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**Fig. 4.5**  Ethylene production by the shoot cultures under hermetic culture conditions. *In vitro* ethylene levels in 60 cm³ culture tube at 0, 3, 4, 8, 10 and 12 day after inoculating shoots in MS basal medium supplemented without (control) and with STS (4 μM).

**Fig. 4.6**  Relative growth of potato shoot cultures on MS basal medium with (+) and without (-) STS (4 μM) at (a) 0; (b) 4; (c) 8 and (d) 14 dai; (e), inset shows epinasty (Ep) and aerial roots (Ar) at 8 dai when ethylene is produced in medium without STS.

**Fig. 4.7**  q-RT-PCR analysis. Relative transcript levels of *StERS1* of *in vitro* plants growing in the STS (4 μM) supplemented MS basal medium under ethylene atmosphere (S4) and *in vivo* plants growing in the field (SF). Values are the average fold difference of *in vitro* (STS treated) and *in vivo* (field grown) plants relative to *in vitro* plants growing in MS basal medium. Relative transcript values are mentioned on the top of bars. Standard errors of the means are represented by the error bars. Values on the top of the bar represented relative expression ratio.

**Fig 4.8**  Different combinations of auxin and cytokinin alone and in combinations tried to assess regeneration system in potato cv. KG. Values around the segment portion represents number of variable concentrations corresponding to each pair of PGRs used.

**Fig. 4.9**  Internodal explants showing friable callus at the cut ends in cv. Kufri Giriraj (a) and Kufri Sutlej (b) on MS basal medium. Arrow indicated appearance of friable callus at cut end of internodal explants.

**Fig. 4.10**  HPLC Chromatogram of IAA Standard (a) IAA from internodes of cv Kufri Sutlej (KS) (b) IAA from internodes of cv. Kufri Giriraj (KG) (c) IAA content (μg g⁻¹ FW) in the internodes of cvs. KG and KS (d) endogenous IAA content in cvs. KG and KS. Vertical bars are the mean of 3 replicates and value above the bars are the amount of IAA (μg g⁻¹ FW).

**Fig. 4.11**  Adventitious shoot regeneration in potato cv. KG. Shoot regeneration in MS basal medium supplemented with: (a) BAP (0.05 mg l⁻¹) and 10% coconut milk, (b) TIBA (0.50 mg l⁻¹) along with zeatin (0.10 mg l⁻¹), (c) shoot regeneration in NAA and zeatin media combination after giving a pulse of TIBA and zeatin to induce shoot bud formation. Abbreviations used: RS, regenerated shoots.

**Fig 4.12**  Schematic presentation of a two step regeneration protocol for cv. KG.
Colony PCR analysis of *Agrobacterium tumefaciens* strain GV3101 transformed with binary vector pCAMBIA1302. (a) Confirmation of presence of *CsTLP* gene in transformed *Agrobacterium tumefaciens* strain GV3101. Lane 1-8, 681 bp amplicon of *CsTLP* gene in all the eight colonies and B, Negative control (pCAMBIA1302 without *CsTLP* gene); P, plasmid harboring *CsTLP* construct treated as positive control. (b) Confirmation of presence of *CsPPO* in transformed *Agrobacterium tumefaciens* strain GV3101. Lane M, 1 kb DNA ladder; lane 1-8, 1.8 kb amplicon of *CsPPO* gene in all the eight colonies; P, plasmid harbouring *CsPPO* gene treated as positive control and B, negative control (pCAMBIA1302 without *CsTLP* gene).

Effect of mode of infection on the appearance of *Agrobacterium* growth around the internodal explants. Upper panel: Immersion method of infection, lower panel: Touch-dry method of infection. Abbreviations: PRM1 and PRM2, step 1 and 2 regeneration medium, respectively, cefotaxime (500 mg l\(^{-1}\)), CF, cefotaxime and CC, co-cultivation. *Agrobacterium* growth represented by ‘+’ signs

Determination of hygromycin concentration to maintain an effective selection pressure. Growth of internodal explants on regeneration medium supplemented with hygromycin concentration: (a) 0 mg l\(^{-1}\); (b) 5 mg l\(^{-1}\); (c) 10 mg l\(^{-1}\); (d) 15 mg l\(^{-1}\) and (e) 20 mg l\(^{-1}\)

Rooting of putative *CsTLP* transgenic plants in hygromycin (20 mg l\(^{-1}\)) containing MS medium. WT (+) represented un-transformed (WT) growing in the presence of hygromycin; WT (-); un-transformed (WT) growing in the absence of hygromycin; TL1, TL2, TL3 and TL4 were putative *CsTLP* plants growing in the presence of hygromycin

PCR analysis to confirm the presence of *CsTLP* in transgenic plants. Lane P, amplification of 681 bp *CsTLP* in plasmid (+ve control); lane M, 100 bp DNA ladder; lane C, DNA from WT plants; lane TL1-TL4, DNA from *CsTLP* transgenic plants showing 681 bp amplification. The amplification and size indicated by arrow

Slot blot analysis of PCR positive *CsTLP* transgenic potato plants with 45ng of total DNA loaded onto hybond N+ membrane. Lane1-4, genomic DNA of TL1-TL4 transgenic potato pants showing positive signal of hybridization; lane 5; genomic DNA from untransformed plant as negative control; lane 6, plasmid harboring *CsTLP* as positive control.

Southern blot analysis of genomic DNA digested with *Hind* III and probed with biotin labeled eluted *CsTLP* PCR product. Lanes represented: WT, untransformed potato plants; TL1–TL4, *CsTLP* transgenic potato plants; UP, un-cut plasmid harboring *CsTLP*; EF, eluted gene specific *CsTLP* fragment
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Fig. 4.21 Semi-quantitative PCR analysis of *CsTLP* transgenic plants. Expression of *CsTLP* in transgenic lines (TL1-TL4) using CsTLP gene specific primers. 26SrRNA was used as an internal control of expression to show that equal amounts of RNA were used in the analysis

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Fig. 4.26 Phylogenetic tree showing relationship among *Macrophomina phaseolina* strain FIHB 1579 and representatives of some related taxa, based on Internal Transcribed Spacer (ITS) region sequence. The numbers on the nodes indicated how often (number of times, %) the species to the right are grouped together in 1000 bootstrap samples. Bar = 0.2 substitution per site

Fig. 4.27 Tuber bioassay for resistance against charcoal rot caused by *Macrophomina phaseolina*, in WT and *CsTLP* transgenic potato tubers. In the photograph, (a) showing the health of tubers at the beginning of the experiment i.e. on day 0. (b) Physical condition of the excised tubers after 3 weeks of *M. phaseolina* inoculation. Abbreviations: C1, tuber skin control; C2, assay control; IN, tuber inoculated with *M. phaseolina*; WT, un-transformed potato; TL1, *CsTLP* transgenic line 1; TL2, *CsTLP* transgenic line 2. Arrow indicates the ingress or and damage caused by the *M. phaseolina*. Necrotized lateral eyes (%) and discoloration of tuber pith (%) were shown in Table 4.10
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Fig. 4.29  qRT-PCR analysis. (a) Relative expression of *CsTLP* in transgenic potato tubers (TL1 and TL2) relative to WT tubers. Values were the average fold difference of non-inoculated tubers of TL1 and TL2 relative to non-inoculated WT tubers. Relative expression of *StLOX*, *StPAL*, and *StTLP* in tubers of WT (b); TL1 (c) and TL2 (d) at 2, 4 and 8 dpi with *M. phaseolina* relative to their respective mock inoculated control tubers. Standard errors of the means were represented by the error bars. Values on the top of the bar represented relative expression ratio

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Fig. 4.31  *Phytophthora infestans* inoculum preparation. (a) Growth of *P. infestans* over the slices of Kufri Chander Mukhi (KCM), white arrow showing cottony growth of the *P. infestans*; (b) Intact sporangia detached from *P. infestans* mycelia marked by yellow arrow; (c) zoospores released from the sporangium by cold shock treatment and red color arrow showing release of zoospores; (d) empty sporangium shown by white color arrow

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Fig. 4.46 DNA slot blot analysis of PCR positive TP plant with 45ng of total DNA loaded onto hybond N+membrane. Lane1: plasmid harboring CsPPO as positive control; lane 2, genomic DNA of TP transgenic potato pant showing positive signal of hybridization; lane 3, genomic DNA from WT plant

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