APPENDIX

1. **MS Sulphates (1X stock)**

   For one litre

$MgSO_4.H_2O$	-	370mg
$MnSO_4.H_2O$	-	22.3mg
$ZnSO_4.7H_2O$	-	8.6mg
$CuSO_4.5H_2O$	-	0.025mg

2. **MS non-sulphates (1X stock)**

   For one litre

NH_4NO_3	-	1650mg
KNO_3	-	1900mg
KH_2PO_4	-	170mg
$CaCl_2.2H_2O$	-	440mg
KI	-	0.83mg
H_3BO_3	-	6.2mg
$CoCl_2.6H_2O$	-	0.025mg
Na_2MoO_4	-	0.25mg

3. **Iron-EDTA (200X stock)**

$FeSO_4.7H_2O$	-	557mg
Na_2 EDTA	-	745mg

Heat both solutions with 40ml double distilled water with stirrer to dissolve separately.Add ferrous sulphate solution slowly to the EDTA solution by warming and mix well.Make upto 100ml and store in refrigerator.

4. B_5 Vitamins (100X)

For 100ml

Myoinositol	-	1gm
Nicotinic acid	-	100mg
Pyridoxine HCl	-	10mg
Thiamine HCl	-	100mg

Store in freezer space.

5. Benzylaminopurine (BAP) 1mM stock

22.52mg of BAP is dissolved in 1N NaOH (0.3-0.5ml).Add slowly double distilled water with stirring and make the final volume to 100ml.Store in the refrigerator.

6. Napthalene acetic acid (NAA) 1mM stock

18.62mg of NAA is dissolved in 0.5ml of DMSO. Slowly double distilled water is added with
stirring and volume is made upto 100ml. Store in the freezer. Note: If precipitation occurs, add 1 or 2 drops of HCl.

7. Indole butyric acid (IBA) 1mM stock

17.52mg of IBA is dissolved in 1N NaOH and double distilled water is added slowly and final volume is made upto 100ml. Store in the freezer.

8. Hoagland's solution

Macronutrients

$MgSO_4$	-	0.52gm

$Ca(NO_3)_2.H_2O$	-	0.94gm
KNO_3	-	0.66gm
$NH_4H_2PO_4$	-	0.12gm
Iron chelate	-	0.07ml

Micronutrients

H_3BO_3	-	28.0gm
$MnSO_4$	-	34.0gm
$CuSO_4$	-	1.0gm
$ZnSO_4$	-	2.2gm
$(NH_4)_6MO_7O_2$	-	1.0gm
H_2SO_4	-	5ml/l

0.1ml of micronutrient solution is added to 1 litre of macronutrient solution.

9. LB Medium

Yeast extract	-	0.5%
Tryptone	-	1%
Sodium chloride	-	1%

Adjust the pH to 7.2 with 2M NaOH solution.

10. Assay of β-glucuronidase (*GUS*) activity

Reagents

Preparation of Sodium phosphate buffer for 100ml (50mM)

This is made by mixing two stock solutions and pH adjusted to 7.

Stock A

0.05M $NaH_2PO_4.H_2O$

It is prepared by dissolving 0.78 gm/100ml.

Stock B

0.05mM $Na_2HPO_4.H_2O$

It is prepared by dissolving 0.7 gm/100ml.

39ml of stock A was mixed with 61ml of stock B and pH was adjusted to 7.

0.5M MES (pH 5.6)

For 100 ml, dissolve 9.76g of MES in 80 ml of dH_20. Adjust pH to 5.6 with NaOH andmake to volume. Store at room temperature.

0.3% Formaldehyde, 10mM MES (pH 5.6), 0.3M mannitol

Formaldehyde 750.0 ml, 0.5M MES 2.0 ml, Mannitol 5.46g.

Preparation of X-gluc (5 Bromo-4 chloro- 3 indolyl-β-D-glucuronide) for 10ml (2mM)

5.2mg of X-gluc was dissolved in 100µl of dimethyl sulphoxide (DMSO) in a glass tube. 8ml of phosphate buffer was added to it slowly, otherwise precipitation occurs. 2ml of methanol was added to it (final concentration 20%).

70%Ethanol

70ml of ethanol is mixed with 30ml of water.

Acetone : Methanol

1: 3 volumes of acetone and methanol are mixed and used.

50% Glycerol

50ml of glycerol is mixed with 50ml of water.

11. DNA isolation and analysis of gene incorporation

DNA extraction buffer

Component and final concentration	**Amount to add per 500ml**
1.4M NaCl	140ml of 5M
20mM EDTA	20ml of 0.5M (pH 8.0)
100mM Tris-Cl	50ml of 1M (pH 8.0 at 25 °c)
3% (w/v) CTAB	15gm
H_2O	to make 500ml
1% (w/v) ß-mercaptoethanol	5ml of 14.4M

The components were combined together and β-mercaptoethanol was added just before use.

1M Tris (pH 8.0)

121.1gm of tris base is dissolved in 800ml of water.Adjust the pH value by adding concentrated HCl and make upto 1000ml.Dispense into aliquots and sterilize by autoclaving.

0.5M EDTA (pH 8.0)

186.1gm of disodium EDTA is added to 800ml water.Stir vigorously on magnetic stirrer and adjust the pH to 8.0 with sodium hydroxide (20gm of NaOH pellets).Dispense into aliquots and sterilize by autoclaving.

5M NaCl

292.2gm is dissolved in 1000ml.Dispense into aliquots and sterilize by autoclaving.

Phenol : Chloroform

Equal volumes of phenol and chloroform are mixed and the pH is adjusted to above 7.5 by extracting several times with 0.1M Tris.Cl (pH 7.6)as DNA will get degenerated at acid pH.The equilibrated mixture is stored under equal volumes of 0.01M Tris.Cl (pH 7.6)at 4°c in dark glass bottle.

Chloroform : Isoamylalcohol

Equal volumes of chloroform and isoamylalcohol are mixed and stored at 4°c.

Isopropanol

70%Ethanol

70ml of ethanol is mixed with 30ml of water.

Resuspension buffer 2 (RB_2)

Stock	**Working standard**	**for 100ml**
1M Tris	10mM Tris pH 8	add 1 ml from stock
0.5m EDTA	1mM EDTA pH 8	add 0.2ml from stock

12. Polymerase Chain Reaction

Materials

Genomic DNA

10ng of genomic DNA.

Primers

Primers were obtained from Promega Ltd., and the primers used were 21 nucleotide sequence primers.

P5CS - 5'-ACA TTA TAC TCG TCT CCT CTG G-3'

5'-AAT TTT GTT TCC TTT CCT CTG A-3'

*hpt*II - 5'- GAG GCT ATT CGG CTA TGA CTG- 3'

5'-ATC GGG AGC GGC GAT ACC GTA-3'.

Reaction mixture

Finnzymes PCR kit was used.

PCR buffer	-	10X buffer
$MgCl_2$	-	50mM
dNTP's conc	-	10mM

Primers	-	100µM
Taq polymerase	-	0.5 U

PCR machine

Eppendorf Mastercycler PCR system.

Concentration of agarose gel

0.8 % of agarose gel was used to view the amplified product after polymerase chain reaction.

13. Agarose Gel

50X TAE buffer

Tris	-	24.2gms
0.5M EDTA (pH 8.0)	-	10.0ml
Glacial acetic acid	-	5.71ml

All the reagents are mixed and the final volume was made up to 100ml.It is sterilized by autoclaving and is diluted 1 to 50 with double distilled water for use.

Tracking dye or Loading buffer

0.25% Bromophenol blue

40%(w/v)Sucrose in water.

The volume is made up to 100ml and is stored at 4°c.

Ethidium bromide - 10mg/ml

14. Determination of proline content

3% Sulphosalycylic acid

3g of sulphosalicylic acid is dissolved in 100ml of distilled water.

6N Orthophosphoric acid

38.1ml is made upto 100ml with distilled water to get 6N orthophosphoric acid.

Acid ninhydrin

1.25g of ninhydrin was dissolved in a blend of 30ml of glacial acetic acid and 20ml of 6N orthophosphoric acid. Store at 4°c and use within 24hr

15. Southern blotting

Digestion of genomic DNA

Genomic DNA	40.0µl
Sterile water	4.0µl
10X buffer	4.0µl
*ECoR*I	1.0µl
Rnase	1.0µl
Total volume	50.0µl

The samples are digested at 37^{o}C for overnight.

Alkali buffer (0.4M NaOH)

16gms of sodium hydroxide was dissolved in one litre of water.

Hybridization with probe

Hybridization buffer

1.4625gm of NaCl and 2gm of blocking reagent were added to 50ml of hybridization buffer (0.5M NaCl as final concentration and 4% (w/v) as final concentration of blocking reagent).The mixture was made to a fine suspension by stirring for 2hrs on magnetic stirrer and used immediately.

Primary wash buffer (200ml)

Urea	-	24gm
SDS	-	0.2gm

Sodium dihydrogen phosphate (pH 7.0)	-	20ml
Sodium chloride	-	1.74gm
Magnesium chloride	-	0.2ml (1M)
Blocking reagent	-	0.4gm

All the reagents were mixed before use.

Secondary wash buffer-20X stock (100ml)

Tris base	-	12.1gm
Sodium chloride	-	11.2gm

Both were mixed and the final volume was made upto 100ml with the pH adjusted to 10.0 and stored at 2-8°c.

Working concentration

5ml of 20X stock solution of secondary wash buffer and 0.2ml of 1M $MgCl_2$ are added to 95ml of water,which was used immediately.

2X SSC

NaCl	-	17.532gm
Trisodium Citrate	-	8.823gm

Both the chemicals are mixed well.The pH was adjusted to 7.0-7.2 and the final volume is made upto 1 litre.

16. Evaluation of tolerance to salinity in transgenic (T_1) plants

200mM NaCl

1.16g of NaCl was dissolved in 100ml of water.

17. Lipid peroxidation

0.1% w/v TCA

0.1g of Trichloroacetic acid was dissolved in 100ml of water.

20% (w/v) TCA

20g of Trichloroacetic acid was dissolved in 100ml of water.

0.5% TBA diluted in 20% (w/v) TCA

0.5g of Thiobarbituric acid was dissolved in 20% (w/v) TCA.

18. Estimation of Chlorophyll

80% Ethanol

80ml of ethanol is mixed with 20ml of water.