Figure S1. Expression profiles of genes related to translation occurring in plastid during isolated microspore culture of cvs. ‘Jersey’ and ‘Mercada’.

a,b The relative expression level of genes encoding proteins of large (a) and small (b) ribosomes subunits. c Genes involved in initiation (infA and InfB) and termination (RF1) of translation. Rpl11, InfB and RF1 are encoded in nuclear genome. Graphs show mean values of n ≥ 3 with SEM. Relative expression level normalised to ML microspores of cv. ‘Jersey’. An asterisk presents a value significantly different between cultivars at a certain day of culture. A hash indicates a value significantly different from the preceding day of culture within cultivar (Tukey’s test, $P < 0.05$). ML – mid-to-late microspore, PM – pre-treated microspores, dC – day of culture.
Figure S2. The relative expression profile of DPD1 gene encoding Mg$^{2+}$-dependent organelle exonuclease during microspore development of cvs. 'Jersey' and 'Mercada'.

Graph shows mean values of n ≥ 3 with SEM. Relative expression level normalised to E microspores of cv. ‘Jersey’. An asterisk presents a value significantly different between cultivars at a certain day of microspore development. A hash indicates a value significantly different from the preceding day of microspore development within cultivar (Tukey’s test, $P < 0.05$). Stages of pollen development: E – early, EM – early-mid, ML – mid-to-late.
Figure S3. Plastid DNA content during regeneration of androgenic plants of cvs. ‘Jersey’ and ‘Mercada’.

a Average plastome copy number in relation to nuclear genome in subsequent days of regenerating plants of ‘Jersey’ and ‘Mercada’ cultivars. b The individual copy number of genes localised in the plastid genome in subsequent days of isolated microspore culture of ‘Jersey’ and ‘Mercada’ cultivars in ‘Jersey’ and ‘Mercada’ cultivars. c The relative expression profile of Poly (Organellar DNA polymerase I) gene. Graphs show mean values of n ≥ 3 with SD in a and b or SEM in c. Relative expression level normalised to 43dC of cv. ‘Jersey’. An asterisk presents a value significantly different between cultivars at a certain day of culture. A hash indicates a value significantly different from the preceding day of culture within cultivar (Tukey’s test, P < 0.05). A $ indicates value significantly different from the calculated average plastome copy number at a particular day of culture within cultivar (Student’s t-test, P < 0.05). Red lines show the average copy numbers calculated from individual copies of presented genes. LSC – long single copy, IR – inverted repeat, SSC – short single copy, ML – mid-to-late microspore, PM – pre-treated microspores, dC – day of culture.
Figure S4. The expression profiles of genes related to chloroplast differentiation during regeneration of plants of cvs. ‘Jersey’ and ‘Mercada’.

Gene encoding transcription factor HY5 and involved in synthesis (PGP1) and docking (RABA5e) of thylakoids in ‘Jersey’ and ‘Mercada’ cultivars. Graphs show mean values of n ≥ 3 with SEM. Relative expression level normalised to 43dC of cv. ‘Jersey’. An asterisk presents a value significantly different between cultivars at a certain day of culture. A hash indicates a value significantly different from the preceding day of culture within cultivar (Tukey’s test, P < 0.05). dC – day of culture.
Figure S5. The plastids observed in converting embryos on 46dC of cvs. ‘Jersey’ and ‘Mercada’. ch- chloroplast, et – etioplast, etl – etioplast-like plastid, Ty – thylakoid. Chloroplasts that were observed only in cv. ‘Jersey’ are characterised by well-developed grana, whereas etioplast contain prolamellar body. Etioplast-like plastid in cv. ‘Mercada’ was more advanced in development and contained single perforated thylakoids and incipient grana without organized structure.
Figure S6. The relative expression level of genes related to plastid biogenesis, chloroplast differentiation and photosynthesis in albino regenerants of cv. ‘Mercada’ compared to albino regenerants of cv. ‘Jersey’.

**a-c** The relative expression level of genes involved in transcription (a), protein import to plastid (b) and translation (c).

**d** The relative expression level of genes related to chloroplast differentiation.

**e** The relative expression level of genes related to photosynthesis. Graphs show mean values of n ≥ 3 with SEM for relative expression level normalised to albino regenerants (GP) of cv. ‘Jersey’. An asterisk presents a value significantly different between green regenerants of ‘Jersey’ and ‘Mercada’ cultivars (t-Student test, P < 0.05).
Figure S7. The normalized expression level of genes important for plastid biogenesis during embryo formation and regeneration of androgenic plants.

Graphs show mean values of n ≥ 3 with SEM for expression level normalised to reference genes. An asterisk presents a value significantly different between ‘Jersey’ and ‘Mercada’ cultivars (t-Student test, P < 0.05). ML – mid-to-late microspore, PM – pre-treated microspores, dC – day of culture.
| Process                     | Genome | Gene  | Gene product                                      | Gene ID or accession number | Primers 5'-3'                                      | Ta [ºC] | Product [bp] |
|-----------------------------|--------|-------|--------------------------------------------------|----------------------------|---------------------------------------------------|--------|-------------|
| **Transcription**           |        |       |                                                  |                            |                                                   |        |             |
| Plastid                     |        | rpoA  | RNA polymerase alpha subunit                     | 4525081                    | pf aacgcttttaggctttgc ggttacgctttgc              | 58     | 114         |
|                             |        | rpoB  | RNA polymerase beta subunit                      | 4525093                    | pf gtttagcaagtgctacag gctttctctctctctctctctctc  | 58     | 101         |
|                             |        | rpoC1 | RNA polymerase beta' subunit                     | 4525121                    | pf ttttagtaagcagcatca ggtttacgctttgc            | 58     | 95          |
|                             |        | rpoC2 | RNA polymerase beta'' subunit                   | 4525122                    | pf ggtttgaggaagacgca ggtttacgctttgc           | 58     | 86          |
|                             |        | tRNA-Glu | tRNAe-UUC                                      | 4525087                    | pf gacctcgttcgtgagctg ggtttacgctttgc          | 58     | 69          |
| Nuclear                     |        | RpoYp | RNA polymerase T phage-like                     | AJ507397.1                  | pf ttgcagaaagatgctg ggtttacgctttgc          | 58     | 114         |
|                             |        | Sig2  | Sigma factor2                                   | AK361731.1                  | pf tacatgcaagcagccag ggtttacgctttgc          | 58     | 91          |
|                             |        | Sig6  | Sigma factor6                                   | AK367382.1                  | pf ctgctcggcctgctgtgc ggtttacgctttgc          | 58     | 92          |
|                             |        | 23S rRNA | 23S RNA                                       | 4525068                    | pf ggttgctttacgctttgc ggtttacgctttgc          | 58     | 103         |
|                             |        | 16S rRNA | 16S RNA                                      | 4525179                    | pf atttggccgtgctttgc ggtttacgctttgc          | 58     | 106         |
|                             |        | infA  | Translation initiation factor1                  | 4525109                    | pf nacccagaaagacgcaag ggtttacgctttgc          | 58     | 103         |
|                             |        | rpl16 | 50S ribosomal protein L2                        | 4525117                    | pf gtttagcttgcagctgac ggtttacgctttgc        | 58     | 115         |
|                             |        | rps1  | 30S ribosomal protein S1                        | 4525112                    | pf ggtgcgctgctgtgag ggtttacgctttgc        | 58     | 111         |
|                             |        | rps4  | 30S ribosomal protein S4                        | 4525135                    | pf cctgaaagatgctttgc ggtttacgctttgc          | 58     | 86          |
|                             |        | rps7  | 30S ribosomal protein S7                        | 4525104                    | pf ttggtcagcagctgctgc ggtttacgctttgc          | 58     | 96          |
|                             |        | rps8  | 30S ribosomal protein S8                        | 4525110                    | pf gacccgccaagcttgaatt ggtttacgctttgc        | 58     | 112         |
|                             |        | cpEF-G | Translation elongation factorG                 | AK353897.1                  | pf tgttgggctgctgctgag ggtttacgctttgc        | 58     | 108         |
|                             |        | lsoB (cpEF2) | Translation initiation factor2               | AK362018.1                  | pf cagatgcaagctgctgtgc ggtttacgctttgc        | 58     | 86          |
|                             |        | cpRF-1 | Peptide release factor1                        | AK360613.1                  | pf cctgaaagatgctttgc ggtttacgctttgc        | 58     | 109         |
|                             |        | Rpl11 | 50S ribosomal protein L11                       | AK369397.1                  | pf tggctcagcagctgcttc ggtttacgctttgc        | 58     | 109         |
| **Translation**             |        | Tic21 | Translocon at inner membrane of chloroplasts21 | AK359467.1                  | pf ttcgctttgcattgct ggtttacgctttgc        | 58     | 95          |
| Nuclear                     |        | Toc159| Translocon of chloroplast 159                   | AK371279.1                  | pf tgttgggctgctgctgag ggtttacgctttgc        | 58     | 96          |
|                             |        | Pol1  | Organellar DNA polymerase gamma                 | KM236205.1                  | pf gttgctttgcattgct ggtttacgctttgc        | 58     | 127         |
| **Replication**             |        |       |                                                  |                            |                                                   |        |             |
| Nuclear                     |        | DPD1  | Defective in pollen organelle DNA degradation1  | MLOC__11431.8               | pf cagatgcaagctgctttgc ggtttacgctttgc        | 58     | 118         |
| **Plastid DNA degradation** |        |       |                                                  |                            |                                                   |        |             |
| Nuclear                     |        | PDR1  | Plastid-dividing ring1                          | AK354365.1                  | pf ggtttgaggaagacgca ggtttacgctttgc        | 58     | 99          |
|                             |        | AgsS  | Glucose-1-phosphate adenylytransferase small subunit | FN179369.1                   | pf ttcggctttgcattgct ggtttacgctttgc        | 58     | 96          |
|                             |        | AgsL  | Glucose-1-phosphate adenylytransferase large subunit | X62242.1                    | pf ttgcagaaagatgctg ggtttacgctttgc        | 58     | 81          |
|                             |        | Dpe2  | 4-alpha-glucanotransferase2                     | FN179406.1                  | pf ggtttgaggaagacgca ggtttacgctttgc        | 58     | 104         |
|                             |        | GBSSI | Granule-bound starch synthasel                 | AF486514.1                  | pf gctgctttgcattgct ggtttacgctttgc        | 58     | 111         |
|                             |        | Ske1  | Starch branching enzyme1                       | AY308454.1                  | pf ggtttgaggaagacgca ggtttacgctttgc        | 58     | 94          |
|                             |        | SSII  | Starch synthasell                             | AK133249.1                  | pf ggtttgaggaagacgca ggtttacgctttgc        | 58     | 87          |
| **Reserve starch biosynthesis** |        |       |                                                  |                            |                                                   |        |             |
| Nuclear                     |        | GBSSIb | Granule-bound starch synthaselb               | AK368223.1                  | pf ggtttgaggaagacgca ggtttacgctttgc        | 58     | 83          |
|                             |        | SSIIb | Starch synthasellb                           | FN179376.1                  | pf ggtttgaggaagacgca ggtttacgctttgc        | 58     | 102         |
|                             |        | Gki1  | Golden 2-like1                                | MLOC 43537                  | pf ggtttgaggaagacgca ggtttacgctttgc        | 58     | 69          |

Table S1. List of genes and primers used to perform RT-qPCR analysis
**Photomorph.**

| Gene      | Description                  | Accession | Start | End |
|-----------|------------------------------|-----------|-------|-----|
| *Gh2*     | Golden 2-like2               | AK353571  | 58    | 115 |
| *HY5*     | Long Hypocotyl5              | HORV1681r | 58    | 91  |
| *PHYA*    | Phytochrome photoreceptorsA  | MLOC 81684| 58    | 107 |
| *PHYB*    | Phytochrome photoreceptorsB  | MLOC 9834 | 58    | 100 |

**Chlorophyll synthesis and photochemistry**

| Gene      | Description                  | Accession | Start | End |
|-----------|------------------------------|-----------|-------|-----|
| *PHF1*    | Phytocrome-interacting factor 3-like5 | HV14044   | 58    | 81  |
| *PGP1*    | Phosphatidylglycerophosphate synthetase 1 | MLOC 5438 | 58    | 107 |
| *R4BA5e*  | Rab GTase protein 5e         | MLOC 73105| 58    | 101 |
| *psbA*    | PSI protein D                | 4525096   | 58    | 110 |
| *psbD*    | PSI protein D2               | 4525151   | 58    | 105 |
| *psaA*    | PSI P700 apoprotein A1       | 4525132   | 58    | 149 |
| *psaF*    | PSI assembly protein ycf4    | 4525190   | 58    | 97  |
| *ycf3*    | PSI assembly protein ycf3    | 4525133   | 58    | 113 |

**Photosystem II**

| Gene      | Description                  | Accession | Start | End |
|-----------|------------------------------|-----------|-------|-----|
| *psaD1*   | PSI-D subunit of PSI         | M98254.1  | 58    | 133 |

**NADH dehydrogenase**

| Gene      | Description                  | Accession | Start | End |
|-----------|------------------------------|-----------|-------|-----|
| *ndhB*    | NADH-PQ oxidoreductase subunit2 | 4525105  | 58    | 126 |
| *ndhF*    | NADH-PQ oxidoreductase subunit5 | 4525163  | 58    | 137 |

**ATP synthase**

| Gene      | Description                  | Accession | Start | End |
|-----------|------------------------------|-----------|-------|-----|
| *atpB*    | ATP synthase CFI beta subunit | 4525186  | 58    | 118 |
| *atpC*    | ATP synthase CFI epsilon subunit | 4525185  | 58    | 107 |
| *atpH*    | ATP synthase CFI subunit III  | 4525125  | 58    | 114 |

**Cytochrome**

| Gene      | Description                  | Accession | Start | End |
|-----------|------------------------------|-----------|-------|-----|
| *petA*    | Cytochrome f                  | 4525192   | 58    | 87  |
| *petD*    | Cytochrome b/f complex subunit IV | 4525080  | 58    | 107 |

**Rubisco**

| Gene      | Description                  | Accession | Start | End |
|-----------|------------------------------|-----------|-------|-----|
| *rbcL*    | Rubisco large subunit        | 4525187   | 58    | 90  |
| *RbcS*    | Rubisco small subunit        | AB020943.1| 58    | 100 |

**Reference genes**

| Gene      | Description                  | Accession | Start | End |
|-----------|------------------------------|-----------|-------|-----|
| *ARF1*    | ADP-ribosylation factor 1-like protein | AJ508222.8| 58    | 61  |
| *EF1*     | Translation elongation factor 1-α | AJ472912  | 58    | 60  |
Table S2. Composition of media used in isolated microspore culture

| Component (mg/L) | SMB1 (Starvation Medium Barley1) | KBP (Kumlehn’s Barley Pollen) | KBPD (Kumlehn’s Barley Pollen Differentiation) | K4NB |
|------------------|-----------------------------------|-------------------------------|-----------------------------------------------|------|
| **Major elements** |                                   |                               |                                               |      |
| KNO₃             | -                                 | 2020                          | 2020                                          | 3640 |
| NH₄NO₃           | -                                 | 80                            | 80                                            | 320  |
| KH₂PO₄           | -                                 | 340                           | 340                                           | 340  |
| MgSO₄·7H₂O       | -                                 | 246                           | 246                                           | 246  |
| CaCl₂·2H₂O       | 147                                | 441                           | 441                                           | 441  |
| **Trace elements** |                                   |                               |                                               |      |
| NaFeEDTA         | -                                 | 27.5                          | 27.5                                          | 20.6 |
| MnSO₄·H₂O        | 8.4                               | 8.4                           | 8.4                                           | 8.4  |
| H₂BO₃            | 3.1                               | 3.1                           | 3.1                                           | 3.1  |
| ZnSO₄·7H₂O       | 7.2                               | 7.2                           | 7.2                                           | 7.2  |
| CoCl₂·6H₂O       | 0.024                             | 0.024                         | 0.024                                         | 0.024|
| CuSO₄·5H₂O       | 0.025                             | 0.025                         | 0.025                                         | 1.25 |
| Na₂MoO₄·2H₂O     | 0.12                              | 0.12                          | 0.12                                          | 0.12 |
| KI               | 0.17                              | 0.17                          | 0.17                                          | 0.17 |
| **Gamborg B5 vitamin mixture** (Duchefa Biochemie) | | | |
| Nicotinic acid   | -                                 | -                             | -                                             | 1    |
| Pyridoxine-HCl   | -                                 | -                             | -                                             | 1    |
| Thiamine-HCl     | -                                 | -                             | -                                             | 10   |
| Myo-inositol     | -                                 | -                             | -                                             | 100  |
| **Kao and Michayluk vitamin mixture** (Sigma) | | | |
| Retinol          | -                                 | 0.01                          | 0.01                                          | -    |
| Thiamine-HCl     | -                                 | 1                             | 1                                             | -    |
| Nicotinic acid   | -                                 | 1                             | 1                                             | -    |
| Riboflavin       | -                                 | 0.2                           | 0.2                                           | -    |
| Ca-pantothenate  | -                                 | 1                             | 1                                             | -    |
| Folic acid       | -                                 | 0.4                           | 0.4                                           | -    |
| Pyridoxine-HCl   | -                                 | 1                             | 1                                             | -    |
| Cobalamine       | -                                 | 0.02                          | 0.02                                          | -    |
| Ascorbic acid    | -                                 | 2                             | 2                                             | -    |
| Calciniferol     | -                                 | 0.01                          | 0.01                                          | -    |
| Biotin           | -                                 | 0.01                          | 0.01                                          | -    |
| Choline chloride | -                                 | 1                             | 1                                             | -    |
| p-Aminobenzoic acid | -                                 | 0.02                          | 0.02                                          | -    |
| Myo-inositol     | -                                 | 100                           | 100                                           | -    |
| **Casein hydrolysate** | -                                 | 250                           | -                                             | -    |
| **Organic acids** |                                   |                               |                                               |      |
| Citric acid      | -                                 | 40                            | -                                             | -    |
| Fumaric acid     | -                                 | 40                            | -                                             | -    |
| Na-pyruvate      | -                                 | 20                            | -                                             | -    |
| NH₄Cl            | 53.4                              | -                             | -                                             | -    |
| Glutamine        | -                                 | 439.3                         | 439.3                                         | 146.4 |
| BAP              | 0.9                               | 0.9                           | 0.224                                         | 0.224|
| Maltose          | 144000                            | 90000                         | 90000                                         | 36000|
| MES              | 424.4                             | -                             | -                                             | -    |
| Phytagel         | -                                 | -                             | 8000                                          | 6000 |
| Cefotaxime       | 250                               | 250                           | -                                             | -    |
| **pH**           | 5.5                               | 5.9                           | 5.9                                           | 5.8  |
Table S3. List of genes, genome localisation and primers used to evaluate plastid DNA copy number using qPCR

(a) Plastid genes

| Gene   | Encoded factor               | Plastome localisation | Region | Gene ID | Primers 5'-3'                     | Product [bp] |
|--------|------------------------------|-----------------------|--------|---------|-----------------------------------|-------------|
| psbA   | Photosystem II protein D1    | 619..1680, cmpl       | LSC    | 4525096 | pF ttggaagctgcattgcgttt          | 110         |
|        |                              |                       |        |         | pR tcccaaaagcagccat              |             |
| matK   | Maturase K                    | 2206..3741 cmpl       | LSC    | 4525145 | pF cgataccatagtccccgct            | 88          |
|        |                              |                       |        |         | pR ggtttaactaatagattagc          |             |
| psbD   | Photosystem II protein D2    | 9159..10220           | LSC    | 4525151 | pF gagttgccaagagattaggcc          | 105         |
|        |                              |                       |        |         | pR aagcacggaaagttttcga           |             |
| atpI   | ATP synthase CF0 subunit IV  | 31348..32091          | LSC    | 4525124 | pF gatccaaacacgcatccga           | 111         |
|        |                              |                       |        |         | pR ggacccaggagcacagcttc          |             |
| clpP   | ATP-dependent Clp protease   | 68250..68900, cmpl    | LSC    | 4525074 | pF cctgagatcgagagtaggc           | 88          |
|        | proteolytic subunit          |                       |        |         | pR cgaacaactcttctgacct          |             |
| infA   | Translation initiation factor1| 76802..77143 cmpl    | LSC    | 4525109 | pF aacccgagaaaaagctgaa          | 103         |
|        |                              |                       |        |         | pR ggctagatgagttctga            |             |
| ndhB*  | NADH-plastoquinone oxidoreductase subunit2 | 86790..89034, cmpl | IR     | 4525060 | pF aggtagtttggtaagcctaa         | 105         |
|        |                              |                       |        |         | pR cggaggtgagtgagcttta          |             |
| 16S rRNA* | 16S rRNA                    | 92685..94176          | IR     | 4525063 | pF atgcattgagatccaa            | 106         |
|        |                              |                       |        |         | pR etgggcgtctatccatctt          |             |
| ndhH*  | NADH-plastoquinone oxidoreductase subunit7 | 102501..102707, cmpl | IR     | 4525162 | pF tagctgattttggcgccctca         | 82          |
|        |                              |                       |        |         | pR tcacacacacacacacatc          |             |
| ndhF   | NADH-plastoquinone           | 102776..104995, cmpl  | SSC    | 4525163 | pF tgggcctaaacacatcgg            | 137         |
|        | oxidoreductase subunit5      |                       |        |         | pR ttcattatctttctctctcct          |             |

(b) Nuclear genes

| Gene   | Encoded factor               | Number of copy in nuclear genome | Accession number | Primers 5'-3' | Product [bp] | Reference      |
|--------|------------------------------|---------------------------------|------------------|---------------|-------------|----------------|
| ARF1   | ADP-ribosylation factor 1-like protein | 1                        | AJ508228.2       | pF etggaagctgcattgcgttt | 61          | Rapacz et al., 2012 |
| EF1    | Translation elongation factor 1-a | 1                          | AJ472912         | pF ccctccttggctggggg | 60          |                |

LSC – long single copy, IR – inverted repeat, SSC – short single copy.