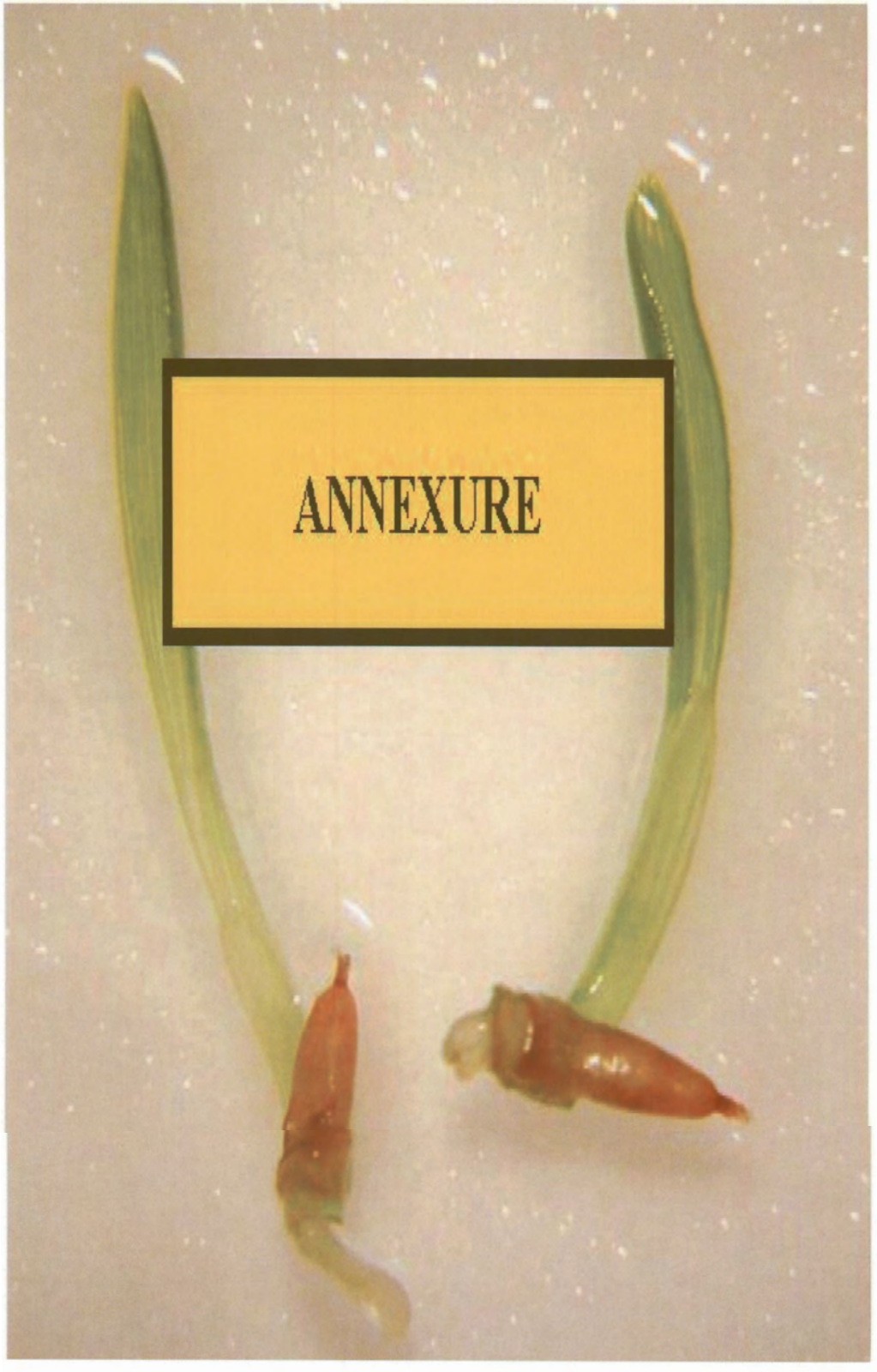

ANNEXURE

Buffers for DNA work

Plant genomic DNA

Extraction buffer

100 mM Tris-HCl (pH 8); 1.4 mM sodium chloride (NaCl); 20 mM ethylenediamine tetra-acetic acid, disodium salt ($Na_2EDTA.\ 2H_2O$); 1% 2-mercaptoethanol and 3% hexadecyltrimethyl-ammoniumbromide (CTAB).

To make 500 ml buffer solution the following was added: 6.05 g Tris-base; 810 mg of NaCl; 2.92 g of $Na_2EDTA.\ 2H_2O$ and 15 g of CTAB was added to 400 ml of dsH_2O. The pH was adjusted to pH 8 with 10 N NaOH. 2-mercaptoethanol was diluted from a 50 mM stock solution on the day of use. 2-mercaptoethanol (350 µl) was added to 100 ml dsH_2O and 1.75 ml of the stock solution was added to 400 ml of the buffer solution and finally dsH_2O was added to make up a 500 ml extraction buffer.

Table A.1: DNA extraction buffer (pH 8)

Chemical	Concentration	Mass/volume
Tris-HCl	100 mM	6.05 g/500 ml buffer
NaCl	1.4 mM	0.81 g/500 ml buffer
EDTA	20 mM	2.92 g/500 ml buffer
ß-mercaptoethanol	50 mM	1.75 ml/500 ml buffer
CTAB	3%	15 g/500 ml buffer
Total		500 ml

The buffer was pre-heated on the day of use in a water bath at 60°C.

Bacterial DNA isolation

Resuspension buffer

50 mM glucose; 25 mM Tris-HCl, pH 8; 10 mM $Na_2EDTA.2H_2O$

Glucose (4.5 g), $Na_2EDTA.2H_2O$ (1.46 g) and ice-cold Tris-base (7.5 ml) were dissolved in dsH_2O (400 ml). The pH was set up with 10 N NaOH to pH 8 and the final volume was made up with dsH_2O to 500 ml. The mixture was autoclaved for 20 minutes and 100 µg/ml of RNAase A was added after cooling down to room temperature and the buffer was stored at 4°C.

Alkaline lysis buffer

200 mM NaOH; 1% SDS

NaOH (8.0 g) pellets were dissolved into dsH_2O (950 ml) and 25 ml of a 10% sodium dodecyl sulphate (SDS; sodium lauryl sulfate) solution was added.

10% SDS stock solution

SDS (10%) was made up the day before use by dissolving SDS (100 g) into dsH_2O (900 ml) using a protection shield to avoid breathing the dust. The mixture was heated to 68°C to assist the dissolution. The pH was adjusted to 7.2 by adding a few drops of concentrated HCl and the volume was adjusted to 1 l with dsH_2O. The 10% SDS solution was not further sterilized.

Neutralization buffer

3 M potassium acetate (pH 5.5)

Potassium acetate (294.5 g) was dissolved in sdH_2O (500 ml). The pH was adjusted to 5.5 with glacial acetic acid (~110 ml) and the volume adjusted to 1 l with dsH_2O.

Low TE buffer

10 mM Tris; 0.1 mM Na_2 EDTA. $2H_2O$ (pH 8)

Tris-base (18 mg) and Na_2EDTA. $2H_2O$ (121 mg) were added to dsH_2O (75 ml), mixed well and 10 N of NaOH was used to set the pH to 8 and then dsH_2O was added to 100 ml. The buffer was made up the day before use.

Precipitation solution

3 M sodium acetate (NaAc) (pH 4.8)

Sodium acetate (40.8 g) was dissolved first in 90 ml dsH_2O, the pH was adjusted to 6.8 with acetic acid and then dsH_2O was added to a final volume of 100 ml.

Buffers for Southern blotting

Denaturation solution for DNA transfer

1.5 M NaCl; 0.5 M NaOH

Sodium chloride (43.83 g, NaCl) and sodium hydroxide (10 g, NaOH) were dissolved in dsH_2O (400 ml) and made up to a final volume of 500 ml by adding dsH_2O. The solution was sterilized by autoclaving.

Neutralization solution for DNA transfer

1.4 M NaCl; 0.5 M Tris-HCl

Sodium chloride (43.83 g) and Tris-base (30.27 g) were dissolved in dsH_2O (400 ml). The pH was adjusted to 7.5 by adding concentrated HCl slowly and carefully under stirring. Finally the volume was made up to 500 ml with dsH_2O and the mixture was autoclaved.

Hybridization buffer

5×SSC; 0.1% (w/v SDS); Dextran sulfate sodium salt; liquid block (Amersham life science, UK)

Into dsH_2O (800 ml), NaCl (175.3 g) and Na_3-citrate $2H_2O$ (88.2 g) were dissolved to produce a 20×SSC stock solution. The pH was adjusted to 7.0 with a few drops of 10 **N** NaOH and dsH_2O was added to a final volume of 1 l. Aliquots were sterilized by autoclaving.

For the preparation of the hybridization buffer, dsH_2O (26.6 ml), 20×SSC (10 ml), 10% SDS (0.4 ml) and liquid block (2 ml) were mixed to make up a hybridization stock solution in which dextran sulfate sodium salt (2 g) was dissolved at 60°C in a total volume of 40 ml.

Wash buffer

Buffer 1

1×SSC; 0.1% (w/v) SDS

To make up 1×SSC, 20×SSC (20 ml) and 10% SDS (4 ml) was added to 376 ml of dsH_2O for a total volume of 400 ml.

Buffer 2

0.5×SSC; 0.1% (w/V) SDS

20×SSC (10 ml) (stock solution) and 10% SDS (4 ml) (stock solution) were added to dsH_2O (386 ml) for a total volume of 400 ml. Both buffers were autoclaved for 20 minutes at 105 kPa to avoid any contamination.

Incubation and blocking buffer

100 mM Tris-HCl; 300 mM NaCl (pH 9.5)

NaCl (58.76 g) and Tris-base (6.05 g) were dissolved in dsH_2O (400 ml). The pH was adjusted to 9.5 with concentrated HCl and dsH_2O was added to a total volume of 500 ml and autoclaved in a 1 l bottle for 20 minutes at 105 kPa.

Table A.2: Buffers/Southern blotting

Required solutions	**Description**	**Concentration**	**Mass/Volume**
HCl	250 mM	250 mM	73 ml/500 ml H_2O
dsH_2O	Distilled, sterile water		
Denaturation buffer	0.5 N NaOH 1.5 M NaCl	0.5 N 1.5 M	10 g/500 ml buffer 43.83 g/500 ml buffer
Neutralization buffer	0.5 M Tris-HCl pH 7.5 1.5 M NaCl	0.5 M 1.5 M	30.27 g/500 ml buffer 43.83 g/500 ml buffer

20×SSC buffer	3 M NaCl 300 mM sodium citrate, pH 7.0	3 M 300 mM	97.66 g/1000 ml buffer 88.2 g/1000 ml buffer
5×SSC	750 mM NaCl 75 mM sodium citrate, pH 7.0	750 mM 75 mM	43.83 g/1000 ml buffer 22.05 g/1000 ml buffer
10%SDS	High SDS buffer	10%	100 g/900 ml H_2O
Hybridization buffer	5×SSC 0.1% (w/v SDS) Dextran sulfate liquid block		500 µl/40 ml buffer 400 µl/40 ml buffer 2 g/40 ml buffer 2 ml/40 ml buffer
2×wash buffer 0.5×wash buffer	2×SSC 0.1% SDS 0.5×SSC 0.1% SDS	 0.1% 0.1%	

Buffers for DNA colony hybridization

Denaturation buffer

0.5 N NaOH; 1.5 M NaCl

NaOH (10 g) pellets and NaCl (43.8 g) were dissolved in dsH_2O (500 ml) and the mixture was sterilized by autoclaving for 20 minutes.

Neutralization buffer

10 M Tris-HCl (pH 7.5); 1.5 M NaCl

Tris-base (60.5 g) and NaCl (43.8 g) were dissolved in dsH_2O (400 ml). The pH was adjusted by adding concentrated HCl slowly and carefully with stirring to 7.5. Finally, the volume was made up to 500 ml by adding dsH_2O and autoclaved for sterilization.

Buffers for DNA electrophoresis

Tris -acetate (TAE) buffer

Stock solution (50×TAE)

50×TAE: contained 2 M Tris-base; 0.5M $Na_2EDTA.2H_2O$ and glacial acetic acid (pH 8). To make up a 1 l stock solution, Tris-base (242 g) was added to 0.5 M $Na_2EDTA.2H_2O$ solution (100 ml) and dsH_2O (800 ml) were added. The pH was adjusted to 8 with 57.1 ml of glacial acetic acid and the volume made up to 1 l with dsH_2O. The stock solution was stored at room temperature in a glass bottle after autoclaving.

1×TAE buffer for DNA electrophoresis

50×TAE (200 ml) was diluted with dsH_2O (9.8 l) in a total volume of 10 l for a final concentration of 0.04 M Tris-acetate and 1 mM $Na_2EDTA.2H_2O$ .The buffer was stored at room temperature, away from light, for further uses.

Table A.3: TAE buffer (50×stock)

Chemical	Concentration	Mass/Volume
Tris	2 M	242 g
EDTA disodium salt	0.5 M	37.2 g
Glacial acetic acid	5.71% (w/v)	57.1 ml
Total volume		1000 ml

DNA loading buffer (agarose gel)

Table A.4: DNA loading buffer

Chemical	Concentration	Mass/Volume
Glycerol	50%	5 ml
TAE buffer	1×	200 µl of 50×stock
Bromophenol blue	1%	0.1 g
Xylene cyanol	1%	0.1%

Agarose gel composition

Agarose gels had the following composition:

Table A.5: Agarose gel composition

Tray	1×TAE buffer	1% agarose	1.5% agarose
7×10 cm	50 ml	0.50 g	0.75 g
15×10 cm	100 ml	1 g	1.50 g
15×15 cm	150 ml	1.5 g	2.25 g

Polymerase chain reaction buffer

10×PCR buffer

PCR reaction buffer consisted of a 10×PCR buffer (Takara, Japan) containing 500 mM KCl; 25 mM $MgCl_2$; 100 mM Tris-HCl (pH 8.3).

Buffers for RDA technique

Ligation buffer

10×ligase buffer contained 66 mM Tris-HCl (pH 7.6); 6.6 mM $MgCl_2$; 10 mM dithiothreitol (DDT) and 66 mM ATP. The supplier of ligase supplied the ligation buffer (Amersham, UK).

Elution TE-buffer

10 mM Tris-HCl (pH 8); 0.1 mM $Na_2EDTA.\ 2H_2O$

Tris- base (605 mg) and $Na_2EDTA\ 2H_2O$ (9 mg) were dissolved in dsH_2O (400 ml). The pH was adjusted with 10 N HCl to 8 and the volume was adjusted to 500 ml with dsH_2O.

Buffer for subtractive hybridization

30 mM EPPS [(N-[2-hydroxyethyl] piperazine)-N'-(3-propane sulfonic acid; HEPPS)] (pH 8.0) at 20°C; 3 mM $Na_2EDTA.\ 2H_2O$; 5 M NaCl.

EPPS (1.51 g), $Na_2EDTA\ 2H_2O$ (220 mg) and NaCl (58.43 g) were dissolved in dsH_2O (150 ml). The pH was adjusted to 8 by stirring the solution at 20°C and the total volume was set up to 200 ml with dsH_2O.

10 M Ammonium acetate

Ammonium acetate (770 g) was dissolved in dsH_2O (800 ml). The volume was adjusted with dsH_2O to 1 l and the mixture sterilized by filtration.

10×Mung bean nuclease buffer

50 mM Tris-HCl (pH 8.9)

Tris-base (300 mg) was dissolved in dsH_2O (90 ml) and the pH adjusted to 8.9 dsH_2O was added to a total volume of 100 ml.

Cloning reagents

Isopropyl-β-D-thiogalactopyranoside; IPTG (0.1 M stock solution)

IPTG (1.2 g) was dissolved in dsH_2O (50 ml) and the stock mixture was filter-sterilized and stored at -20°C.

5-bromo-4-chloro-3-indolyl- β-D-galactoside; X-gal (2% stock solution)

X-gal (20 mg) was dissolved in dimethylformamide (DMF) (1 ml). The stock mixture was covered with aluminum foil and stored at -20°C.

Ampicillin50

Ampicillin (50 mg) was dissolved in dsH_2O (1 ml). The mixture was filter-sterilized and stored at -20°C.

Growth media

Bacteria growth medium (Luria–Bertani broth)

To dsH_2O (1 l), Tryptone (10 g); Yeast extract (5 g) and NaCl (10 g) were added and the pH was adjusted to 7.4 with NaOH. For a solid medium LB medium, agar (15 g) was added. Both media were sterilized by autoclaving to avoid any contamination.

LB plates with ampicillin

LB-agar medium (30-35 ml) was poured into 85 mm petri dishes before adding ampicillin (20 or 40 µl/plate) to a final concentration of 50 µg or 100 µg/ml. The medium was allowed to cool down to 50°C. After agar hardened, the plates were stored at 4°C for up to one month or at room temperature for up to one week.

LB plates with ampicillin/ IPTG/X-gal

LB plates containing ampicillin were produced as outlined above but then supplemented with IPTG (20-100 µl of 0.1 M stock solution) and X-gal (20-35 µl of a 2% stock solution). The LB agar was mixed with the reagents and plates were dried for 30 minutes at room temperature.

Table A.6: Growth medium composition

Reagents	Concentration	Quantity/plates	Mass/volume
IPTG	100 mM	20-100 µl	1.2 g/50 ml dsH_2O
X-gal	2%	20-35 µl	0.02 g/1 ml DMF
Ampicillin	50-100 µg/ml	20-35 µl	50 mg/1 ml dsH_2O
Tryptone			10 g in 1 l dsH_2O
Yeast extract			5 g/1 in1 l dsH_2O
NaCl			10 g/1 in 1 l dsH_2O
Agar			15 g/1 in 1 l LB broth
LB medium		25-35 ml	

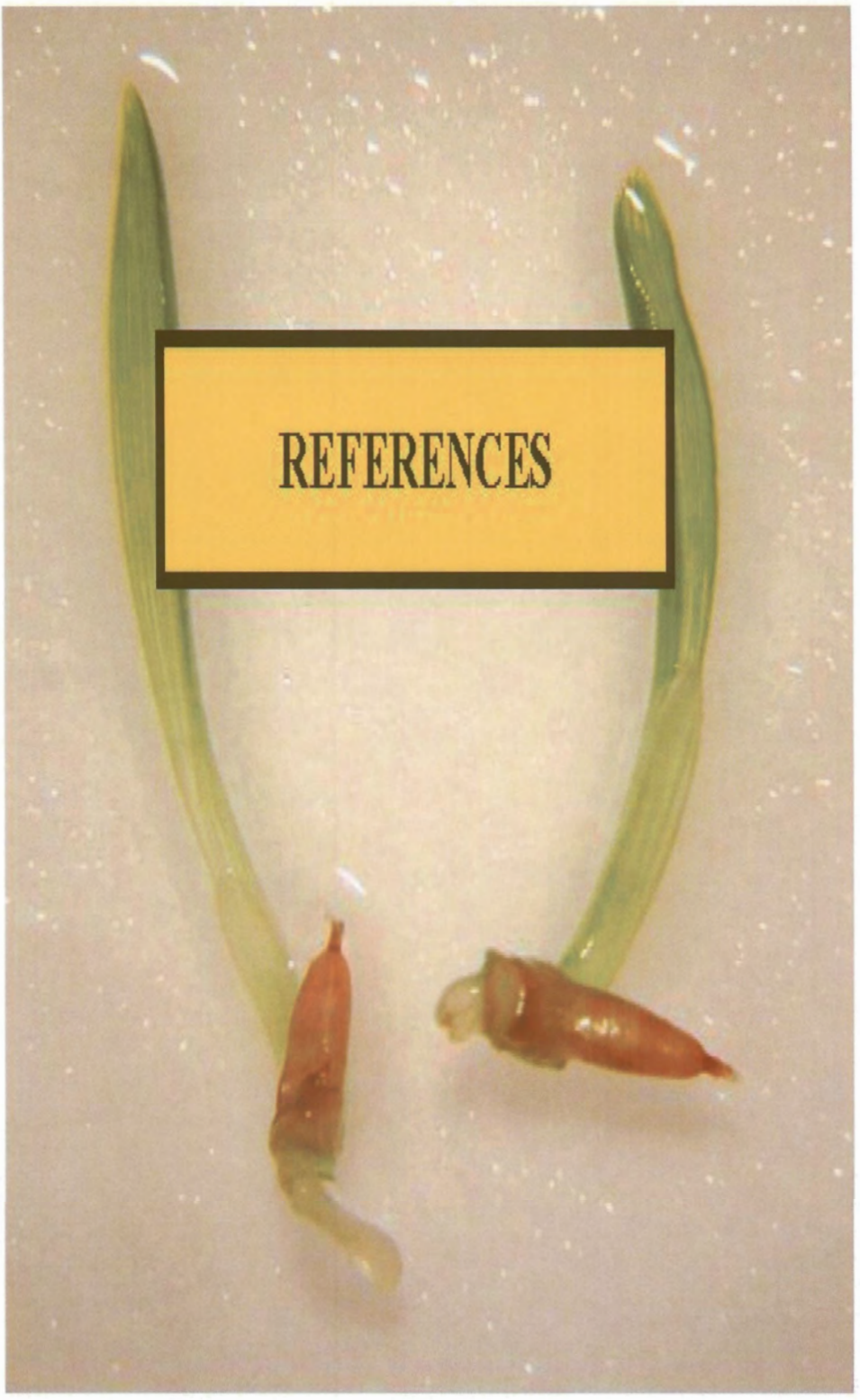
Sequence of primers used in experiments

Table A.7: Sequences of all the primers used for amplification of the DNA regions." ITS" represents the sequence of the primer set used to amplify the internally transcribed spacer sequence (ITS region); "NTS" primer set used to amplify the non-transcribed spacer (NTS region); "Retrotransposon" primer used to amplify a retrotransposon like region from the grass species *Monocymbium ceresiiforme*. "DP510" primer set used to amplify a fragment with homology to *Bacillus halodurans* region and "*Bacillus subtilis*" the primer set used to amplify the *Bacillus subtilis* 16s rRNA region.

DNA regions	Primer	Primer sequence
ITS	ITS1	5'-TCCGTAGGTGAACCTGCGGG-3'
	ITS4	5'-GCTGCGTTCTTCATCGATGC-3'
NTS	NTS1	5'-TTTAGTGCTGGTATGATCGC-3'
	NTS2	5'-TTGGAAGTCCTCGTGTTGCA-3'
Retrotransposon	S3Cl2L	5'-CTCGGTATCGAGGGAGA-3'
	S3CL2R	5'-TTTCAAGAATGCTCTGCAGG-3'
DP 510	Bhal5R	5'-CCGCGCTTGAACAAAGTATT-3'
	Bhal3L	5'-TTCACATTGGAGTTTTGGGA-3'
	Bhal5A	5'-ACCGACGTCGACTATCCATGAACAA-3'
	Bhal3A	5'-AAGCTTGTTCATGGATAGTCGACGTCGGT-3'
Bacillus subtilis	Bsub3R	5'-CCAGTTTCCATTGACCCTCCCC-3'
	Bsub5F	5'-AAGTCGAGCGGACAGATGG-3'

Table A.8: Sequence of the three adaptor sets used for execution of the RDA.

RDA adaptor sets	Adaptor sequence
Set 1	
RHind12	5'-AGCTTCGGGTGA-3'
RHind24	5'-AGCACTCTCCAGCCTCTCACCGCA-3'
Set 2	
JHind12	5'-AGCTTGTTCATG-3
JHind24	5'-ACCGACGTCGACTATCCATGAACA-3'
Set 3	
NHind12	5'-AGCTTCTCCCTC-3'
NHind24	5'-AGGCAGCTGTGGTATCGAGGGAGA-3'



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SOMMAIRE

Le génome des graminées diffère en taille; degré de ploïdy; et nombre de chromosome. Depuis le siècle dernier, les methodes d´ identification et de charactérisation des génomes ont dramaticallement changées dans la reproduction des plantes. Elles sont passées des croisements simples aux croisements retour jusqu'aux techniques moleculaires actuelles. L'analyse des différences représentatives de deux génomes qui en est une des techniques moleculaires, a été appliquée sur l'avoine sauvage collectée á différents endroits en Afrique du Sud pour isoler une unique fraction de son génome. Cinq séries d'hybridation soustractive ont été appliquée. Après la deuxième série, un produit différentiel obtenu etait homologue à une séquence connue de 'rétrotransposon'du maïs et aussi à une région chromosomique du riz. Ce produit de soustraction n'était pas unique à une seule des échantillons testées. En plus, ce produit avait aussi un nombre élevé de copies dans le genome de la plante. La troisième, quatrième et cinquième tour d'hybridation soustractive ont été aussi appliquées. La cinquième étape d'hybridation soustractive a été appliquée sur un quatrième produit ayant subi une digestion enzymatique au *Mse*I reconnue active pour couper l'ADN repetitif. Ce cinquième produit de soustraction analysée etait homologue a une séquence de l'ADN bactérienne, ainsi qu'a une séquence partielle d'ADN de riz et de mil. L'homologie du produit de différence génomique à une séquence d'ADN bactérienne nous a fait penser à la contamination de l'ADN de départ par une bactérie endophyte de la plante. Pour s'assurer de la pureté de notre matériel de départ, l'ADN isoler des plantes a été utilisée pour amplifier un fragment de 595 bp caractéristique de la région 16S de l'ADN ribosomal du *Bacillus subtilis*. Cette réaction a été négative. De même l'identification du *Bacillus subtilis* comme endophyte spécific de la même plante a donné plutôt lieu à d'autres espèces bactériennes.