b. If no or few blue spots were observed after x-gluc staining, it may mean that DNA coating or bombardment parameters (rupture pressure, distance between the sample and the stopping plate, and the macrocarrier travel distance, and so on) were not optimal.

16. Imposition of selection can be earlier or later than the time specified here. The earliest selection may be applied 2 d after bombardment. The late selection may be applied at the plantlet stage, which is about 6–8 wk after bombardment. The early selection normally provides a tight procedure to catch the transformants, but it may reduce regeneration frequency as a result of suppressing the proliferation of the majority of the untransformed cells. The late selection gives all the cultured tissues a maximum opportunity to regenerate, but unavoidably allows some untransformed shoots to escape the selection.
17. Observation of root formation from the preselected shoots on the 1/2 MS medium with 10 mg/L of L-PPT helps to recognize the true T_o plantlets. The roots of the real transformants will grow into the medium and develop lateral roots at the bottom of the culture vessel; the escapes or chimeric transformants will have poor initiation of roots, which cease to grow when the root tips touch to the surface of the selection medium. At this stage, small sections of leaves or roots from the plantlets can be collected for GUS histological assay, which will provide further information about the stable transformation status.
18. Although wheat is an inbred species, to avoid possible genetical complication in the transgenic progeny resulting from crosspollination between individual transgenic plants, it is necessary to bag the flowering spikes.
19. Further genetical analysis of the T_1 plants by crossing them with the wild-type plants is sometimes necessary. This test is particularly useful in obtaining genetic information from those T_1 plants that have multiple copies of the transgene integrated into different positions on the nuclear genome.
20. In the Southern hybridization analysis (*see* Chapters 41 and 43) of transformed plants derived from the direct DNA delivery, it is often observed that common rearrangement patterns of transgenes obscures the hybridization pattern of the integration. The *Dpn*1-aid Southern hybridization technique ***(12)*** may be used to remove all the possible N^6-methyladenine DNA.

References

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