbic acid utilization in Ragi Co. 5.
Ragi Co. 5

Germination Stages

Ascorbic Acid Utilization - Percent

Embryo

Endosperm

Revised (after 5 days)

Desiccated (10 days)

Revived (after 10 days)
ASCORBIC ACID UTILIZATION - PERCENT
SESAMUM N.P. 6

STAGES
DESICCATION PRETREATMENT

■ ■ UNDESICCATED
■ ■ DESICCATED (5 DAYS)
■ ■ REVIVED (AFTER 5 DAYS)
■ ■ DESICCATED (10 DAYS)
■ ■ REVIVED (AFTER 10 DAYS)

GERMINATION STAGES
f. Ascorbigen content (ASG):

Data of ascorbigen content of Wheat, Ragi and Sesamum are presented as histograms in Figures 17, 18 and 19.

Effects of single factors and also their interactions are brought out clearly by grouping the data in different ways as described previously. The results indicate the following facts:

(i) Histograms for the germination stages shown in the upper left hand corner of all the three figures point out that in the case of embryo axis of Wheat, there is a slight rising trend in the ascorbigen content during different stages of germination. In endosperm, however, a gradual rise in ASG content is observed with the advance of growth. In the case of embryo axis of Ragi, ASG content increases gradually up to the fifth stage of germination. Afterwards it remains at more or less the same level and in the eighth stage it decreases. In the endosperm ASG content increases gradually with the advance in growth stages. In the whole seed of Sesamum, the value of ascorbigen content seems to be fluctuating in the beginning and in later stages of germination, it remains more or less at the same level.
(ii) Embryo axis of Wheat and Ragi revived after 5-day and 10-day desiccation treatments, register a higher ascorbigen content as compared to those subjected to 5-day and 10-day desiccation treatments and also the undesiccated ones. ASG is the highest in embryo axis of both the crops revived after 10-day desiccation treatment. Ascorbigen content is also increased to a considerable extent in Ragi endosperm revived after the two desiccation treatments. Endosperm of Wheat, follows an uneven trend. In the case of Sesamum, ASG content increases appreciably in seedlings revived after 2-day and 4-day desiccation treatments.

(iii) In the case of embryo axis of wheat pretreatment with ascorbic acid is more beneficial for ascorbigen content as compared to the other two pretreatments. In endosperm, pretreatments do not show any noticeable effect on the ASG content. In the case of embryo axis as well as endosperm of Ragi, pretreatments with ascorbic acid as well as distilled water are more advantageous for ASG content than the untreated series. In the seedling of Sesamum pretreatments have no effect on the ascorbigen content of the seedling.
(iv) The enhancing effect of AA-pretreatment in the embryo axis as well as endosperm of Wheat over the other two pretreatments on ascorbigen content is again clearly brought out when the data are considered separately for the five desiccation treatments (Central set of histograms). In the case of Ragi, advantageous effect of both AA-pretreatment as well as distilled water pretreatment is quite evident in both embryo and endosperm. Pretreatments do not show any noticeable effect on the ascorbigen content of the whole seedling of Sesamum.

(v) Studying the results of ascorbigen content for the five desiccation treatments separately for the eight stages of germination (lower set of histograms), it becomes clear that there is a rising trend in ASG content of embryo axis of Wheat and Ragi, especially those revived after 5-day and 10-day desiccation treatments. Ragi endosperm also follows the same trend. In endosperm of Wheat, however, the trend is uneven. In the whole seedling of Sesamum, there is a progressive rise in the ASG content with the advance in growth stages of seedlings revived after 2 and 4-day desiccation treatments. In all the three crop plants ASG content decreases in the undesiccated seedlings during later stages of germination.
Statistical significance of the data

The data of ascorbigen content in wheat, Regi and Sesamum was subjected to analysis of variance using the same method as described previously. The analysis is presented in Tables 7, 8 and 9 of Appendix IV.

Effects of pretreatment, desiccation and germination stages were highly significant ($P > 0.01$) in all the three crop plants.
FIGURE 17. Ascorbigen content of Wheat N.P. 710.
WHEAT N.P. 710

**EMBRYO**

- **STAGES** I - VIII
- **DESICCATION PRETREATMENT**
- **STAGES** I - VIII

**ENDOSPERM**

- **STAGES** DESICCATION PRETREATMENT
- **STAGES** DESICCATED (5 DAYS)
- **STAGES** REVIVED (AFTER 5 DAYS)
- **STAGES** REVIVED (AFTER 10 DAYS)

**GERMINATION STAGES**

**ASCORBIGEN**

- **D. Wt.**
- **F** 5D 5R 10D 10R

**CONTROL**
- **DIST. WATER**
- **ASCORBIC ACID**

**UNDISICATED**
- **DESICATED (5 DAYS)**
- **REVIVED (AFTER 5 DAYS)**
- **DESICATED (10 DAYS)**
- **REVIVED (AFTER 10 DAYS)**
FIGURE 18. Ascorbigen content of Ragi Co. 5.
STAGES OF DESiccATION PRETREATMENT

EMBRYO

STAGES OF DESiccATION PRETREATMENT

ENDOSPERM

GERMINATION STAGES

- CONTROL
- DIST. WATER
- ASCORBIC ACID

DESiccATED (5 DAYS)
- REVIVED (AFTER 5 DAYS)
- DESiccATED (10 DAYS)
- REVIVED (AFTER 10 DAYS)
FIGURE 19. Ascorbic acid content of Sesamum N.P. 6.
**SESAMUM N.P. 6**

Diagram showing germination stages and ascorbic acid content under different pretreatment conditions.

- **Stages of Desiccation (I-VII)**
- **Pretreatment Conditions**
  - Undesiccated
  - Desiccated (5 days)
  - Revived (after 5 days)
  - Desiccated (10 days)
  - Revived (after 10 days)

**Germination Stages**

**Ascorbic Acid Content (mg/g D. wt.)**

Control, Dist. Water, Ascorbic Acid
In Wheat, Ragi and Sesamum, drought coefficients calculated for the above mentioned various metabolic activities by the procedure outlined on pp. 34 of Experimental Procedure are presented in Tables 1-10 of Appendix X. These results also fully corroborate the observations made in the preceding pages.