APPENDIX 1 : General plan of the field

REP 3	A1	A1	B1	B1	B1	B1	A3	A3	B4	B4	A4	A4	C3	C3	B3	B3	C1	C1	C4	C4	A2	A2	B2	B2	A1	A1
REP 2	B4	B4	C4	C4	A2	A2	A1	A1	C3	C3	B1	B1	C1	C1	A4	A4	A3	A3	B2	B2	C2	C2	C5	C5	B3	B3
REP 1	A2	A2	A4	A4	C3	C3	B1	B1	A3	A3	C5	C5	C2	C2	A1	A1	B3	B3	C4	C4	B4	B4	B2	B2	C1	C1

- Each rectangle represents a row of 25 trees
- * - buffer trees surrounding the plot

APPENDIX 2

DNA extraction (modified CTAB method)

1. Wash leaf samples in 0.1% SDS and rinsed several times in distilled water.
2. Macerate leaf tissue in liquid nitrogen in a mortar using a pestle. Transfer approximately 0.5 g of the frozen leaf powder to 1.5 mL tube containing 1 mL CTAB extraction buffer. Incubate at 65 °C for 30 minutes.
3. Allow the tubes to cool to ambient. Add 200 µL wet chloroform and gently mix contents in the tubes to a single phase. Centrifuge at 13 000 rpm for 4 minutes.
4. Remove upper (aqueous) layer to a clean tube. Perform a second chloroform extraction as in 3 above, but using 500 µL instead of 200 µL wet chloroform.
5. Incubate with 0.2 mg/mL RNAse at 37 °C for 1 hour.
6. Add equal volume (500 µL) of phenol-chloroform-isoamyl alcohol (25:24:1) and transfer the epiphase to a new sterile 1.5 mL tube.
7. Add equal volume (500 µL) wet chloroform and remove the aqueous layer to a clean tube.
8. Precipitate the DNA by adding 0.1x volume of 3 M sodium acetate (pH 5.2) and 2 x volume ice-cold 98% ethanol. Leave either at –20 °C overnight or –80 °C for 2 hours.
9. Centrifuge for 15 minutes at 14 000 rpm.
10. Wash pellet twice with 70% ethanol. Pour away the supernatant and invert tubes to dry at room temperature or spin dry in a Speedvac for 4 minutes. Dissolve pellets overnight at 4 °C in 50 µL TE buffer.

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APPENDIX 3

***Eco*RI/*Mse*I mixture**

Components	Volume (μL)
EcoR I (10 U/μL) Mse I (5 U/μL) R/D buffer	0.125 0.250 1.625
	2

Restriction digestion buffer

Components	Volume (μL)
Tris-Hac pH 7.5 (1 M)	5.0
MgAc (0.1 M)	50.0
KAc (1 M)	25.0
DTT (100mM)	25.0
BSA (10 mg/L)	2.5
dH_2O	392.5
	500.0

Adapter ligation solution

Sequence of EcoRI and MseI adapters

Name	Sequence
EcoRI Adapter1	5'-CTCGTAGACTGCGTACC-3'
EcoRI Adapter2	3'-CTGACGCATGGTTAA-5'
MseI Adapter1	5'-GACGATGAGTCCTGAG-3'
MseI Adapter2	3'-TACTCAGGACTCAT-5'

Components	Volume (μL)
EcoRI Adapter1 (58.48 μM)	4.10
EcoRI Adapter2 (62.97 μM)	3.80
MseI Adapter1 (53.99 μM)	44.45
MseI Adapter2 (44.14 μM)	54.38
ATP (100 mM)	4.80
TrisHCl pH7.5 (1M)	12.00
KCl (1M)	12.00
Glycerol (50%)	600.00
dH_2O	464.47
	1200.00

Preamplification mixture

Sequence of EcoRI-PA and MseI-PA

Name	Sequence
EcoRI-PA	5'-AGACTGCGTACCAATTC/C-3'
MseI-PA	5'-GACGATGAGTCCTGAGTAA/A-3'

Components	Volume (µL)
EcoRI-PA (43.17 M)	3.4
MseI-PA (59.19 M)	2.4
dNTPs (10mM)	1.0
dH_2O	33.2
	40.0

Selective amplification primers

Name	Designation	Primer sequence
EcoRI + 3-ACT	E-ACT	5'-GACTGCGTACCAATTC/ACT-3'
EcoRI + 3-ACG	E-ACG	5'-GACTGCGTACCAATTC/ACG-3'
EcoRI + 3-AGG	E-AGG	5'-GACTGCGTACCAATTC/AGG-3'
EcoRI + 3-ACA	E-ACA	5'-GACTGCGTACCAATTC/ACA-3'
EcoRI + 3-AAG	E-AAG	5'-GACTGCGTACCAATTC/AAG-3'
EcoRI + 3-AGC	E-AGC	5'-GACTGCGTACCAATTC/AGC-3'
EcoRI + 3-AAC	E-AAC	5'-GACTGCGTACCAATTC/AAC-3'
EcoRI + 3-ACC	E-ACC	5'-GACTGCGTACCAATTC/ACC-3'
MseI + 3-CAA	M-CAA	5'-GATGAGTCCTGAGTAA/CAA-3'
MseI + 3-CAC	M-CAC	5'-GATGAGTCCTGAGTAA/CAC-3'
MseI + 3-CAT	M-CAT	5'-GATGAGTCCTGAGTAA/CAT-3'
MseI + 3-CTA	M-CTA	5'-GATGAGTCCTGAGTAA/CTA-3'
MseI + 3-CTC	M-CTC	5'-GATGAGTCCTGAGTAA/CTC-3'
MseI + 3-CTG	M-CTG	5'-GATGAGTCCTGAGTAA/CTG-3'
MseI + 3-CTT	M-CTT	5'-GATGAGTCCTGAGTAA/CTT-3'
MseI + 3-CAG	M-CAG	5'-GATGAGTCCTGAGTAA/CAG-3'

Rox-500 internal standard

Components	Volume (μL)
Deionised formamide	90
Blue loading dye	45
GS-500 Rox	15
	150

TE buffer

- Add 0.2 mL EDTA (0.5M) to 1 mL Tris HCl (1M)
- Adjust to pH 8.0 and bring to 100mL with dH_2O

10 x TBE buffer

- 108 g Tris
- 55.0 g boric acid
- 8.3 g EDTA (Na_2)
- Bring to 1 L with water

Loading dye

Components	Final Concentration	Volume (μL)
Glycerol	50 %	500
EDTA (0.5M)	1 mM	2
Bromophenol Blue	0.4%	4

Bring to 1 mL with dH_2O and store at –20 °C

APPENDIX 4 : ANOVA on effect of various concentrations of BAP on number of shoot buds and shoots formed

Number of shoots

Concentration (mgL^{-1})	Count	Sum	Average	Variance
0.1	20	22	1.1	0.94
0.2	20	34	1.7	0.96
0.5	20	42	2.1	0.73
1	20	37	1.9	1.08
2	20	34	1.7	0.64

Source Variation	SS	df	MS	F	P-value	F crit
Between Groups	10.84	4	2.71	3.118716	0.018561	2.467495
Within Groups	82.55	95	0.868947			
Total	93.39	99				

Number of buds

Concentration (mgL^{-1})	Count	Sum	Average	Variance
0.1	20	98	4.9	11.5
0.2	20	98	4.9	9.1
0.5	20	125	6.3	5.7
1	20	156	7.8	4.8
2	20	111	5.6	10.4

Source Variation	SS	df	MS	F	P-value	F crit
Between Groups	117.06	4	29.265	3.530381	0.009891	2.467495
Within Groups	787.5	95	8.289474			
Total	904.56	99				

APPENDIX 5 : ANOVA on effect of various concentrations of kinetin on number of shoot buds and shoots formed

Number of shoots

Concentration (mgL^{-1})	Count	Sum	Average	Variance
0	4	2	0.5	1
0.1	4	19	4.75	2.92
0.2	4	15	3.75	12.92
1	4	14	3.5	3
2	4	12	3	2.67
5	4	11	2.75	2.92

Source Variation	SS	df	MS	F	P-value	F crit
Between Groups	40.70833	5	8.141667	1.921967	0.140454	2.77285
Within Groups	76.25	18	4.236111			
Total	116.9583	23				

Number of shoot buds

Concentration (mgL^{-1})	Count	Sum	Average	Variance
0	4	4	1	0.67
0.1	4	41	10.25	72.92
0.2	4	36	9	47.33
1	4	27	6.75	24.25
2	4	25	6.25	8.92
5	4	28	7	8

Source Variation	SS	df	MS	F	P-value	F crit
Between Groups	202.7083	5	40.54167	1.500771	0.238781	2.77285
Within Groups	486.25	18	27.01389			
Total	688.9583	23				

APPENDIX 6 : ANOVA on effect of various concentrations of NAA or IBA on number of roots formed

NAA

Concentration (mgL^{-1})	Count	Sum	Average	Variance
1	10	95	9.5	6.94
2	10	95	9.5	19.61
5	10	111	11.1	17.21
10	10	102	10.2	15.07

ANOVA (among treatments)

Source Variation	SS	df	MS	F	P-value	F crit
Between Groups	17.275	3	5.758333	0.391501	0.759829	2.866265
Within Groups	529.5	36	14.70833			
Total	546.775	39				

IBA

Concentration (mgL^{-1})	Count	Sum	Average	Variance
1	10	30	3	4.67
2	10	64	6.4	8.04
5	10	77	7.7	15.34
10	10	93	9.3	2.23

ANOVA (among treatments)

Source Variation	SS	df	MS	F	P-value	F crit
Between Groups	215	3	71.66667	9.464417	9.54E-05	2.866265
Within Groups	272.6	36	7.572222			
Total	487.6	39				