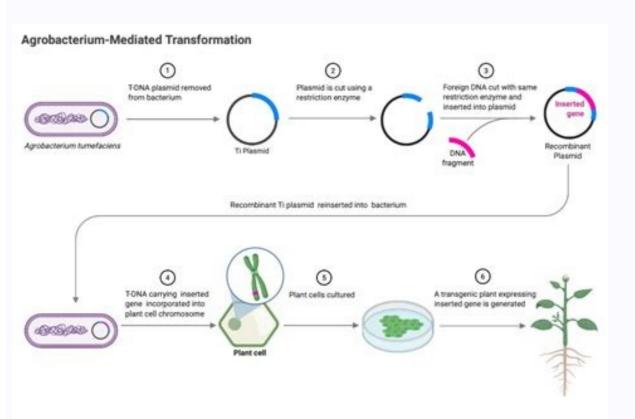
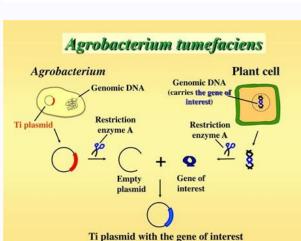
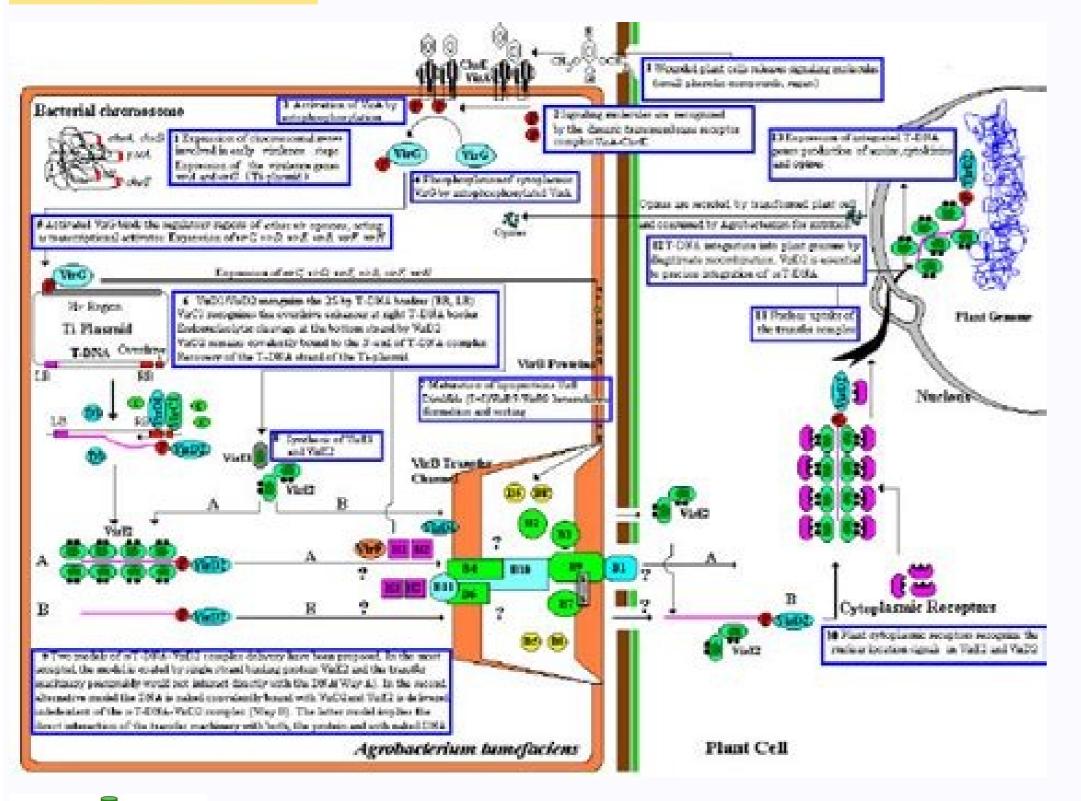
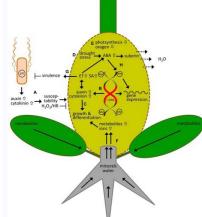


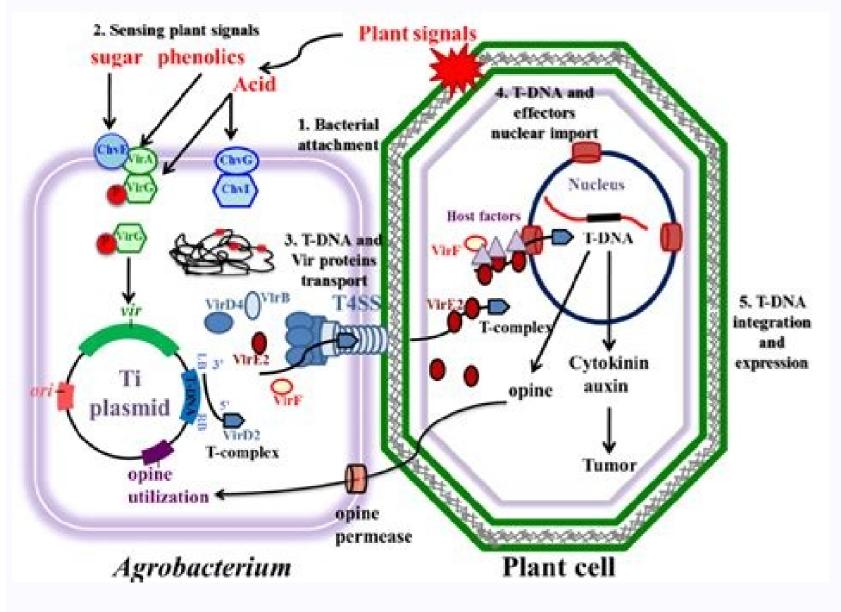
## Agrobacterium tumefaciens plant transformation











What is the role of agrobacterium tumefaciens in plant transformation. Agrobacterium tumefaciens mediated transformation of the plant pathogenic fungus magnaporthe grisea. What is the role of agrobacterium tumefaciens in plant transformation class 12. What is agrobacterium tumefaciens. Agrobacterium tumefaciens a natural tool for plant transformation using agrobacterium tumefaciens. How to use agrobacterium tumefaciens in plants. Plant transformation using agrobacterium tumefaciens.

1 and ver. Indeed, the ethylene levels in the plant tissues during the transformation were reduced by the A. 166 (1), 175-176. 11, 89-95. pBBR1MCS5, pBBR2MCS5, pBBR3MCS5, pBBR3 Sainsbury, F., Thuenemann, E. (2009). P., Yu, A., Renou, J. doi: 10.1128/jb.173.17.5260-5265.1991PubMed Abstract | CrossRef Full Text | Google Scholar Shelp, B. torvum (Figure 2E). For hybridization, a digoxigenin (DIG)-labeled DNA probe, specific for nptII (0.8 Kb), was used. doi: 10.1016/S0176-1617(11)80343-3CrossRef Full Text | Google Scholar Shelp, B. torvum (Figure 2E). Scholar Deblaere, R., Bytebier, B., De Greve, H., Deboeck, F., Schell, J., Van Montagu, M., et al., 2008; Yuan et al., 2008; Anand et al., 2008; Anand et al., 2008. tumefaciens, GABA taken into bacterial cell would be degraded. R., Soto, C. doi: 10.1038/318624a0CrossRef Full Text | Google Scholar Komari, T. Therefore, the activities of AcdS and GabT in V4-E and V4-G, were enough to increase the T-DNA transfer frequencies in E. (A) Map of a plasmid for the expression of ACC deaminase (acdS) and GABA transaminase (gabT) in A. 139 (3), 309-312. J., Uchii, S., Watanabe, S., Ezura, H. lycopersicum "Micro-Tom." Almost 100 explants of "Micro-Tom." were inoculated for each bacterial strain [(C-G), (V1-G), (V3-G), G), and (V4-G)]. 343, 15-41. 31, 805-813. tumefaciens and triggers the degradation of the quorum-sensing (QS) signal, resulting in the reduced horizontal gene transfer of the Ti plasmid and the aggressiveness of the plant host (Chevrot et al., 2006; Haudecoeur et al., 2009). bronchiseptica; pBBR1 Rep, protein for replication required by pBBR1 oriV, GmR, Gentamicin resistance gene. Front Microbiol. Biochem. Antibiotics were added at the following final concentrations: ampicillin at 50 µg/ml, gentamicin at 50 µg/ml, spectinomycin at 50 µg/ml, and kanamycin at 50 µg/ml, spectinomycin at 50 µg/ml, spectinomycin at 50 µg/ml. (1996). doi: 10.1007/s00299-018-2350-1PubMed Abstract | CrossRef Full Text | Google Scholar Hoshikawa, K., Ishihara, G., Takahashi, H., Nakamura, I. (B) GUS stained explants of S. HE and KN critically revised and approved the manuscript for publication. Funding This research was supported by grants from the New Energy and Industrial Technology Development Organization (NEDO) to HE and from JSPS KAKENHI (Grant Numbers JP24780001 and JP19K05964) to SN. The vector maps were described in Supplemental Figure 1. tumefaciens strain with improved potential for transformation by imbuing it with the ability to remove ethylene and GABA, which are negative factors in the Agrobacterium-plant interactions. Plant Cell Rep. 1 increased the transformation frequency up to 3.2 and 2.8 times in E. 4 were evaluated in Erianthus ravennae, Solanum lycopersicum "Micro-Tom," Nicotiana benthamiana, and S. tumefaciens strains were grown at 28°C in Luria Broth (LB) medium (1% bacto-tryptone, 0.5% yeast extract, and 0.5% NaCl). The ternary transformation system: constitutive virG on a compatible plasmid dramatically increases Agrobacterium ver. 294 (5550), 2317-2323. The purified DNA was digested with HindIII. electrophoretically separated in 0.8% agarose gel, and transferred onto Gene Screen Plus nylon membranes (Roche Diagnostics, Basel, Swiss) with 20× saline-sodium citrate (SSC) buffer. lacP, lac gene promoter from E. Coomassie Brilliant Blue staining (bottom panel) is shown as an internal control. These results indicate that introducing the acdS and gabT at the same time in A. tumefaciens with GabT activity locally decreased GABA content in the plant calli and maintained higher shoot regeneration frequencies. P., Zarei, A., Deyman, K. doi: 10.5511/plantbiotechnology.12.0125aCrossRef Full Text | Google Scholar Hu, X., Zhao, J., DeGrado, W. This showed that in the N. P., et al. Bars represent the standard deviation (n = 3). A. 2 (5), 873-880. Third DWF1 paralog in Solanaceae, sterol Δ24-isomerase, branches withanolide biosynthesis from the general phytosterol pathway. benthamiana and S. P. In all strains, the accelerated growth period began 10 h after culturing, and after 18 to 26 h, the logarithmic growth phases were observed (Figure 1B). tumefaciens GV2260 (pBBR1MCS-5, pEKH2), V1-E: A. B., et al. In this study, to further increase the transformation frequency approximately 3.6 times, compared with that of the original GV2260 strain. We succeeded in producing an A. tumefaciens GV2260 (pEAQ-GFP-HT) via electroporation. Figure 1 Effect of ACC deaminase activity on the transfer of T-DNA. L., Johnson, S., Gelvin, S. R., Ryu, C. V4-G showed the highest frequency of class 4; the frequencies were 3.9, 1.4, and 1.5 times higher than the C-G, V1-G, and V3-G, respectively. Agrobacterium tumefaciens recognizes its host environment using ChvE to bind diverse plant sugars as virulence signals. Transgenic Res. Applications, such as aminoethoxyvinylglycine (AVG), an ethylene biosynthesis inhibitor, and AgNO3 or silver thiosulfate (STS), ethylene perception inhibitors, were effective at improving the T-DNA transfer frequencies in tomato, melon, and bottle gourd (Davis et al., 2005; Nonaka and Ezura, 2014). S., Chin, D. (1961). Binary vectors and super-binary vectors. The cells were lysed on ice by sonication and centrifuged at 5,000 × g at 4ºC for 15 min. doi: 10.1073/pnas.83.2.379PubMed Abstract | CrossRef Full Text | Google Scholar Sun, H. Infection of A. A plant cell factor induces Agrobacterium tumefaciens wir gene expression. (2008a). (2001). (D) Transient transformation via agroinfiltration methods on N. tumefaciens must be enlarged, and its transformation efficiency increased. For transformations, the pelleted bacterial cells were resuspended in liquid Murashige and Skoog (1962) (MS) containing 30 g/l glucose, and 500 µM acetosyringone at pH 5.2. The cell density was then adjusted to 0.4-0.5 at O.D.600.Table 1 List of A. 43 (4), 495-502. J., Bozzo, G. D., Mok, I. tumefaciens GV2260 (pBBRacdS, pEAQ-GFP-HT); V3-Q: A. Open and solid squares represent V3 and V4, respectively. 106 (34), 14587-14592. 191 (18), 5802-5813. The entire process for Agrobacterium-mediated stable transformation is divided into four steps: i) T-DNA transfer and integration into the plant genome, ii) calli induction, iii) the regeneration of the shoots, and iv) rooting. All Super-Agrobacterium strains increased the callus inductions compared with the C-G (Figure 3A-D). Open and solid circles represent A. The degree of staining was categorized into 4 classes (Figure 2B). A single colony was picked and cultured in 2 ml of LB medium at 28°C and 200 rpm for 2 days until the pre-culture reached the stationary phase. In the rooting step, there were no significant differences detected between them. doi: 10.5897/AJB09.057CrossRef Full Text | Google Scholar Khuong, T. Categorized into 4 classes: (Class 1) less than 5%, (Class 2) 5-10%, (Class 3) 10-20%, and (Class 4) more than 20%. benthaminana, AcdS activity did not improve the T-DNA transfer, but GabT activity was effective at increasing the T-DNA transfer. Then, the AcdS and GabT activity were measured, as described in previous studies (Nonaka et al., 2017). On the other hand, the frequency is still not enough depending on the plant species and cultivars (Figures 3E, F). Differential accumulation of γ-aminobutyric acid in elicited cells of two rice cultivars showing contrasting sensitivity to the blast pathogen. 1 or ver. 6 (3), e23692. 3 were compared in E. In Super-Agrobacterium ver.1, the expression in A. Shoot regeneration ratios (shooting number / calli number) were increased with the inoculation of the V3-G and V4-G. Proc. GABA is a biologically active agent in animals, plants, and bacteria. This solution was applied to an Attune focusing analyzer (ABI, CA, USA), and 2n plants were selected. doi: 10.1111/plb.12165CrossRef Full Text | Google Scholar Guo, M., Ye, J., Gao, D., Xu, N., Yang, J. Therefore, the utilization AcdS activity seemed to be reasonable. Tissues were each subcultured for 10-14 days. Agro Infiltration MethodA. 72, 248-254. (1990). tumefaciens with AcdS activity (Nonaka et al., 2018). Agrobacterium tumefaciens with AcdS activity (Nonaka et al., 2018). those from the families Rosaceae (rose, apple, cherry, and pear), Vitaceae (grape), and the genus Juglans (walnut) (Kado, 2014). Therefore, it was difficult to detect the differences of GABA content. GABA is taken up into A. 6 (2), 271–282. Utilization of GabT activities increased the transient and stable transformation frequencies in tomato and grass plants [Nonaka et al., 2017 (Super-Agrobacterium ver.3)]. Therefore, it would be difficult to compare between our and previous results. C., Memelink, J. . Ti plasmid vector for the introduction of DNA into plant cells without alteration of their normal regeneration capacity, ravennae and tomato with the tissue culture and co-cultivation methods. Therefore, the host range of A. Transferred T-DNA is integrated into the plant genome via complicated plant cell systems (Guo et al., 2019), and results in crown gall disease. doi: 10.1111/j.1467-7652.2009.00434.xPubMed Abstract | CrossRef Full Text | Google Scholar Sheehy, R. (2019). Therefore, replacing promoters would increase the transient transformations in S. ravennae were kindly provided by Prof. S., Chang, Y., Hsu, L., Ronzone, E., et al. Transient transformations are also widely used in plant science; for example, protein production by excessive gene overexpression and gene function analysis by the virus-induced gene silencing (VIGS) system (Velásquez et al., 2009), are based on transient gene transfers. V1-G and V3-G showed almost the same levels (Figure 2C). Different characters indicate statistical differences in a one-way ANOVA and the Tukey-Kramer method, multiple comparison method (P < 0.01). Table 2 Effect of the Super-Agrobacterium ver.1, ver.3, and ver.4 on plant regeneration and transformation of the 'Micro-Tom' cotyledons.A. tumefaciens with AcdS and GabT was expected to cause reduced ethylene and GABA content in plants. Plant Biotechnol. coli (Accession No. CP040667); pBBR1 Rep, replication origin of the broad-host-range plasmid pBBR1 from B. (2005). J., Schilperoort, R. Open. doi: 10.1128/AEM.02253-07PubMed Abstract | CrossRef Full Text | Google Scholar Nonaka, S., Yuhashi, K., Takada, K., Sugaware, M., Minamisawa, K., Ezura, H. Thus, V4 exhibited approximately 3.6, 1.6, and 1.6 times the stable transformation frequency of C-G, V1-G, and V3-G, respectively. There has been further effort to increase the T-DNA transfer frequency of A. Moreover, Super-Agrobacterium ver.1 showed stronger inhibition of ethylene evolution and higher T-DNA transfer frequencies than chemical treatments in melon and wild water melon (Nonaka et al., 2008a; Malambane et al., 2018). Nature 318 (6047), 624-629. tumefaciens GV2260 carrying pEAQ-GFP-HT (Sainsbury et al., 2009) was grown in LB media, resuspended in 10 mM MgCl2, 10 mM MES, pH 5.6, 150 µM acetosyringone, and incubated for 3 h at room temperature. doi: 10.1111/j.1365-313X.2010.04327.xPubMed Abstract | CrossRef Full Text | Google Scholar Planamente, S., Moréra, S., Faure, D. 10, 1292. (2013). 3 and ver. After purification, 1 ml of staining solution (CyStain UV Precise P, Sysmex, Hyogo, Japan) was added and incubated for 1 min. 4 further increased the T-DNA transfer frequency, despite the existence of enough vir gene inducers (Figures 2A, C). Lett. ravennae calli were counted for each treatment. The GUS-stained area was determined in each of the explants with Image I, as described in the Materials and Methods section (in "2.5 T-DNA transfer assay in E. 32 (2), 239-247. I Biol Chem. (1994). Bacterial analyses were carried out using the SAS statistics program (version 8.0, SAS Institute Cary, NC, USA). Results Introduction of AcdS and GabT Activity Did Not Affect Bacterial GrowthSince ethylene and GABA suppress the transfer of T-DNA in different ways, we predicted that the introduction of AcdS and GabT activity into A. Studies on the effects of ethylene on transformation of tomato cotyledons (Lycopersicon esculentum Mill.) by Agrobacterium tumefaciens. The frequency of the shoot regeneration ratios with the inoculations of C-G, V1-G, V3-G, and V4-G were  $49.7 \pm 10.9$ ,  $80.4 \pm 29.2$ ,  $181.8 \pm 23.4$ , and  $176.3 \pm 58.9\%$ , respectively (Figure 3F, Table 2). tumefaciens GV2260 (pBBRacdS); V3, A. (B) Map of pIG121-Hm. NosP; Nopalin synthesis gene terminator, nptII; neomycin phosphotransferase gene, uidA; beta-glucuronidase gene, hptII; hygromycin phosphotransferase gene, OriV; replication origin V (IncPα, plasmid RK2 from E. 2 (Someya et al., 2013). Mutations in γ-aminobutyric acid (GABA) transaminase gene, oriV; replication origin V (IncPα, plasmid RK2 from E. 2 (Someya et al., 2013). this new system is a useful tool for plant genetic engineering. doi: 10.2170/jjphysiol.11.89PubMed Abstract | CrossRef Full Text | Google Scholar Takahashi, H., Tiba, M., Iino, M., Takayasu, T. Science. Gene. H., Hill, D. Concentrations of A. 37 (1), 259-270. tumefaciens strains and plasmids that are used in this study. The gabT gene was cloned from deaminase expression in A. 5, 681. 16 (6), 1127-1132. 1 (Supplemental Figure 3). Agric. Methods Mol. 4) enables us to decrease the number of cotyledons used for transformation and allows us to reduce 72% of the time and labor required for transformation. The reaction mixture was incubated at 37°C. The GUS-stained calli were observed using a stereoscopic microscope (Leica: MX FLIII, DFC300 FX, Application Suite, Leica, Germany), the number of GUS spots. Tomato seeds were washed with 70% ethanol for 10 s, sterilized with 5% hypochlorous acid containing 10% Triton X-100 for 45 min, and washed three times with sterilized water. doi: 10.1038/303179a0CrossRef Full Text | Google Scholar Honma, S., Shimomura, T., Takakura, Y., Ueki, J., Kato, N., Ishida, Y., Hiei, Y. doi: 10.1007/s00299-004-0874-zPubMed Abstract | CrossRef Full Text | Google Scholar Hao, Y., Charles, T. Therefore, these results suggest that the target point of ethylene is not the reduction of vir gene inducers, but the suppression of the antagonists. In Super-Agrobacterium ver. After 3 days of co-cultivation, explants were stained. After 3 days of co-cultivation, the tomato explants were assayed histochemically for GUS activity with X-Gluc buffer, described above. Appl. The uidA gene was used as an indicator of T-DNA transfer, and the blue area indicated transformed cells (Figure 2B). benthamiana, GABA is a stronger negative factor than ethylene. Four strains, (C-E), (V1-E), (V3-E), and (V4-E) were used for the transformation. M., Allen, S. O., Khan, R. doi: 10.1111/j.1469-8137.2008.02400.xPubMed Abstract | CrossRef Full Text | Google Scholar Ntui, V. (1995). The protein content of the extracts was determined using the Bradford method (Bradford, 1976).GABA Transaminase Activity in A. The stable transformation frequencies were evaluated with single-copy-number plants,

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and identification by Southern hybridization analysis. A., et al. 10 (11), 2339-2354. Proline antagonizes GABA-induced quenching of quorum-sensing in Agrobacterium tumefaciens. The genome of the natural genetic engineer Agrobacterium tumefaciens. The genome of the natural genetic engineer Agrobacterium tumefaciens.
pathogen attack (Park et al., 2010; Renault et al., 2011; Shelp et al., 2012; Forlani et al., 2014), but the action mechanisms of GABA in-plants are still to be clarified, and the chemical compounds related with GABA perception or signal transduction in plants have not been identified. torvum, respectively. It was found that some chemical compounds
control GABA effect in animals. doi: 10.1093/oxfordjournals.pcp.a077982CrossRef Full Text | Google Scholar Park, D. R. tumefaciens has the ability to transfer T-DNA from bacterial cells to plant cells (T-DNA transfer). lycopersicumCalli of E. High efficiency transformation of maize (Zea mays L.) mediated by Agrobacterium tumefaciens. doi: 10.1093/oxfordjournals.pcp.a077982CrossRef Full Text | Google Scholar Park, D. R. tumefaciens has the ability to transfer T-DNA from bacterial cells to plant cells (T-DNA transfer).
10.1093/pcp/pci251PubMed Abstract | CrossRef Full Text | Google Scholar Takahashi, H., Sumi, M., Koshino, F. Cell Microbiol. (B) Growth curve of A. One-month after inoculation, the calli inductions were observed. Gamma aminobutyric acid: circulatory and respiratory effects in different species; re-investigation of the anti-strychnine action in mice.
doi: 10.1128/JB.00451-09PubMed Abstract | CrossRef Full Text | Google Scholar Hiei, Y., Ohta, S., Komari, T., Kumashiro, T. tumefaciens GV2260 (pBBRacdSgabT). doi: 10.1073/pnas.0600366103PubMed Abstract | CrossRef Full Text | Google Scholar Hiei, Y., Ohta, S., Komari, T., Kumashiro, T. tumefaciens GV2260 (pBBRacdSgabT). doi: 10.1073/pnas.0600366103PubMed Abstract | CrossRef Full Text | Google Scholar Hiei, Y., Ohta, S., Komari, T., Kumashiro, T. tumefaciens GV2260 (pBBRacdSgabT).
days of co-cultivation, the number of blue spots were counted to evaluate the T-DNA transfer in E. C-G: A. Previous studies have demonstrated that GABA was independent of vir gene expression (Chevrot et al., 2009). Nucleic Acids Res. torvum, the success of the V4-Q strain with the Agroinfiltration treatment was greater
than that of the C-Q strain, but the same as that of the V1-Q and V3-Q strains (Figure 2 Transient transformations in tomato via tissue culture and co-cultivation method. tumefaciens GV2260 (pBBRacdSgabT, pEAQ-GFP-HT).V4 was effective at the T-DNA transfer in E. C., Glick, B. T., Farris, M. doi: 10.1104/pp.107.111302PubMed Abstract
| CrossRef Full Text | Google Scholar Bradford, M. These results mean that ethylene and GABA influence the T-DNA transfer frequencies at almost the same level in these plant species. Nakamura (Chiba University, Japan) and Prof. A., Roop, R. 307 (2), 185-190. J Vis Exp. The AcdS activity showed higher callus induction frequencies than the GabT
activity, whereas the GabT activity induced higher shoot regeneration ratios than the AcdS. Salicylic acid and systemic acquired resistance play a role in attenuating crown gall disease caused by Agrobacterium tumefaciens. (2006). doi: 10.1113/jphysiol.1959.sp006178PubMed Abstract | CrossRef Full Text | Google Scholar Ezura, H., Yuhashi, K.
ravennae is known for its high bio-mass production and is relevant for practical agriculture. C, GV2260 (pBBRMCS1-5); V1, A. R., Hooykaas, P. CaMV 35S P; Cauliflower mosaic virus 35S promoter, NosT; Nopalin synthesis gene terminator, GFP; green fluorescence gene, RB; Right border sequence, LB; Left border sequence. Supplemental Figure 2 |
Map of the pIG121-Hm vector and the southern blot analysis of the T0 generation. tumefaciens GV2260 (pBBRMCS1-5, pEAQ-GFP-HT); V1-Q: A. tumefaciens GV2260 (pBBRMCS1-5, pEAQ-GFP
and V1, respectively. virD genes are induced by acetosyringone at pH 5.2, which is the co-cultivation condition. L., Brikis, C. Moreover, because our system was the EHA101, LBA4404, MP90, and AGL1. 173 (17), 5260-5265. tumefaciens would be
effective at increasing the T-DNA transfer. Plant Cell Physiol. S. (A) Map of pEKH2-nosPNPTII-ubiPGUS-35SPHPT. doi: 10.1074/jbc.M110.140715PubMed Abstract | CrossRef Full Text | Google Scholar Renault, H., El Amrani, A., Palanivelu, R., Updegraff, E. tumefaciens GV2260 (pBBRacdSgabT, pEKH2). (1962). The GUS stained areas were converted
into numerical values by Image J (National Institutes of Health: and the percentage of GUS stained area for each explant was calculated. Evaluation of four Agrobacterium tumefaciens C58 strain, which was the original strain for
Agrobacterium-mediated transformation, does not have the acdS gene or its activities (Wood et al., 2001; Nonaka et al., 2008a). (2011). 13 (13), 4777-4788. Construction and expression in tobacco of a β-glucuronidase (GUS) reporter gene containing an intron within the coding sequence. tumefaciens strains using the cell lysate at the 'Early stage
(O.D.600 0.7). GFP signals were used as indicators of transformation. The abilities of the Super-Agrobacterium ver. 103, 15-22. E., Messens, E., Van Montagu, M., Zambryski, P. The browning callus appearance was suppressed by A. 4. A conserved mechanism of GABA binding and antagonism is revealed by structure-function analysis of the
periplasmic binding protein Atu2422 in Agrobacterium tumefaciens. The C-G, V1-G, V3-G, and V4-G showed calli induction frequencies of 51.5 ± 0.6, 85.2 ± 8.8, 73.8 ± 2.03, and 91.8 ± 3.7%, respectively (Figure 3E, Table 2). N. S., Kim, C. C-Q: A. (1983). Plant Sci. Biotechnol Adv. Plant Physiol. 3, and ver. Commun Integr Biol. NPTII probes were
used in. doi: 10.1111/j.1574-6968.2010.01977.xPubMed Abstract | CrossRef Full Text | Google Scholar Haudecoeur, E., Planamente, S., Cirou, A., Tannières, M., Shelp, B. 4 showed higher level of T-DNA transfer than GV2260 and Super-Agrobacterium ver. 64 (2), 318–330. These results imply that all of the lines we obtained were independent and did
not contain a cloned plant. 1, but the level of T-DNA transfer was same in ver. ravennae calli. E., Honma, M., Yamada, M., Sasaki, T., Martineau, B., Hiatt, W. On the other hand, research has shown that the ethylene target points are involved with vir gene expression, and the ethylene perceiving plant would reduce vir gene inducers or release
antagonists of the vir gene inducers (Nonaka et al., 2008b). One-way analysis of variance (ANOVA) and Tukey Kramer's multiple range test, with P < 0.01 or P < 0.05, were carried out to determine the significant differences. GABA controls the level of quorum-sensing signal in Agrobacterium tumefaciens. FEMS Microbiol. 8, 195. Therefore, the
additional effort should have been required to adapt Agrobacterium-mediated transformation for a wide variety of plants. doi: 10.1385/1-59745-130-4:15PubMed Abstract | CrossRef Full Text | Google Scholar Anand, A.
Uppalapati, S. doi: 10.1002/mbo3.123CrossRef Full Text | Google Scholar Cangelosi, G. In our study, the transformation frequency was calculated using regenerated rooting shoot
with diploid and a single copy per inoculated segment. tumefaciens with AcdS and GabT increased the T-DNA transfer and stable transformation frequency. After regenerated diploid shoots (2n) were selected, the exogenous T-DNA was detected by PCR (data not shown) and Southern hybridization analysis (Supplemental Figure 2). Classification of
GUS-stained cotyledon explants. doi: 10.1073/pnas.87.17.6708PubMed Abstract | CrossRef Full Text | Google Scholar Chetty, V. The bars indicate the standard deviation (n = 3). Biotechnol. The calli were induced directly from the seeds on MS medium, containing 1 g/l casamino acids, 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 0.2 mg/l 6-
benzylaminopurine (BAP), 30 g/l 4-O-α-D-glycopyranosyl-D-glycopyranose (maltose H) (Wako, Tokyo, Japan), were subcultured for 2 weeks before inoculation. Other steps showed different responses to AcdS and/or GabT activity (Figure 3 and Table 2). (2000). tumefaciens with AcdS (Super-Agrobacterium ver. The
protein concentration of the lysate was measured with a BCA Protein Assay Kit (Novagen, MA, USA). Environ. In this study, with the tissue culture and co-cultivation methods, 500 µM of acetosyringone, which was enough to induce vir gene expression (Nonaka et al., 2008b), was used during co-cultivation. The transcription terminal sequence of the
tumefaciens GV2260 (pBBRgabT, pEAQ-GFP-HT); V4-Q: A. To evaluate the ability of the T-DNA transfer in C-G, V1-G, V3-G, and V4-G, the frequency of class 4 was compared. Different characters indicate values that were statistically different in the one-way ANOVA and Tukey-Kramer method, multiple comparison method (P < 0.01). Cells were
collected and washed twice with 100 mM Tris-HCl (pH 8.5) and resuspended in 1.5 ml of lysate buffer. P., Okura, V. The sterilized tomato seeds were sown on MS medium, containing 15 g/L sucrose (Wako, Tokyo, Japan) and 0.3% Gelrite (Wako, Tokyo, Japan). An alternative strategy was the utilization of ACC deaminase (AcdS) activity, which cleaves
ACC, the ethylene precursor, to ammonia and α keto-butyrate. Comparative transcriptomic analysis reveals that ethylene/H2O2-mediated hypersensitive response and programmed cell death determine the compatible interaction of sand pear and Alternaria alternata. 115 (34), E8096–E8103. E., Kitajima, J. E. 23 (10-11), 692-698. tumefaciens strains
(Figure 31). Figure 3 Effect of AcdS and GabT activity on regeneration and stable transformation. 5, 334-341. E., Nester, E. We would like to thank Editage (www.editage.jp) for English language editing. Supplementary Material for this article can be found online at: Figure 1 | Map of binary vectors. 103 (19), 7460-7464.
(1978). doi: 10.1073/pnas.0808005106PubMed Abstract | CrossRef Full Text | Google Scholar He, F., Nair, G. Microbiol. From this, 15 µl of culture was harvested and added to 15 ml of LB medium and cultured at 28°C and 200 rpm. 7, 42649. In N. Plasmid pEAQ-GFP-HT was used as a binary vector in Agroinfiltration method. lycopersicum "Micro-
Tom" (Figures 2A, C), however this is one step of the stable transformation process. tumefaciens GV2260 (pBBRgabT, pIG121-Hm); V4-G: A. Plant Breed. Cotyledons from 7-day-old tomato seedlings were cut into four pieces and used to generate two locations for inoculations for inoculation of V4-E increased the T-DNA transfer frequency.
by 7.2, 2.4, and 1.7 times, compared to the C-E, V1-E, and V3-E, respectively (Figure 2A). Therefore, using these promoters would be effective in E. torvum. The activity of AcdS and GabT increased step (i) (Figures 2A, C). On the other hand, in N. RB; Right
border sequence, LB; Left border sequence. doi: 10.1016/j.biotechadv.2018.12.008PubMed Abstract | CrossRef Full Text | Google Scholar Han, J. 2 (12), 2143-2150. lycopersicum "Micro-Tom," which has a well-established regeneration system for processes (ii) to (iv) (Sun et al., 2006). Then, the two fragments were combined by fusion-PCR with the
primer's amp_ter-for2 and gabTR. doi: 10.1111/j.1462-5822.2008.01215.xPubMed Abstract | CrossRef Full Text | Google Scholar Zambryski, P., Joos, H., Genetello, C., Leemans, J., Van Montagu, M., Schell, J. (1985). Indeed, A. Both activities were detected in the V4 strain, but the AcdS and GabT activities in V4 were one-third of the V1 and V3,
respectively (Figures 1C, D). Evaluation of the Super-Agrobacterium for T-DNA Transfer in PlantsTo examine whether the AcdS and GabT activities were enough to increase the transfer in E. Identification of the signal molecules produced by wounded plant cells that activate T-DNA transfer in Agrobacterium
tumefaciens. J. 82 (3), 433-441. doi: 10.2170/jjphysiol.5.334CrossRef Full Text | Google Scholar Vaghchhipawala, Z., Radke, S., Nagy, E., Russell, M. ACC deaminase activity and GabT activity in A. The expression of both genes was under the control of the lacZ gene promoters (pBBRacdSgabT, Figure 1A). Four new derivatives of the broad-host-range
cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. H., Mirabella, R., Bronstein, P. coli, GeneBank accession #J01780), KanR; Kanamycin resistance gene, RB; Right border sequence, LB; Left border sequence. V1-G was slightly higher than that of the C-G. benthamiana and 1 at O.D.600 for S. C., Hobbiger, F. Historical
account on gaining insights on the mechanism of crown gall tumorigenesis induced by Agrobacterium tumefaciens. Mii (Chiba University, Japan) for kindly providing the E. tumefaciens and W1-Q (Figure 2D), but it was the
same level as the V3-Q. U. The increased endogenous concentrations of GABA seem to be the reason for impaired cell elongation in the Arabidopsis thaliana mutants, pop2, and her1, and the corresponding phenotypes (Renault et al., 2011). Enhanced resistance to gray mold (Botrytis cinerea) in transgenic potato plants expressing thionin genes
isolated from Brassicaceae species. The frequencies for rooting from the shoots (rooting number) were similar for all strains (Figure 3G, Table 2). This value was calculated as the ratio between independently transformed plants with diploid and single copy number in soil (checked by a ploidy analyzer and Southern blot analysis) and
the total number of explants infected with A. After co-cultivation, the β-glucuronidase (GUS) activity of the E. YK performed the experiments about Agroinfiltration protocol for Micro-Tom, a model cultivar for tomato functional genomics.
tumefaciensThe pellet of A. Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. Efficient octopine Ti plasmid-derived vectors for Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. Efficient octopine Ti plasmid-derived vectors for Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. Efficient octopine Ti plasmid-derived vectors for Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. Efficient octopine Ti plasmid-derived vectors for Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. Efficient octopine Ti plasmid-derived vectors for Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium ver. The amount of AcdS protein in the Super-Agrobacterium v
10 mM GABA, and a proteinase inhibitor cocktail. (1992). The frequency of regenerated rooting shoot with single copy was same level in all A. doi: 10.1046/j.1439-0523.2000.00438.xCrossRef Full Text | Google Scholar Forlani, G., Bertazzini, M., Giberti, S. (2008b). It was not clear if the V4 affected these other steps. Plant Mol. If stronger promoters
were used, the expression levels would be increased. Effect of ethylene on Agrobacterium tumefaciens-mediated gene transfer to melon. In S. The additional effects of AcdS and GabT under the acetosyringone indicate that in the T-DNA transfer, the ethylene and GABA affect different from the level of vir gene inducer. Thirty explants were subjected
to each treatment. The V1-E, V3-E, and V4-E showed higher T-DNA transfer frequencies than the control, C-E. 4 by introducing both AcdS and GabT activity to the GV2260 strain, which has similar abilities, compared with other strains such as EHA101, LBA4404, and MP90 (Sun et al., 2006 and Chetty et al., 2013). H., Hirschi, K. torvumvia
the agroinfiltration method.In this study, the stable transformation frequency was 15.2 ± 1.1%. doi: 10.1038/nbt0696-745CrossRef Full Text | Google Scholar Liu, P., Nester, E. M., 2nd, et al. In fact, our results showed that the Super-Agrobacterium
ver. Efficient transient protein expression in tomato cultivars and wild species using agroinfiltration-mediated high expression system. doi: 10.1007/s00299-012-1358-1PubMed Abstract | Google Scholar Yuan, Z.
The leaves were then syringe infiltrated with the A. tumefaciens for research purposes, there has been a great deal of effort to remove its oncogenesis characteristics, and to develop a binary vector system (Zambryski et al., 1983; Bevan, 1984; Komari et al., 2006). doi: 10.1046/j.1365-313X.1994.6020271.xPubMed Abstract |
CrossRef Full Text | Google Scholar Hoekema, A., Hirsch, P. doi: 10.1080/09168451.2018.1431516PubMed Abstract | CrossRef Full Text | Google Scholar Murashige, T., Skoog, F. 8, 6068-6076. tumefaciens GV2260 (pBBRacdS, pIG121-Hm); V3-G: A. To ascertain this information, the frequency of each process was observed in S. To create a
transgenic plant, it is important to avoid somaclonal variation and multiple copies, which our method did. The process of Agrobacterium-mediated stable transformation contains four steps: i) the T-DNA transfer into plant cells and integration into the host genome, ii) callus induction, iii) the regeneration of shoots, and iv) rooting. In animals, GABA is
particularly well known as an effector, lowering blood pressure (Elliott and Hobbiger, 1959; Takahashi et al., 1961; Takahashi et al., 1961; Takahashi et al., 1965), and its mechanism of action has been well studied. 146 (2), 703-175. (1959). In this study, we used the lac promoter to drive both the acdS and gabT genes. The application of vir gene inducers (Stachel et al.,
1985; Stachel et al., 1986; Cangelosi et al., 1990; He et al., 2013), using ternary system (van der Fits et al., 2000), utilization of the Ori of the binary vector (Ye et al., 2011; Vaghchhipawala et al., 2018), have subsequently improved its
transformation frequencies. Another strategy to increase T-DNA transfer frequencies was the removal of the lac promoter, the virD promoter was cloned from A. Thus, the inhibition of GABA further increased the
transformation. tumefaciens GV2260 (pBBRacdSgabT, pIG121-Hm). tumefaciens takes the GABA from the plant into the bacterial cell through a kind of ABC transporter (Planamente et al., 2013), if the GabT activity introduced A. Enhanced production of single copy backbone-free transgenic plants in multiple crop species
using binary vectors with a pRi replication origin in Agrobacterium tumefaciens. 9 (6), 303-306. (1986). lycopersicum, respectively, even with the application of the vir gene inducers. These results suggest that V1-G had positive effects on step (ii) and (iii) in the
Agrobacterium-mediated stable transformation process. tumefaciens with AcdS (Figures 3A-D), as the ethylene induced hypersensitive responses and programmed cell death (Wang et al., 2017); infection of A. tumefaciens GV2260 that had AcdS activity introduced into it, was efficacious in the suppression of ethylene evolution from plant tissues
during co-cultivation and increasing T-DNA transfer [Nonaka et al., 2010; Hao et al., 2010]. A DIG-labeled probe was generated by DIG-High Prime, and the DIG signal was detected according to the manufacturer's protocol (Roche Diagnostics, Basel, Swiss). Statistical Analysis The average values were
obtained from three biological replicates. GabT metabolizes GABA to glutamate assay kit (Yamaki, Tokyo, Japan) (Akihiro et al., 2008).T-DNA Transfer Assay in E. In planta fitness-cost of the Atu4232-regulon encoding
for a selective GABA-binding sensor in Agrobacterium. (A) Present the maps of the T-DNA region in the pIG121-Hm expression vectors used for the stable transformation. This enzyme was found in some plant growth promoting bacteria (PGPBs), such as Pseudomonas sp., which were found on the plant surface (Sheehy et al., 1991), and these bacteria
utilize ACC as a nitrogen source. These two genes were introduced by pBBR1MCS-5, the broad host range plasmid (Kovach et al., 2008a; Nonaka et al., 2017). Plant Cell Tiss. (E) Transient transformation via Agroinfiltration methods on S. M., Wise,
A. The C-G, V1-G, V3-G, and V4-G showed the stable transformation efficiencies of 4.3 ± 1.9, 9.7 ± 0.4, 9.8 ± 1.6, and 15.2 ± 1.1%, respectively (mean ± SD of three repetitions) (Figure 3H and Table 2). Optimization of regeneration and transformation parameters in tomato and improvement of its salinity and drought tolerance. TS constructed the
plasmid pBBRacdSgabT and did the western blot analysis. 1-Aminocyclopropane-1-carboxylate deaminase enhances Agrobacterium tumefaciens-mediated gene transfer into plant cells. On the other hand, significant differences in GABA content during the co-cultivation were not observed between A. 4) with the ability to remove ethylene, suppressed
the browning phenomena. After ultraviolet (UV) cross-linking, the membranes were hybridized in a solution containing 7% sodium dodecyl sulfate (SDS), 50% deionized formamide, 50 mM sodium phosphate (pH 7.0), 2% blocking solution, 0.1% N-lauroylsarcosine, 0.75 M NaCl, and 75 mM sodium citrate at 42°C overnight. doi: 10.1007/s11248-010-
9458-6PubMed Abstract | CrossRef Full Text | Google Scholar Yuan, Z. The effect of γ-aminobutyric acid on blood pressure. Nature 303 (5913), 179-180. The transformation frequency might depend on the bacterial strain, binary vector and the selection method. 42 (10), 1825-1831. In brief, after 3 days of co-cultivation, tomato cotyledon segments
were placed on a callus-induction medium [MS medium containing 0.3% Gelrite (Wako, Tokyo, Japan), 1.5 mg/l zeatin, 100 mg/l kanamycin, and 375 mg/l Augmentin (GlaxoSmithKline, London, UK)] for 4 weeks. Plant Biol. C., Lomonossoff, G. Next, we evaluated the abilities of Super-Agrobacterium V4 using S. A binary plant vector strategy based on
separation of vir- and T-region of the Agrobacterium tumefaciens Ti-plasmid. J Bacteriol. 4 was one third of that found in Super-Agrobacterium wer. From these results, the effect of the Super-Agrobacterium was found to be different, dependent on the plant species, thus the selection of the most suitable strain is important for the successful application
of the technology. Even under conditions where the vir gene is sufficiently expressed, our Super-Agrobacterium strains could further improve T-DNA transfer. ACC deaminase was probed with an anti-ACC deaminase antibody. doi: 10.1038/srep42649PubMed Abstract | CrossRef Full Text | Google Scholar Nonaka, S., Sugawara, M., Minamisawa, K.,
Yuhashi, K., Ezura, H. Efficient transformation of rice (Oryza sativa L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA. The number of GUS-stained spots per 1 g of E. Natl. 5, 340. tumefaciens GV2260 (pEKH2-nosPNPTII-ubiPGUS-35SPHPT; pEKH2), A. coli, GeneBank accession #J01780), KanR; Kanamycin
resistance gene. The ligated fragment was inserted into pBBRacdS (Nonaka et al., 2008a) and digested with EcoRI and XbaI (New England Biolabs, Hirchin, UK). doi: 10.3791/1292CrossRef Full Text | Google Scholar Wang, H., Lin, J., Chang, Y., Jiang, C. 83 (2), 379-383. tumefaciens was re-suspended in 100 µl of BugBuster Master mix (Novagen, MA
USA) for lysate preparation. New Phytol. Chem. 103 (12), 4658-4662. coli; acdS, ACC deaminase gene from E. The plant signal salicylic acid shuts down expression of the vir regulon and activates quormone-quenching genes in Agrobacterium.
Cooperative Research Grant from Plant Transgenic Design Initiative (PTraD), Gene Research Center in Tsukuba Innovation Plant Research Center in Tsukuba, Japan supported this research Center in Tsukuba Innovation Plant Research Center in Tsukuba, Japan supported this research Center in Tsukuba Innovation Plant Research Center in Tsukuba, Japan supported this research Center in Tsukuba, Japan supported this research Center in Tsukuba Innovation Plant Research Center in Tsukuba, Japan supported this research Center in Tsukuba, Japan supported this research Center in Tsukuba Innovation Plant Research Center in Tsukuba, Japan supported this research Center in Tsukuba, Japan supported this research Center in Tsukuba Innovation Plant Research Center in Tsukuba, Japan supported this research Center in Tsukuba, Japan s
could be construed as a potential conflict of interest. Acknowledgments We appreciate the help of Prof. 38 (1), 75-84. ravennae calli were assayed histochemical with X-Gluc buffer containing 100 mM phosphate buffer, 10 mM EDTA, 2.5 mM potassium ferricyanide, 2.5 mM potassium ferrocyanide, 0.1% Triton X-100, and 0.5 mg/l X-glucuronide.
Therefore, to expand plant spices and cultivars adapting Agrobacterium-mediated transformation, multiply suppress of these negative factors would be also effective. Data Availability Statement and the experiments, analyzed the
data, and wrote the manuscript. doi: 10.1002/j.1460-2075.1983.tb01715.xPubMed Abstract | CrossRef Full Text | Google Scholar strain ACP gene encoding 1-aminocyclopropane-1-carboxylate deaminase. W., Setubal, J. Comparative transcriptome analysis of Agrobacterium tumefaciens in response to plant signal salicylic acid, indole-3-acetic acid and
gamma-amino butyric acid reveals signalling cross-talk and Agrobacterium-plant co-evolution. (2017). J., Ceballos, N., Garcia, D., Narváez-Vásquez, J., Lopez, W., Orozco-Cárdenas, M. Each experiment was repeated three times. Ploidy Analysis The ploidy of the rooting shoots was checked with flow cytometry. P., Liu, P., Saenkham, P., Banta, L. E.
Elzer, P. I., Yasuta, T., Minamisawa, K. However, some plants have significantly lower transient gene transfer rates, creating a limitation in plant science research that should be resolved by increasing the transfermation (T-DNA transfer) frequency in a wide variety of plant hosts. Ethylene production in plants during transformation
suppresses vir gene expression in Agrobacterium tumefaciens. To utilize this unique ability of A. Therefore, in N. lycopersicum "Micro-Tom." Additionally, we evaluated the ability of T-DNA transformation frequencies (Supersicum "Micro-Tom." Additionally, we evaluated the ability of T-DNA transformation with V4 in the Agroinfiltration method. PLoS One. G., Nester, E. tumefaciens, resulting in increased T-DNA transformation frequencies (Supersicum "Micro-Tom." Additionally, we evaluated the ability of T-DNA transformation with V4 in the Agroinfiltration method.
Agrobacterium ver. M., Haring, M. Calli that formed segments were cultured on shoot-induction medium [MS medium containing 0.3% Gelrite (Wako, Tokyo, Japan), 1.0 mg/l zeatin, 100 mg/l kanamycin, and 375 mg/l Augmentin (GlaxoSmithKline, London, UK)] for 4 weeks. To measure the AcdS and GabT activity, cells were collected by
centrifugation, and the lysate was prepared. pEAQ: versatile expression vectors for easy and quick transformations. Materials and MethodsBacterial Strains, Vectors, and Culture ConditionsAll bacterial strains and vectors, which were used in
this study, were listed up in Table 1. Masahiro Mii from Chiba University, Japan. 29, 87-93. 49 (9), 1378-1389. The shoots were then placed on rooting medium, which consisted of half-strength MS medium, 0.3% Gelrite (Wako, Tokyo, Japan), 100 mg/L kanamycin, and 375 mg/l Augmentin, for 2 weeks. If the target point of the ethylene was the
reduction of the vir gene inducer, the effect of the Super-Agrobacterium ver. Different characters indicate values that were significantly different according to the one-way analysis of variance, multiple comparison method (P < 0.01). Cult. C., Edlind, M. tumefaciens with GabT activity and the control (data not shown). Plant J. doi:
10.1371/journal.pone.0200972. V1-G and V4-G showed slightly higher calli induction ratios (calli number / segments number) than the V3-G. C., Kaul, R., Monks, D. 7 (7), 682-693. For the further improvement of Super-Agrobacterium ver.1, a stronger promoter was used to drive acdS. Physiol. Transformation of cultured cells of Chenopodium quinoa
by binary vectors that carry a fragment of DNA from the virulence region of pTiBo542. 74 (8), 2526-2528. Different characters indicate values that were statistically different in a one-way ANOVA and the Tukey-Kramer method, multiple comparison method (P < 0.01). 1 would be masked by acetosyringone. Regenerated shoots from "Micro-Tom" calli
inoculated with (A) C-G, (B) V1-G, (C) V3-G, and (D) V4-G. Afr. Sugars induce the Agrobacterium virulence genes through a periplasmic binding protein and a transmembrane signal protein. As A. We also thank Prof. In contrast, most previous studies calculated this frequency from the PCR-positive tomatoes (Sun et al., 2006; Khoudi et al., 2009;
Khuong et al., 2013; Chetty et al., 2013; Chetty et al., 2013). With the Agroinfiltration method, the same tendency was observed in S. doi: 10.1080/00021369.1978.10863261CrossRef Full Text | Google Scholar Hoshikawa, K., Fujita, S., Renhu, N., Ezura, K., Yamamoto, T., Nonaka, S., et al. Indoleacetic acid, a product of transferred DNA, inhibits vir gene expression and
growth of Agrobacterium tumefaciens C58. Previous studies have reported transformation frequencies that differ from ours (Sun et al., 2013). Indeed, replacement of the promoter increased the acdS gene expression and the activity in A. G., Trobacher, C. Red bars represent the position of
probes used in the southern blot analysis. One square centimeter of leaf was cut from the rooting shoots, chopped, and added to 250 µl of nucleus-extraction solution (CyStain UV Precise P, Sysmex, Hyogo, Japan). 32 (9), 1441-1454. doi: 10.1111/j.1399-3054.1962.tb08052.xCrossRef Full Text | Google Scholar Nonaka, S., Ezura, H. The amount of
protein was adjusted to 100 µg per reaction mixture. Sci. (1991). lycopersicum "Micro-Tom." Explants were prepared from 7 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 7 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 7 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 7 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 7 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 7 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 7 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 7 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 7 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 7 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 7 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 7 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lycopersicum "Micro-Tom." Explants were prepared from 8 days old seedlings. lyco
Google Scholar Velásquez, A. This promoter showed higher gene expression levels than the lac promoter during co-cultivation (Someya et al., 2013), resulting in higher T-DNA transfer frequencies in Super-Agrobacterium ver. 178 (3), 647-656. To purify the nucleus-extraction solution, 1 mm2 mesh was used. tumefaciens GV2260 (pBBRgabT, pEKH2),
V4-E: A. (1976). Increased 1-aminocyclopropane-1-carboxylate deaminase activity enhances Agrobacterium tumefaciens-mediated transformation frequency of commercial canola cultivars. Agrobacterium tumefaciens-mediated transformation of bottle gourd
(Lagenaria siceraria Standl.). 87 (17), 6708-6712. (2008). (B) Southern blot analysis of the T0 generation. An Agrobacterium tumefaciens strain with gamma-aminobutyric acid transaminase activity shows an enhanced genetic transformation ability in plants. Acad. Front. ravennae. T., Crété, P., Robaglia, C., Caffarri, S. F., Nester, E. Plant. 285 (39),
30294-30303. A revised medium for rapid growth and bio assays with tobacco tissue cultures. doi: 10.4161/cib.23692PubMed Abstract | CrossRef Full Text | Google Scholar Planamente, S., Vigouroux, A., Mondy, S., Nicaise, M., Faure, D., Moréra, S. J., Ron, E., et al. pEKH2), V3-E: A. torvum were inoculated with four strains [(C-Q), (V1-Q), (V3-Q), and
(V4-Q)]. To estimate the T-DNA transfer, the frequency of more than 20% was calculated. Tomato Stable Transformations followed the protocol by Sun et al. 2) (Someya et al., 2013). Virus-induced gene silencing (VIGS) in Nicotiana benthamiana and tomato. HindIII indicates the restriction enzyme sites that were used in the
southern hybridization. tumefaciens; one effective strategy was to upregulate its vir gene expression levels. To characterize each strain, C-G, V1-G, V3-G, and V4-G were used. GFP fluorescence was detected 3 and 5 days after infiltration for N. F., Binns, A. The 2n plants were then planted on solid medium and acclimatized. Southern Blot
AnalysisGenomic DNA was extracted from young tomato leaves using Maxwell 16 System DNA Purification kits (Promega, WI, USA). doi: 10.1126/science.1066804PubMed Abstract | CrossRef Full Text | Google Scholar Ye, X., Williams, E. 3 was also effective in the agro-infiltration method (Hoshikawa et al., 2019; Knoch et al., 2019). Stable
transformation techniques are important as they are used for breeding GM crops. Previous research compared the vir gene promoter (someya et al., 2013). Jpn. doi:
10.1016/j.plantsci.2012.06.001PubMed Abstract | CrossRef Full Text | Google Scholar Someya, T., Nonaka, S., Nakamura, K., Ezura, H. (2007). After the third wash, the seeds were kept in water for 2 days. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. (C) ACC
deaminase activity in A. No differences were observed between the strains in E. W. R., Lineberger, R. Anal. doi: 10.3389/fmicb.2014.00340PubMed Abstract | CrossRef Full Text | Google Scholar Khoudi, H., Nouri-Khemakhem, A., Gouiaa, S., Masmoudi, K. Expressing multiple genes using the same promoter may reduce the expression levels of each
gene. Optimisation of tomato Micro-tom regeneration and selection on glufosinate/Basta and dependency of gene silencing on transgene copy number. Hypothesis/review: contribution of putrescine to 4-aminobutyrate (GABA) production in response to abiotic stress. (E) Appearance of calli, (F) Frequency of regeneration, (G) Rooting ratio, (H)
Frequency of calli regeneration. H. GUS stained tomato cotyledon explants were observed and images were taken using a stereoscopic microscope system (Leica: MX FLIII, DFC300 FX, Application Suite, Leica, Wetzlar, Germany).
GABA as a signaling compound in plant growth and development. C., Haudecoeur, E., Faure, D., Kerr, K. (C) Appearance ratio Class 4 in tomato cotyledons. M. 14 (6), 745-750. NosP; Nopalin synthesis gene promoter, uidA;
beta-glucuronidase gene, hptII; hygromycin phosphotransferase gene, OriV; replication origin V (IncPα, plasmid RK2 from E. Comparative effects of ethylene inhibitors on Agrobacterium-mediated transformation of drought-tolerant wild watermelon. GUS stained tomato cotyledons were categorized depending on the stained area. To ascertain this, the
T-DNA transfer abilities of Super-Agrobacterium ver. According to the results, GUS stained tomato explants were categorized into 4 classes: (Class 1) less than 5%, (Class 2) 5-10%, (Class 3) 10-20%, and (Class 4) more than 20%. tumefaciens GV2260 (pBBRacdS. Isolation, sequence, and expression in Escherichia coli of the Pseudomonas sp. Biosci
Biotechnol Biochem. A., Hoge, J. tumefaciens GV2260 (pBBRacdSgabT, pIG121-Hm). References Akihiro, T., Koike, S., Tani, R., Tominaga, T., Watanabe, S., Iijima, Y., et al. doi: 10.1007/s11240-010-9748-yCrossRef Full Text | Google Scholar Ohta, S., Hattori, T., Nakamura, K. 4, the enzymatic activity was one third of the Super-Agrobacterium
ver. tumefaciens were effective at increasing the T-DNA transfer frequency (Nonaka et al., 2010; Hao e
Zhou, S., Takayama, M., Nakamura, K., Ezura, H. GABA accumulation causes cell elongation defects and a decrease in expression of genes encoding secreted and cell wall-related proteins in Arabidopsis thaliana. tumefaciens did not affect its bacterial growth. Mitsui (Tohoku University, Japan) for the gift of the pEKH2 plasmid and pBBR1MCS-5,
respectively. 47 (3), 426-431. An efficient Agrobacterium tumefaciens-mediated genetic transformation of "Equsi" melon (Colocynthis citrullus L.). To estimate whether these two genes affect bacterial growth or not, growth curves were compared for the four strains [(C), (V1), (V3), and (V4)]. Z. E., Miller, A. W., Zambryski, P. lycopersicum"). Effect of
gamma-aminobutyric acid (GABA) on normotensive or hypertensive rats and men. J., Shen, J., Johnson, S., Lowe, B., Radke, S., et al. (I) Frequency of appearence for transgenic tomato plants which have single copy of T-DNA. (2014). doi: 10.1073/pnas.1215033110PubMed Abstract | CrossRef Full Text | Google Scholar Ishida, Y., Saito, H., Ohta, S.,
Hiei, Y., Komari, T., Kumashiro, T. J., Moréra, S., et al. RepB C-terminus mutation of a pRi-repABC binary vector affects plasmid copy number in Agrobacterium and transgene copy number in Agrobacte
ChvE function in sugar binding, sugar utilization, and virulence in Agrobacterium tumefaciens. doi: 10.1007/s00299-013-1456-8PubMed Abstract | CrossRef Full Text | Google Scholar Knoch, E., Sugawara, S., Mori, T., Poulsen, C., Fukushima, A., Harholt, J., et al. When the O.D.600 of the culture reached 0.7 to 0.9, the cells were then centrifuged,
collected, and checked for enzymatic activity. tumefaciens. EMBO J. Biol. I. doi: 10.1093/nar/13.13.4777PubMed Abstract | CrossRef Full Text | Google Scholar Elliott, K. tumefaciens were 0.3 at O.D.600 for N. 15, 473-497. Especially in tomato, this newly bred bacterium (Super-Agrobacterium ver. doi: 10.3389/fpls.2017.00195PubMed Abstract |
CrossRef Full Text | Google Scholar Wood, D. The inoculated explants were cultured on co-cultivation medium (pH 5.2) containing MS salts, 30 g/L glucose, 500 µM acetosyringone, and 0.3% Gelrite (Wako, Tokyo, Japan) at 25°C, for 3 days in the dark. tumefaciens GV2260 (pBBRMCS1-5, pIG121-Hm); V1-G: A. doi: 10.1073/pnas.0600313103PubMed
Abstract | CrossRef Full Text | Google Scholar Davis, M. PubMed Abstract | CrossRef Full Text | Google Scholar van der Fits, L., Deakin, E. 146, 70-84. Rep. tumefaciens GV2260 (pIG121-Hm), or A. (2012). The strength of the GFP signal was used as an indicator of the frequency of the T-DNA transfer. Biochemical mechanism on GABA accumulation
during fruit development in tomato. K., Park, S. Some bacteria are known to harbor GABA transaminase (gabT), a GABA degradation enzyme. tumefaciens strains were then cultured on solid LB medium at 28°C for 2 days. 2 than in ver.1 (Someya et al., 2013). Gamma-aminobutyric acid (GABA), an amino acid, was determined to be another negative
factor in Agrobacterium-plant interactions (Chevrot et al., 2006; Haudecoeur et al., 2009; Nonaka et al., 2017). The AcdS activity was measured according to a modified protocol based on that of Honma and Shimomura (1978).
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