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| <b>(51) International Patent Classification <sup>6</sup>:</b><br><b>A01H 1/06</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | <b>A1</b> | <b>(11) International Publication Number:</b> <b>WO 99/03326</b><br><b>(43) International Publication Date:</b> 28 January 1999 (28.01.99)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>(21) International Application Number:</b> PCT/US98/14504<br><b>(22) International Filing Date:</b> 14 July 1998 (14.07.98)<br><br><b>(30) Priority Data:</b><br>08/892,735 15 July 1997 (15.07.97) US<br><br><b>(71) Applicant:</b> UNITED STATES OF AMERICA, as represented by THE SECRETARY OF AGRICULTURE [US/US]; Washington, DC 20250-1400 (US).<br><b>(72) Inventors:</b> BURKE, John, J.; 5412 79th Street, Lubbock, TX 79424 (US); OLIVER, Melvin, J.; 5728 72nd Street, Lubbock, TX 79424 (US); VELTEN, Jeffrey, P.; 5722 70th Place, Lubbock, TX 79424 (US).<br><b>(74) Agents:</b> FIGG, E., Anthony et al.; Rothwell, Figg, Ernst & Kurz, Suite 701 East, Columbia Square, 555 13th Street N.W., Washington, DC 20004 (US).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |           | <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).<br><br><b>Published</b><br><i>With international search report.</i> |
| <b>(54) Title:</b> POLLEN-BASED TRANSFORMATION SYSTEM USING SOLID MEDIA<br><br><b>(57) Abstract</b><br><br>A medium is disclosed that supports pollen germination and pollen tube growth in the presence of <i>Agrobacterium</i> , the medium comprising agarose, sucrose, NO <sub>3</sub> , MnSO <sub>4</sub> , H <sub>3</sub> BO <sub>3</sub> , MgSO <sub>4</sub> and gibberellic acid. A method is disclosed for the genetic transformation of plants and lines by a pollen-based <i>Agrobacterium</i> -mediated transformation. The method comprises the steps of obtaining pollen from a first plant, applying a lawn of <i>Agrobacteria</i> to a solid pollen culture medium, the <i>Agrobacterium</i> comprising at least one heterologous gene sequence capable of being transferred to a plant cell, applying the pollen to the solid medium, allowing the pollen to germinate and grow on the medium, whereby the <i>Agrobacterium</i> attaches to the pollen tube or mediates transfer of the heterologous gene sequence to the germinating pollen to obtain transgenic pollen, applying the transgenic pollen to the stigma of a second plant capable of being fertilized by the pollen of the first plant, thereby fertilizing the second plant, obtaining transgenic seed from the second plant and germinating the transgenic seed to obtain a transgenic plant. The method is particularly suited for use with so-called "dry stigma" pollen.<br><br><div style="text-align: center;"><br/>*162162*<br/><br/><br/>*G00001*</div> |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |

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