

Article

# Genetic Evaluation of In Vitro Micropropagated and Regenerated Plants of *Cannabis sativa* L. Using SSR Molecular Markers

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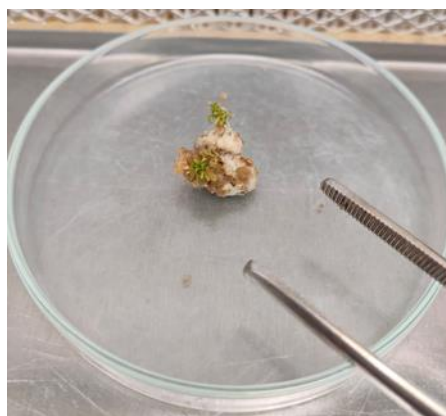


(a)

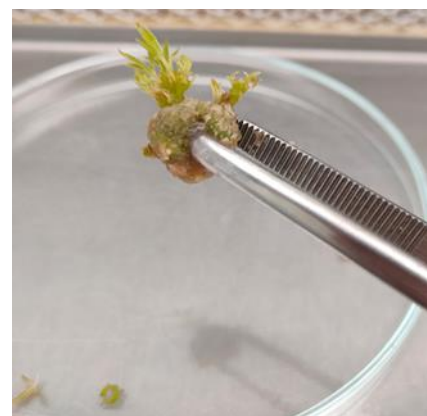


(b)

**Figure S1.** Callus induction and subculturing: (a) stem and leaf explants placed in callus induction medium (1x MS basal medium supplemented with 2.25  $\mu$ M Kin and 4.5  $\mu$ M 2,4 D). Petri dish diameter = 9 cm. (b) Calli subculturing in magenta vessel on MS medium containing 4.52  $\mu$ M 2,4-D after 3 weeks of culture. Magenta vessel bottom dimensions = 7 cm X 7 cm.



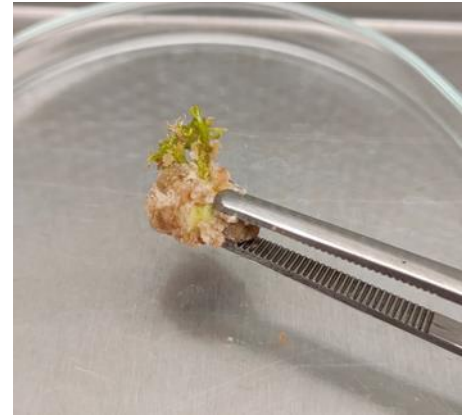
(a)



(b)



(c)



(d)

**Figure S2.** Shoot formation and plant regeneration from callus in stem explants: (a) and (b) shoot organogenesis in stem segment explants of *Cannabis sativa* L. on MS medium containing 4.44  $\mu$ M BA after 2 and 3 weeks of culture respectively; (c) Multiple shoots formation on MS basal medium supplemented with 4.54  $\mu$ M TDZ. Petri dish diameter = 9 cm.



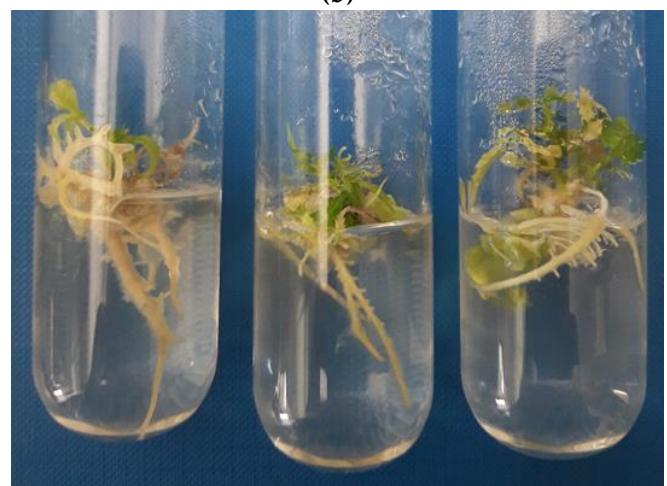
(a)



(b)



(c)

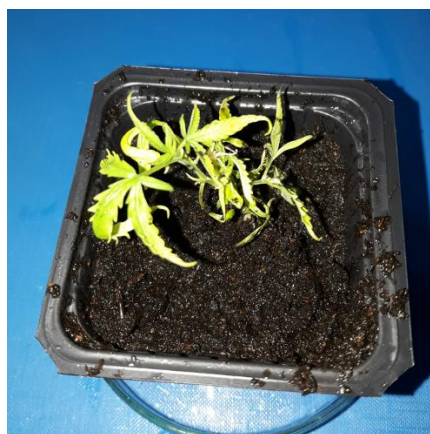


(d)

**Figure S3.** Rooting of shoots regenerated from calli: (a) root induction. Test tube diameter = 25 mm.; (b-d) root elongation on MS basal medium supplemented with several plant growth regulators. Test tube diameter = 25 mm. Magenta vessel bottom dimensions = 7 cm X 7 cm.



**Figure S4.** Rooted shoots regenerated from calli ready for acclimatization.



**Figure S5.** Survived plantlet regenerated from calli placed in plastic pot, containing a sterile mixture of peat and perlite after three weeks of acclimatization.

**Table S1.** Several genetic characteristics of PCR amplicons for used STR loci and mother plant material of the two *Cannabis sativa* L. varieties, i.e. the H-CBD and the H-CBG variety.

| Variety | Locus     | n | Na | Ne    | I     | Ho    | He    | uHe   | F      | PIC   |
|---------|-----------|---|----|-------|-------|-------|-------|-------|--------|-------|
| H-CBD   | ANUCS 202 | 8 | 4  | 2.977 | 1.234 | 0.500 | 0.664 | 0.708 | 0.247  | 0.616 |
|         | ANUCS 301 | 8 | 2  | 1.882 | 0.662 | 0.250 | 0.469 | 0.500 | 0.467  | 0.359 |
|         | B05 CANN1 | 8 | 4  | 3.879 | 1.371 | 1.000 | 0.742 | 0.792 | -0.347 | 0.694 |
|         | B01 CANN1 | 8 | 2  | 2.000 | 0.693 | 0.500 | 0.500 | 0.533 | 0.000  | 0.375 |
|         | ANUCS 303 | 8 | 1  | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | -      | 0.000 |
|         | H09 CANN2 | 8 | 4  | 3.282 | 1.282 | 0.500 | 0.695 | 0.742 | 0.281  | 0.645 |
|         | ANUCS 305 | 8 | 3  | 2.844 | 1.072 | 0.625 | 0.648 | 0.692 | 0.036  | 0.575 |
|         | ANUCS 304 | 8 | 4  | 3.048 | 1.212 | 0.500 | 0.672 | 0.717 | 0.256  | 0.612 |
|         | ANUCS 201 | 8 | 3  | 1.471 | 0.602 | 0.375 | 0.320 | 0.342 | -0.171 | 0.294 |
|         | ANUCS 302 | 8 | 4  | 3.200 | 1.255 | 1.000 | 0.688 | 0.733 | -0.455 | 0.630 |
|         | ANUCS 501 | 8 | 1  | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | -      | 0.000 |
|         | C11 CANN1 | 8 | 3  | 2.667 | 1.040 | 0.500 | 0.625 | 0.667 | 0.200  | 0.555 |
| H-CBG   | ANUCS 202 | 8 | 2  | 2.000 | 0.693 | 0.000 | 0.500 | 0.533 | 1.000  | 0.375 |
|         | ANUCS 301 | 8 | 1  | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | -      | 0.000 |

|           |   |   |       |       |       |       |       |        |       |
|-----------|---|---|-------|-------|-------|-------|-------|--------|-------|
| B05 CANN1 | 8 | 2 | 2.000 | 0.693 | 1.000 | 0.500 | 0.533 | -1.000 | 0.375 |
| B01 CANN1 | 8 | 3 | 2.415 | 0.984 | 0.875 | 0.586 | 0.625 | -0.493 | 0.520 |
| ANUCS 303 | 8 | 2 | 1.280 | 0.377 | 0.250 | 0.219 | 0.233 | -0.143 | 0.195 |
| H09 CANN2 | 8 | 3 | 2.612 | 1.024 | 0.375 | 0.617 | 0.658 | 0.392  | 0.544 |
| ANUCS 305 | 8 | 3 | 2.462 | 0.974 | 0.750 | 0.594 | 0.633 | -0.263 | 0.511 |
| ANUCS 304 | 8 | 3 | 2.909 | 1.082 | 0.250 | 0.656 | 0.700 | 0.619  | 0.582 |
| ANUCS 201 | 8 | 5 | 1.730 | 0.909 | 0.375 | 0.422 | 0.450 | 0.111  | 0.404 |
| ANUCS 302 | 8 | 6 | 4.571 | 1.630 | 1.000 | 0.781 | 0.833 | -0.280 | 0.748 |
| ANUCS 501 | 8 | 1 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | -      | 0.000 |
| C11 CANN1 | 8 | 3 | 2.032 | 0.831 | 0.125 | 0.508 | 0.542 | 0.754  | 0.428 |

n = Number of samples, Na = Number of Different Alleles, Ne = Number of Effective Alleles, I = Shannon's Information Index, Ho = Observed Heterozygosity, He = Expected Heterozygosity, uHe = Unbiased Expected Heterozygosity, F = Fixation Index, PIC = polymorphism information content.

**Table S2.** Several genetic characteristics of PCR amplicons for used STR loci and mother plant material of the total *Cannabis sativa* L. samples.

| Locus     | n  | Ht    | Mean<br>He | Mean<br>Ho | Fis    | Fit    | Fst   | PIC   |
|-----------|----|-------|------------|------------|--------|--------|-------|-------|
| ANUCS 202 | 16 | 0.619 | 0.582      | 0.250      | 0.570  | 0.596  | 0.060 | 0.551 |
| ANUCS 301 | 16 | 0.305 | 0.234      | 0.125      | 0.467  | 0.590  | 0.231 | 0.258 |
| B05 CANN1 | 16 | 0.670 | 0.621      | 1.000      | -0.610 | -0.493 | 0.073 | 0.608 |
| B01 CANN1 | 16 | 0.568 | 0.543      | 0.688      | -0.266 | -0.210 | 0.045 | 0.482 |
| ANUCS 303 | 16 | 0.117 | 0.109      | 0.125      | -0.143 | -0.067 | 0.067 | 0.110 |
| H09 CANN2 | 16 | 0.736 | 0.656      | 0.438      | 0.333  | 0.406  | 0.109 | 0.693 |
| ANUCS 305 | 16 | 0.646 | 0.621      | 0.688      | -0.107 | -0.063 | 0.039 | 0.571 |
| ANUCS 304 | 16 | 0.719 | 0.664      | 0.375      | 0.435  | 0.478  | 0.076 | 0.669 |
| ANUCS 201 | 16 | 0.377 | 0.371      | 0.375      | -0.011 | 0.005  | 0.016 | 0.361 |
| ANUCS 302 | 16 | 0.754 | 0.734      | 1.000      | -0.362 | -0.326 | 0.026 | 0.717 |
| ANUCS 501 | 16 | 0.000 | 0.000      | 0.000      | -      | -      | -     | 0.000 |
| C11 CANN1 | 16 | 0.619 | 0.566      | 0.313      | 0.448  | 0.495  | 0.085 | 0.539 |

n = Number of samples, Ht = Total Expected Heterozygosity, Mean He = Mean Expected Heterozygosity, Mean Ho = Mean Observed Heterozygosity, Fis = inbreeding coefficient within individuals, Fit = inbreeding coefficient within individuals relative to the total, Fst = inbreeding coefficient, PIC = polymorphism information content.

**Table S3.** Mean genetic characteristics of PCR amplicons for used STR loci and mother plant material of the two *Cannabis sativa* L. varieties, i.e. the H-CBD and the H-CBG variety, as well as for total donor plants.

| Variety | Statistic | n | Na   | Ne   | I    | Ho   | He   | uHe  | F    | Percentage of    |       |       |       |
|---------|-----------|---|------|------|------|------|------|------|------|------------------|-------|-------|-------|
|         |           |   |      |      |      |      |      |      |      | Polymorphic Loci | Nei D | Nei I | Fst   |
| H-CBD   | Mean      | 8 | 2.92 | 2.44 | 0.87 | 0.48 | 0.50 | 0.54 | 0.05 | 83.33%           | 0.145 | 0.865 | 0.075 |

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|       |      |    |       |       |       |       |       |       |       |       |        |
|-------|------|----|-------|-------|-------|-------|-------|-------|-------|-------|--------|
|       |      | SE |       | 0.34  | 0.27  | 0.14  | 0.09  | 0.08  | 0.08  | 0.09  |        |
| H-CBG | Mean | 8  | 2.83  | 2.17  | 0.77  | 0.42  | 0.45  | 0.48  | 0.07  |       |        |
|       |      | SE |       | 0.42  | 0.28  | 0.13  | 0.11  | 0.07  | 0.08  | 0.18  | 83.33% |
| Total | Mean | 8  | 2.875 | 2.303 | 0.818 | 0.448 | 0.475 | 0.507 | 0.061 |       |        |
|       |      | SE |       | 0.265 | 0.194 | 0.095 | 0.071 | 0.051 | 0.055 | 0.097 | 83.33% |

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n = Number of samples, Na = Number of Different Alleles, Ne = Number of Effective Alleles, I = Shannon's Information Index, Ho = Observed Heterozygosity, He = Expected Heterozygosity, uHe = Unbiased Expected Heterozygosity, F = Fixation Index, PIC: polymorphism information content, Nei D = Nei's Genetic Distance, Nei I = Nei's Genetic Identity, Fst = inbreeding coefficient.