Production of Transgenic Apple (Malus domestica Borkh.) for Fungal Resistance Improvement

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Abstract

In the present study, g_psa gene from Gerbera hybrida coding for 2-pyrene synthase and utilizes acetyl-coA and 2-malonyl-co-A for the biosynthesis of two types of 6-Methyl-4-hydroxy-2-pyrene derivatives, ‘gerbrin’ and ‘parasorboside’, which contribute for fungal and insect resistance was used. On the other hand, fungal diseases are considered the most dangerous pests that face apple production worldwide, while, genetic transformation can be a helpful alternative and/or complementary solution for improving fungal resistance in crops. The aim was to develop an efficient regeneration and transformation system for apple (Malus domestica Borkh.) cv. ‘Golden Delicious’ and MM111 rootstock to improve their fungal resistance using genetic engineering approaches.

Factors affecting on organogenesis capacity of leaf pieces were studied as a prerequisite for transformation using Agrobacterium with g_psa gene. Young light green leaves were cultured for 3 days on co-cultivation media with Agrobacterium harbouring the g_psa gene. Adventitious shoot formation was achieved using leaf pieces from in vitro-grown shoots. The organogenesis capacity of leaf pieces was dependent on the leaf maturity and the origin of the leaf piece, using the youngest light green expanding leaves, with middle leaf segments showing best regeneration on medium containing: MS+B shoots. The organogenesis capacity of leaf pieces was dependent on the leaf maturity and the origin of the leaf piece, using the youngest light green expanding leaves, with middle leaf segments showing best regeneration on medium containing: MS+B5 vitamins +1.0 g l⁻¹ MES (Morpholinio ethanesulfonic acid) + 2.0 mg l⁻¹ TDZ + 0.2 mg l⁻¹ NAA.

For transformation, Agrobacterium EHA105 pSoup harbouring binary vector pGreenn1-3SS-g_psa was inoculated on Luria–Bertani (LB) media with 50 mg l⁻¹ Kanamycin. Putative transgenic shoots could be obtained on MS media with B5 vitamins, 1 mg l⁻¹ BAP, or 2.0 mg l⁻¹ TDZ with 0.2 mg l⁻¹ NAA in the presence of the selection agent “PPT” at 3.0-5.0 mg l⁻¹. Shoot multiplication of transgenic shoots was achieved on: MS + B5 vitamins+ 1.0 mg l⁻¹ BAP + 0.3 mg l⁻¹ IBA, 0.2 mg l⁻¹ GA3 +1.0 g l⁻¹ MES + 30 g l⁻¹ sucrose + 7.0 g l⁻¹ agar, with the selection agent PPT at 3.0-5.0 mg l⁻¹ and were sub-cultured every 4 weeks in the presence of the “PPT” at 5.0 mg l⁻¹. Six transgenic clones of cv. ‘Golden Delicious’ and one clone of MM111 have been obtained with transformation efficiency of 0.4% and 0.1% respectively. They were confirmed by PCR for the presence of the selection gene “bar” and also for the gene of interest “g_psa”, using the specific primers in all clones obtained. In vitro rooting was achieved easily on 1/5 MS rooting basal medium with 1.0 mg l⁻¹ IBA in the presence of the selection agent “PPT”. Rooted transgenic plantlets were successfully acclimatized and are being kept in the greenhouse to evaluate their performance for fungal resistance under containment conditions.

Materials & Methods


Methods: The system of transformation consisted of the following steps:
1. Establishment of in vitro cultures,
2. Developing an efficient regeneration system via direct organogenesis from in vitro-grown leaf pieces, and
3. Genetic transformation via Agrobacterium tumefaciens harbouring the binary vector pGreenn1-3SS-g_psa,
   (Fig. 1), by co-cultivation and regeneration from leaf pieces in the presence of the selection agent “Glufosinate-ammonium (Phosphinothricin)” (PPT) for herbicide resistance,
4. Multiplication of putative transformants in the presence of “PPT”,
5. Confirmation of transformation by PCR for the presence of “bar” and “g_psa” genes, and
6. Rooting and acclimatization of the transgenic clones obtained.

Results

Regeneration of transgenic shoots
Putative transgenic shoots could be obtained on MS media with BS vitamins, 5.0 mg l⁻¹ BAP or 2.0 mg l⁻¹ TDZ with 0.2 mg l⁻¹ NAA in the presence of the selection agent “PPT” at 3.0-5.0 mg l⁻¹ (Fig. 2). Treating the leaves with the non-traumatic forceps before immersion in the bacterial inoculum resulted in a higher and faster organogenic response, due to severe wounding, in consistence with observation of Norelli et al. 1996.

Evaluation of transformants
Putative transformants subcultured on media with selection agent “PPT” at concentrations of 3-5 mg l⁻¹ could survive, while the non-transformed explants were died (Figs. 2, 3). These were subcultured and proliferated on proliferation media containing PPT (Fig. 3).

Confirmation of transformation by PCR
Confirmation of putative transgenic regenerants was done by PCR using specific primers for detection of the selection gene “bar” and also for the gene-of-interest “g_psa” (Fig 4), while no band was shown in the negative control (not transformed apple). Also, no band were shown in the water control (well containing no DNA). Six transgenic clones of cv. “Golden Delicious” and one clone of “MM111” have been obtained and confirmed by PCR for the presence of the “bar” and “g_psa” using the specific primers in with transformation efficiency of 0.4% and 0.1% respectively.

In vitro rooting and acclimatization of transgenics
Rooting was easily obtained with 85 % efficiency and plantlets were successfully acclimatized to ambient conditions with 70% efficiency and are being kept in the greenhouse under containment conditions to evaluate their performance for fungal resistance (Fig. 5).

Fig. 1: Physical map of the binary vectors used for apple transformation. (dSSI-P: double 35S promoter; SSI-1: terminator; P: promoter, NOS: Agrobacterium nopalin synthese gene, Bar: herbicide resistance selectable marker from Agrobacterium). Fig. 2: Shoot regeneration after transformation on different media containing TDZ or BAP, 8 weeks after transformation
Fig. 3: Transformants Multiplication on Selection Media with PPT
Fig. 4: PCR for Confirmation of “g_psa” gene (1100 bp) and “bar” genes (down, 265 bp) in the Transformants. Lanes1: 100 bps Marker; 2-6: GD-T97; GD-T59; GD-T87; GD-T97, GD-T97; negative control; 8: positive control (plasmid) 9: Water
Fig. 5. In vitro rooting and acclimatization of transgenics

Conclusion

A regeneration system using young light green leaf pieces for the apple studied was worked out.

- Transgenic apples harbouring g2ps1 gene from Gerbera hybrida that confer fungal resistance were produced.

References


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