APPENDIX

7. APPENDIX

1. Assay for xylose (glucose) isomerase enzyme activity

1. **Tris-HCl buffer pH 7.0 (0.1 M)**

2. **Substrate for xylose / glucose isomerase:**

xylose (70mM)	0.105 gm
0.1M Tris-HCl buffer (pH 7.0)	10 ml
Glucose (0.8 M)	1.44 gm
0.1M Tris-HCl buffer (pH 7.0)	10 ml

3 **0.5 M $HClO_4$**

4 **1.5% Cysteine hydrochloride**

Cysteine hydrochloride	1.5 gm
Distilled water	100 ml

5 **70% Sulphuric acid**

6 **0.12% alcoholic solution of carbazole**

Carbazole	0.12 gm
Ethanol	100 ml

2. Estimation of total protein content in the enzyme samples.

1. **Standard Bovine serum albumin (BSA):**
A stock solution was prepared by dissolving 100 mg of BSA in 100 ml of water in a standard flask. 10 ml of the stock was diluted to 100 ml to get a working standard containing 100 μg/ml.

2. **Coomassie Dye reagent**

CBB G-250	100 mg
Methanol	50 ml
Othrophosphoric acid	100 ml

Diluted to 200ml with distilled water.

3. Determination of molecular weight by SDS PAGE (Laemmli, 1970)

1.Acrylamide-bisacrylamide Solution (30%):

Acrylamide	29.2 gm
bis acrylamide	0.8 gm
Distilled water	100 ml

filtered through Whatman No.1 filter paper. The filtrate was stored in a brown bottle.

2. 4X Separating gel buffer (1.5 M Tris , pH 8.8):

Tris (1.5 M) 18.21 gm

Dissolve in about 80 ml of distilled water, adjust the pH to 8.8 with conc. HCl and make up the volume to 100 ml.

3. 4X Stacking gel buffer (0.5M Tris, pH 6.8):

Tris (0.5 M) 6.06 gm

Dissolve in about 80 ml of distilled water adjust the pH to 8.8 with conc. HCl and make up the volume to 100 ml.

4. SDS Solution (10%):

SDS (AR)	10 gm
Distilled water	100ml

5. Initiator : (10% APS):

Ammonium per sulphate	0.1 gm
Distilled water	1.0 ml

Prepare fresh and store on ice

6. Catalyst:

TEMED is supplied in brown bottle and store at 4°C.

7. Bromophenol blue (0.1%)

Dissolve 5 mg bromophenol blue in 5 ml of water.

8. 2X Sample Buffer:

Tris (0.5 M, pH 6.8)	2.5 ml (4X stacking gel buffer)
10% SDS	4.0 ml
Glycerol (100%)	2.0 ml
β-mercaptoethanol	0.8 ml
Bromophenol blue (0.1%)	0.3 ml
Distilled water	10.0 ml

9. Electrophoresis Buffer (pH 8.3):

Tris (0.025 M)	3.02 gm
Glycine (0.192 M)	14.42 gm
SDS (0.1%)	1 gm
Distilled water	1000ml

10. Coomassie Staining solution:

CBB R-250	0.25 g
Methanol: water (1:1)	90 ml
Glacial acetic acid	10 ml

The solution was filtered through Whatman No.1 filter paper.

11. Destaining Solution:

Methanol: water (1:1)	90 ml
Glacial acetic acid	10ml

4. Substrate gel electrophoresis for the determination of enzyme activity

1. xylose substarate (50mM):

xylose	0.75 gm
10mM $MnCl_2$	0.197gm
1mM $CoCl_2$.	0.023gm

dissolved in 20mM Tris HCl, pH 7.0

2. 2,3,5 Triphenyl tetrazolium (0.1%):

Triphenyl tetrazolium	0.1 gm
1N NaOH	4 gm /100ml

3. Hydrochloric acid (2N):
   Dilute concentrated 11.6 N HCl to 2 N by 6X dilution with distilled water.

5. Dot Blot and Western blot Analysis

1. Wash buffer (TST):

50mM Tris base	6.05 gm
150mM sodium chloride	8.76 gm
0.05% Tween 20	0.5 ml
Distilled water	1000 ml

2. Blocking buffer: (5% skimmed milk in TST)

3. Primary antibody (1:250)

4. Secondary antibody (1:1000)

5. Substrate buffer:

100mM sodium chloride	5.84 gm
5mM Magnesium chloride	1.01 gm

100mM Tris-HCl pH 9.5

6. Substrate: TMB/H_2O_2 (10 X)

7. Transferring buffer for western blot analysis pH 8.6:

Tris	3.03 gm
Glycine	14.4 gm
10% SDS	8ml
Methanol	200ml

The solution was made upto one litre

6. DNA Isolation method

1. Extraction Buffer:

0.35 M sorbitol	6.37 gm
5 mM EDTA	0.18 gm
1% mercaptoethanol (added just before use).	
100 mM Tris-HCl pH 8.0	100 ml

2. High- salt CTAB Buffer:

4 M NaCl	0.233 gm
1.8% CTAB	1.8 gm
25 mM EDTA	0.93 gm
50 mM Tris-HCl pH 8.0	100 ml

3. Chloroform: isoamyl alcohol (24:1 v/v)

4. Isopropanol (100%)

5. TE Buffer (pH 8)

6. Ribonuclease A, diluted to 5 mg/ml

7. Sodium acetate solution (3M adjusted to pH 5.2)

8. Phenol:chloroform (1:1 v/v)

9. Chloroform (100%)

10. Ethanol (75% and 100%)

7. Total RNA Extraction

1. Denaturing solution:
   4.5M Guanidine hydrochloride
   0.5M Tris-HCl (pH 7.6)
   2% Sodium-lauryl-sarcosinate

2. Water saturated phenol

3. Chloroform: isoamyl alcohol (49:1)

4. 100% isopropanol

5. 75% ethanol

6. Diethyl pyrocarbonate (DEPC) water

8. Agarose gel electrophoresis

1. **TAE buffer (50 X)**

Tris base	242 gm
Glacial Acetic acid	57.1 ml
EDTA (0.5 M, pH 8.0)	18.6 gm
Distilled Water	1000 ml

2. **Gel loading buffer (6X)**

Bromophenol blue	0.25%
Xylene cyanol	0.25%
Glycerol	30%

3. **Preparation of Agarose solution for casting gel**

50 X TAE	0.6 ml
Distilled water	30.0 ml
Agarose (low EEO)	
1.0 %	0.3 gm
1.5%	0.45 gm
2.0%	0.6 gm