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Transfer of a β-Glucuronidase Reporter Gene to Sugarbeet (*Beta vulgaris* L.) Via Microprojectile Bombardment*

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Abstract: For the transfer of a β-Glucuronidase (GUS) reporter gene to sugarbeet (*Beta vulgaris* L.) callus and leaf explants by microprojectile bombardment, various rupture disk pressures and sample plate distances were tested. By coating the pBI221.23 DNA construct over the gold particles, bombardments were carried out by the DuPont PDS-1000/He system and the results clearly indicated the superiority of leaf explants over the callus structures as targets. The sample plate distances affected the distribution pattern of the particles and the cells expressing the GUS reporter gene were noted to be aggregated in short distances whereas longer distance shots yielded better distribution of transformed cells. For both leaf explants and callus structures the rupture disk pressure of 1350 psi was observed to yield the highest gene expression results and the sample plate distance of 12 cm for the leaf explants and 9 cm for the callus were found to increase the penetration success of the gold particles.

Şekerpancarına (*Beta vulgaris* L.) Partikül Bombardımanı Yöntemiyle β-Glukuronidaz Raportör Geni Transferi

Özet: Şekerpancarı (*Beta vulgaris* L.) yaprak ve kallus eksplantlarına partikül bombardımanı yöntemiyle β-glukuronidaz (GUS) raportör geninin aktarılmasında değişik basınç ve mesafeler denendi. Altın partiküllerine pBI221.23 DNA yapısının bağlanmasıyla ve DuPont PDS-1000/He sisteminden yararlanılarak yapılan atışlar sonucunda yaprakların, kalluslara oranla daha iyi bir hedef doku oldukları belirlendi. Yakın mesafeden yapılan atışlarda partikül dağılımı tam olmadığından gen geçişlerinin dar alanda toplandığı, buna karşın mesafenin artmasıyla partikül dağılımının daha düzenli bir şekilde gerçekleştiği görüldü. şekerpancarında hem yaprak hem kallus eksplantları için 1350 psi'lik basıncın en yüksek geçici gen ifadesi sonuçları verdiği gözlendi ve yaprak eksplantları için 12 cm'lik ve kallus için 9 cm'lik örnek-tabla mesafesinin altın partiküllerinin penetrasyon başarısını artırdığı bulundu.

Introduction

Within the last decade a tremendous amount of effort has been dedicated to develop new techniques of gene transfer to plants. Following the invention of microprojectile bombardment by Klein et al. (1) in 1987, the potential of the technique was rigorously tested and the results have confirmed the applicability of the technique to any living cell regardless of species (2-4). These efforts have also produced transgenic soybean (5), cotton (6), and forest tree species (7) in addition to transgenic recalcitrant monocotyledonous plant species like maize (8), rice (9) and wheat (10) which otherwise would have been difficult to transform with the *Agrobacterium tumefaciens*-mediated gene transfer system.

While significant progress has been achieved in transgenic cereals, the advances in sugarbeet research, especially gene transfer studies have been severely limited by the problems in regeneration potential of this crop species. So far, a handful of published results have demonstrated the feasibility of gene transfer studies in very restricted genotypes. Various explant sources like shoot-base (11) and hypocotyls and cotyledons (12) were used for transformation studies with the *A. tumefaciens* system. However, following the gene transfer, requirement of callus formation and the subsequent regeneration process necessitated the application of direct gene transfer techniques to this crop species.

More recently two independent research groups have successfully demonstrated the feasibility of the microprojectile bombardment system for sugarbeet and reported transient expression of marker genes (13, 14).

The aim of this pioneering study of microprojectile bombardment in Turkey, was to optimise the physical

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variables (i.e. rupture disk pressure and sample plate distance) and determine the superior explant source (leaf vs. callus) with the transient β-Glucuronidase (GUS) expression system (15).

Materials and Methods

For the gene transfer to sugarbeet, the "ELK 345" variety was used due to its superior response to callus formation and its high regeneration capacity. Leaf explants and callus structures developed from leaf explants were used as targets for microprojectile bombardment.

The germinated seeds were used to obtain the leaf explants and 5 explant/petri dish were oriented at the centre of the petri dish 24 h prior to bombardment on B5 medium. Callus formation from these explants was achieved by incubating the explants in B5 medium supplemented with 1 mg/l BAP + 0.5 mg/l NAA + 0.5 mg/l TIBA and the callus material was oriented at the centre of the petri dish 24 h prior to bombardment.

All bombardments were carried out with the Bio-Rad Biolistic®, PDS 1000/He particle delivery system, which was first imported to Turkey in 1996, according to the manufacturer. Bombardments were made under a partial vacuum of 25" Hg pressure and gold particles, 1.0 μm mean diameter, and were coated with 5 μg of pBI221.23 DNA (16) as described in the information supplied by the manufacturer.

Leaves and callus material were subjected to histochemical GUS assay (15) 48 h after bombardment. The assay solution consisted of 0.25 mg / ml X-gluc, 0.1 M $NaPO_4$ buffer, 0.01 M Na_2EDTA, 0.1 % Triton X-100, 0.5 mM $K_4Fe(CN)_6.3H_2O$ and 0.5 mM $K_3Fe(CN)_6$ all at pH 7.0 for 24 h at 37°C. Transient gene expression events were counted under a dissecting microscope.

Results and Discussion

In order to optimise the physical parameters for the efficient transient expression of a GUS reporter gene, the rupture disk pressures and sample plate distances were tested in a wide range. Thus, the bombardments were carried out at 1100 psi (rupture disk pressure) from 6 and 9 cm (sample plate distance), at 1350 psi from 9 and 12 cm, at 1550 psi from 12 and 15 cm and finally at 1800 psi from 12 cm.

One of the fundamental reasons that justified the choice of leaves as the target explant source rests on their large size and their smooth surface structure. Although petioles do form callus structures at a higher rate when compared with the leaf explants, their small size and undulated surface structure have limited their use as ideal target explants for microprojectile bombardment.

The results we obtained following the histochemical GUS assay revealed a general trend of which a lower rupture disk pressure but a longer sample plate distance reduced the transient gene expression events probably due to less penetration of the gold particles because of the higher air friction. Likewise, a higher rupture disk pressure but a shorter sample plate distance also reduced the transient gene expression events probably either, due to extremely deep penetration of the gold particles causing the inability of the assay solution to diffuse to such cell layers, or since the higher velocity of the gold particles, their piercing through the explant material which impeded their delivery of plasmid DNA into leaf cells. As seen in Figure 1, transient gene expression events displayed a tremendous amount of variation when the same rupture disk pressure but a different sample plate distances were used. While at 1100 psi from 6 cm, the smallest number of GUS positive events were scored (14/plate), at the same pressure from 9 cm one of the highest numbers of GUS positive events were observed (647/plate), which demonstrated the importance of the sample plate distance on the optimisation process.

Like many other plant species tested, the rupture disk pressure of 1350 psi was observed to be optimum for sugarbeet and at this pressure we obtained the highest GUS expression events (671/plate). However, above this pressure GUS expression events were observed to decline with a slight improvement at 1800 psi. Another noticeable effect of sample plate distance on GUS expression events was the particle distribution pattern following the bombardment, thus reflected as individual GUS positive blue spots on the leaf surface. In short distance bombardments while only one or two explants were receiving most of the gold particles with an extreme number of GUS expression events (Figure 2) longer distance bombardments revealed a homogenous GUS expression events in most of the explants (Figure 3).

For testing the suitability of the callus structures, developed from leaf explants, sample plate distance - rupture disk pressure combinations of 9 cm-1100 psi, 9 cm-1350 psi, 12 cm- 1550 psi were used. However, following the bombardments, histochemical GUS assays revealed a significantly low number of GUS expression events (8-25/plate) when compared with leaf explants. Nevertheless, 1350 psi rupture disk pressure still yielded the highest GUS expression events. However, in contrast

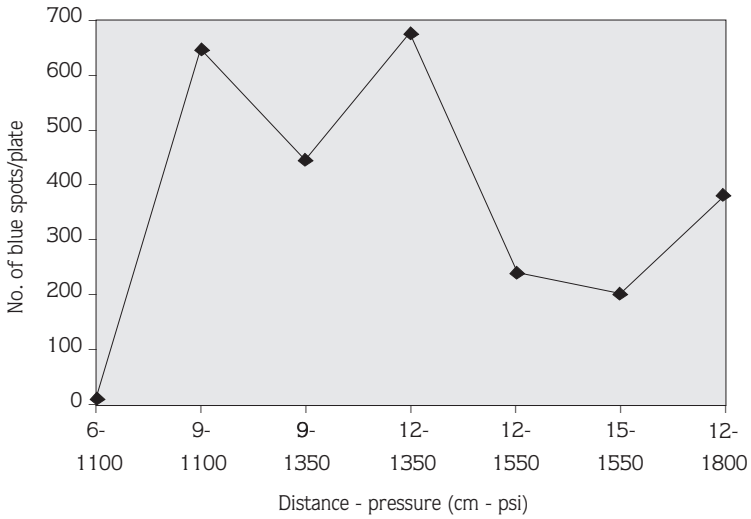

Figure 1. Number of blue spots scored on sugar beet leaf explants, indicative of transient GUS expression, following microprojectile bombardment from various sample plate distances with different rupture disk pressures.

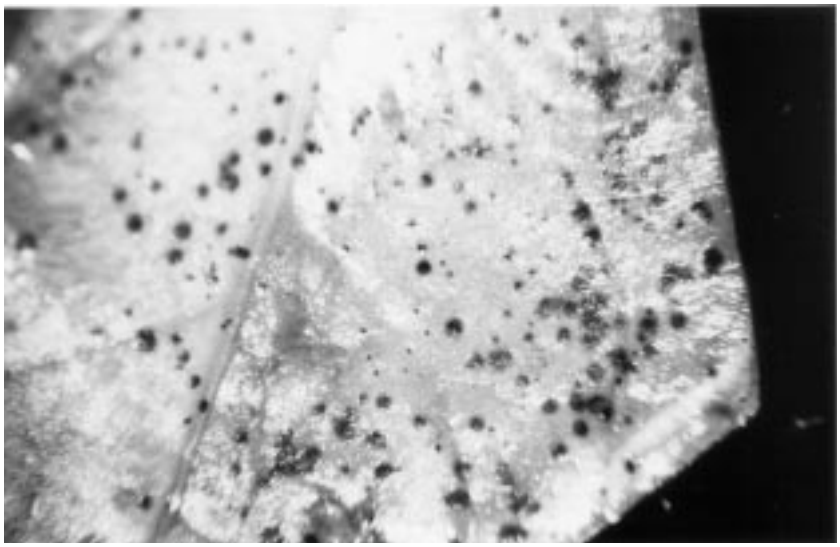

Figure 2. Aggregated distribution of GUS. expression events in leaf explants bombarded from a short distance (9 cm, 1350 psi).

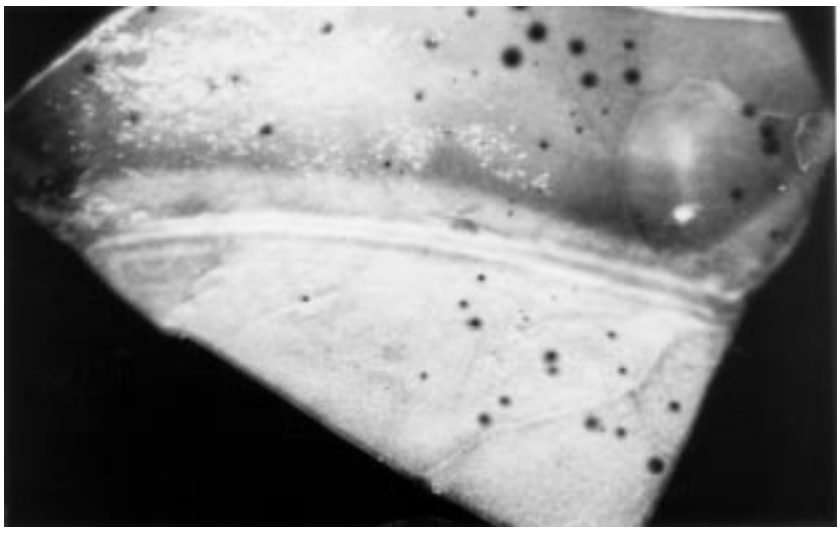

Figure 3. Homogenous distribution of GUS expression events in leaf explants bombarded from a long distance (12 cm, 1350 psi).

to the leaf explants, reducing the sample distance increased the GUS expression events approximately 2.5-fold at 1350 psi from 12 cm to 6 cm. Low GUS expression events in callus explants could be attributed to the limitations in the histochemical GUS assay system. Although not observed in leaf explants, extensive browning was noticed in callus structures following histochemical GUS assay, probably due to the strong oxidation/reduction potential of the Potassium Ferro/Ferri Cyanide couple in the assay mixture and their subsequent oxidation/reduction of the accumulated sugars in callus explants. This browning which could not be bleached out by ethanol treatments probably masked a considerable number of GUS expression events. The effect of distance on the GUS expression events in callus structures might be attributed to their irregular surface characteristics as well as their hard nodular structures.

Our results clearly demonstrated that for both explants a rupture disk pressure of 1350 psi was optimum however, for leaf explants long sample plate distances, and for callus explants short distances yielded highest GUS expression events. Optimisation of the regeneration procedures for this genotype will ultimately lead to the recovery of transgenic sugarbeet plants.

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References

1. Klein, T.M., Wolf, E.D., Wu, R., Sanford, J.C. High velocity microprojectiles for delivering nucleic acids into living cells, Nature 327, 70-73, 1987.
2. Christou, P. Genetic transformation of crop plants using microprojectile bombardment, Plant J. 2, 275-281, 1992.
3. Klien, T.M., Arentzen, R., Lewis, P.A., Fitzpatrick-McElligott, S. Transformation of microbes, plants and animals by particle bombardment, Bio/Technology 10, 286-291, 1992.
4. Luthra, R., Varsha Dubey, R.K., Srivastava, A., Kumar, S. Microprojectile mediated plant transformation-A bibliographic search, Euphytica 95, 269-294, 1997.
5. Sato, S., Newell, C., Kolacz, K., Tredo, L., Finer, J., Hinchee, M. Stable transformation via particle bombardment in two different soybean regeneration systems. Plant Cell Rep. 12, 408-413, 1993.
6. Finner, J., McMullen, M.D. Transformation of cotton (*Gossypium hirsitum* L.) via particle bombardment. Plant Cell Rep. 8, 586-589, 1990.
7. Ellis, D.D., McCabe, D.E., McInnis, S., Ramachandran, R., Russell, D.R., Wallace, K.M., Martinell, B.J., Roberts, D.R., Raffa, K.F., McCown, B.H. Stable transformation of *Picea glauca* by particle acceleration. Bio/Technology 11, 84-89, 1993.
8. Gordon-Kamm, W.J., Spencer, T.M., Mangano, M.L., Adams, T.R., Daines, R.J., D-Start, W.G., O'Brian, J.V., Chambers, S.A., Adams, W.R., Willets, N.G., Rice, T.B., McKey, C.J., Krueger, R.W., Kausch, A.P., Lemaux, P.G. Transfromation of maize cells and regeneration of fertile transgenic plants. Plant Cell Rep. 2, 603-618, 1990.
9. Christou, P., Ford, T.L., Kofron, M. Production of transgenic rice (*Oryza sativa* L.) from agronomically important indica and japonica varieties via electric discharge particle acceleration of exogenous DNA into immature zygotic embryos. Bio/Technology 9, 957-962, 1991.
10. Alpeter, F., Vasil, V., Srivastava, V., Stöger, E., Vasil, K. Accelerated production of transgenic wheat (*Triticum aestivum* L.) plants. Plant Cell Rep. 16, 12-17, 1996.
11. Hayakawa, H., Shimamoto, Y., Asano, S., Iizuka, T. Developments of shoot-base tissue on leaf blade for raising transgenic plants in beets. Proc. Japanese Soc. Sugar Beet Tech., Sapparo, 130-135, Hokkaido, 1994.
12. Krens, F.A., Trifonova, A., Keizer, L.C.P., Hall, R.D. The effect of exogenously-applied phytohormones on gene transfer efficiency in sugar beet (*Beta vulgaris* L.). Plant Sci. 116, 97-106, 1996.
13. Mahn, A., Matzk, A., Sautter, C., Scheimann, J. Transient gene expression in shoot apical meristem of sugar beet seedlings after particle bombardment. J.Exp. Bot. 291, 1625-1628, 1995.
14. Ingersoll, J.C., Heutte, T.M., Owens, L.D. Effects of promoter-leader sequences on the transient expression of reporter gene chimeras biolistically transferred into sugar beet (*Beta vulgaris* L.) suspension cells. Plant Cell Rep. 15, 836-840, 1996.
15. Jefferson, R.A. Assaying chimeric genes in plants : The GUS fusion system. Plant Mol. Biol. Rep. 5, 387-405, 1987.
16. Lonsdale, D., Önde, S., Cuming, A.C. Transient expression of exogenous DNA in intact, viable wheat embryos following particle bombardment. J. Exp. Bot. 41, 1161-1165, 1990.