**TABLE S1** | Characteristics of SSR primers used in the analysis. Annealing temperature ($T_a$) and number of alleles ($N_a$) are indicated.

| SSR locus | GenBank accession no. | Primer sequence (5'-3') | Repeat motif | $T_a$ (°C) | Allelic size range (bp) | $N_a$ | Primer origin (specie) | References | Results for *hylocereus* |
|---|---|---|---|---|---|---|---|---|---|
| AaA3 | DQ314551 | F: GCAAGCAAGAGTATGGTGAATTGG<br>R: AGTTATTTTCACGGTAACACACATGG | $(AAG)_{13}$ | 64 °C | 138-168 | 10 | *Astrophytum asterias* | Terry et al., 2006 | No amplification |
| AaD9 | DQ314553 | F: CTGTTTAGTTCTCTCGTCTTCACC<br>R: CTCCGCTTTTACTGCTAGCACC | $(AG)_{10}$ | 64 °C | 135-143 | 6 | | | No amplification |
| AaG3 | DQ314554 | F: CTAACAGAGAATCCAAGGCTTTTCC<br>R: AATCGCCAGCCGAGGGAGAC | $(CA)_7$ | 64 °C | 127-133 | 4 | | | No amplification |
| mEgR17 | AM746465 | F: ATCGTTGAAAGAGGCGAAA<br>R: TCCCTCTTCTTCGTCAGAGC | $(AG)_{11}$ | 58 °C | 330-347 | 4 | *Echinocactus grusonii* | Hardesty et al., 2008 | Non-specific amplification* |
| mEgR39 | AM746463 | F: GAGCGCAGAATTGAGGTG<br>R: GATGTGGCATTTTCAGAC | $(GA)_9CA(GA)_2$ | 58 °C | 161-171 | 3 | | | No polymorphism♦ |
| mEgR76 | AM746467 | F: TCACAATTTGGAAGGAAGCA<br>R: GTGAGCAAAGGGCTGATTTC | $(AG)_{10}(AAG)_2C(CA)_2$ | 58 °C | 376-396 | 7 | | | No polymorphism |
| mEgR78 | AM746469 | F: AGCCCAAAGCCCAACTTATT<br>R: TGCATGCAATCATAAGGTTTTC | $(AG)_{13}GAG(CA)_3$ | 58 °C | 148-242 | 2 | | | No polymorphism |
| mEgR98 | AM746470 | F: ACCCTAGTGGGGTCGAGAAT<br>R: GTCGCCCAGAACCCTAGTCT | $(AG)_{12}AA(AG)_4$ | 58 °C | 172-187 | 5 | | | No polymorphism |
| Ops.9 | EX720594 | F: AACTGCCTCACACGAGTTCC<br>R: GCTACGAAATCTGCCGAGTC | $(TGA)_9$ | 53 °C | N/A | 17 | *Opuntia ficus indica* | Caruso et al., 2010 | Non-specific amplification |
| Ops.24 | EX720605 | F: TCCTTCCATTTCCACCACAC<br>R: CAAGACCCCTCATTCCAAAG | $(CT)_{24}$ | 53 °C | N/A | 18 | | | Non-specific amplification |
| Pchi21 | AY147837 | F: CGTTTAGCCCCTCTTTCTCC<br>R: GTTCCCAACTGACCGACAAC | $(CT)_5(AT)_3(GT)_8GA(GT)_5$ | 60 °C | 124 | 6 | *Polaskia chichipe* | Otero-Arnaiz et al., 2004 | Non-specific amplification |
| Pchi25 | AY147836 | F: GCCCTTCTAAGGCCATTTCT<br>R: ATTCCGTGTCAAGATTGTGC | $T_5(GT)_{16}A_5$ | 60 °C | 273 | 5 | | | Non-specific amplification |
| Pchi44 | AY147834 | F: ATTCAAACAGGCCCACACAG<br>R: GGGTGTTAGAAGGAATAATAGCTTG | $(CA)_{17}$ | 59 °C | 137 | 4 | | | Showing polymorphism in *H. megalanthus* |
| Pchi47 | AY147832 | F: GTCCTTGTGGCTAGCCCTTT<br>R: CCATTTCTCTCGCCATCTG | $(TG)_{15}$ | 60 °C | 120 | 2 | | | Showing polymorphism in *H. monacanthus* |
| Pchi54 | AY147831 | F: CCTTGAGCTTTGACATTGAGA<br>R: GGAAGGTTTTCATTGGATGAG | $(CA)_5CG(CA)_5TG(CA)_{22}(TA)_3$ | 60 °C | 170 | 8 | | | No polymorphism |
| Ppri02 | KC349893 | F: TTCCATCGTCCCCTCACTTA<br>R: CATTCACCACCGTGAACACT | $(AG)_{11}$ | 66 °C | 115-119 | 2 | *Pachycereus pringlei* | Flores et al., 2014 | No amplification |
| Ppri03 | KC349894 | F: GGTGTTCCTCGCTCTCATTC<br>R: CTCGCAAATCCAAGCAAAAT | $(CT)_{11}$ | 65 °C | 133-155 | 10 | | | No amplification |
| Ppri05 | KC349896 | F: AAACTGCAGGTGTTTCAGGG<br>R: AATGAAGCGAAAGGAAGCAA | $(GTTT)_8$ | 61 °C | 166-186 | 5 | | | No amplification |
| Ppri06 | KC349897 | F: GCTCACGTTGGCAGATTTGT<br>R: GGTGATGACAAAAGGTTTTGC | $(AAAT)_6$ | 50 °C | 138-146 | 3 | | | No amplification |
| Ppri07 | KC349898 | F: TGGACTTCCAAGGGATAATGA<br>R: TCAACTCAAAGTGTCAGTGCTG | $(AAAT)_8$ | 59 °C | 127-143 | 4 | | | No amplification |
| Ppri08 | KC349899 | F: AATAGCGCATGCCTCAAAGG<br>R: CAATAGTCCAGAAATAGGTCAGGTCA | $(CT)_8(CTTT)_2$ | 66 °C | 109-126 | 4 | | | No amplification |
| Ppri09 | KC349900 | F: AAGAGACAGGCCCTGAGACA<br>R: TCGTAGGTTCCATCACCACA | $(TC)_{10}$ | 68 °C | 119-140 | 5 | | | No amplification |
| Pmac102 | HQ667131 | F: TCTATAAGTGCCGATGGATGC<br>R: CACACCTCACTCCCAACCTC | $(AG)_9$ | 54 °C | 188-120 | 2 | *Pilosocereus machrisii* | Perez et al., 2011 | No amplification |

* Amplifications that generated several unstable bands containing partial sequences of forward and/or reverse primers, and primer pairs that did not flank the desired repeat motif.

♦ Amplifications that generated the same band patterns.

**TABLE S2** | DNA sequences of *H. monacanthus* alleles from the microsatellite locus Pchi47. Nucleotides that are underlined are sequences from forward and reverse primers. Nucleotides that are marked with a grey background are tandem repeat units.

| Plant species | Band no./ GenBank no. | Allele size (bp) | DNA sequence (5'-3') | Repeat motif |
|---|---|---|---|---|
| *H. monacanthus* | Band 1 | 118 | GTCCTTGTGGCTAGCCCTTTCGGATCATGTGTCAATCTAT GTTAAGGACTTCATTTGTTTGTGTGTCTATGTGCGTGTTT GTGTGGCATATCAATTTCTCAGATGGCGAGAGAAATGG | $(TG)_3$ |
| | Band 2 | 120 | GTCCTTGTGGCTAGCCCTTTCGGATCATGTGTCAATCTAT GTTAAGGACTTCATTTGTTTGTGTGTGTCTATGTGCGTGT TTGTGTGGCATATCAATTTCTCAGATGGCGAGAGAAATGG | $(TG)_4$ |
| *P. chichipe* | AY147832 | 120 | GTCCTTGTGGCTAGCCCTTTCGGATCGTGTGTCAATCTAT GTTAAAGACTTCATTTGTGTGTGTGTGTGTGTGTGTGTGT GTGTGGGGCATATCAATTTCTCAGATGGCGAGAGAAATGG | $(TG)_{15}$ |

**TABLE S3** | DNA sequences of *H. megalanthus* alleles from the microsatellite locus Pchi44. Nucleotides that are underlined are sequences from forward and reverse primers. Nucleotides that are marked with a grey background are tandem repeat units.

| Plant species | Band no./ GenBank no. | Allelic size (bp) | DNA sequence (5'-3') | Repeat motif |
|---|---|---|---|---|
| *H. megalanthus* | Band 1 | 121 | ATTCAAACAGGCCCACACAGCTACATCAGCCTTTAATTTATGCCTCCCTT CACACACACACACACAACACCAAAGGAATGAAAACAAAGAAATAAACAAG CTATTATTCCTTCTAACACCC | $(CA)_8$ |
| | Band 2 | 123 | ATTCAAACAGGCCCACACAGCTACATCAGCCTTTAATTTATGCCTCCCTT CACACACACACACACACAACACCAAAGGAATGAAAACAAAGAAATAAACA AGCTATTATTCCTTCTAACACCC | $(CA)_9$ |
| | Band 3 | 137 | ATTCAAACAGGCCCACACAGCTACATCAGCCTTTAATTTATGCCTCCCTT CACACACACACACACACACACACACACACACAACACCAAAGGAATGAAAA CAAAGAAATAAACAAGCTATTATTCCTTCTAACACCC | $(CA)_{16}$ |
| | Band 4 | 220 | ATTCAAACAGGCCCACACAGACCCAGAAAATTGCAGTGCGCTCTCCTCAC CGTCTAGCCAGCTACGTTGCTGTGCTTATGTGCTCACGCATGCCTTAAAG CGCATATATTGAAACTCCTACATCAGCCTTTAATTTATGCCTCCCTTCAC ACACACACACACACAACACCAAAGGAATGAAAACAAAGAAATAAACAAGC TATTATTCCTTCTAACACCC | $(CA)_9$ |
| | Band 5 | 234 | ATTCAAACAGGCCCACACAGACCCAGAAAATTGCAGTGCGCTCTCCTCAC CGTCTAGCCAGCTACGTTGCTGTGCTTATGTGCTCACGCATGCCTTAAAG CGCATATATTGAAACTCCTACATCAGCCTTTAATTTATGCCTCCCTTCAC ACACACACACACACACACACACACACACACAACACCAAAGGAATGAAAACAA AGAAATAAACAAGCTATTATTCCTTCTAACACCC | $(CA)_{16}$ |
| *P. chichipe* | AY147834 | 137 | ATTCAAACAGGCCCACACAGCTACATCAGCCTTTAATTTGTGCCTCCCTT CACACACACACACACACACACACACACACACAACACCAAAGGAATGAAAA CAAAGAAGTAAACAAGCTATTATTCCTTCTAACACCC | $(CA)_{16}$ |


**FIGURE S1** | Multiple sequence comparison of the SSR locus (Pchi47) in *H. monacanthus* (HM) and *P. chichipe* (PC). Sequences were aligned using the multiple alignment procedure of Clustal W provided by the program (http://www.ebi.ac.uk/Tools/msa/muscle/). Asterisks indicate sequence identity. Nucleotides that are marked with a grey background are tandem repeat units.

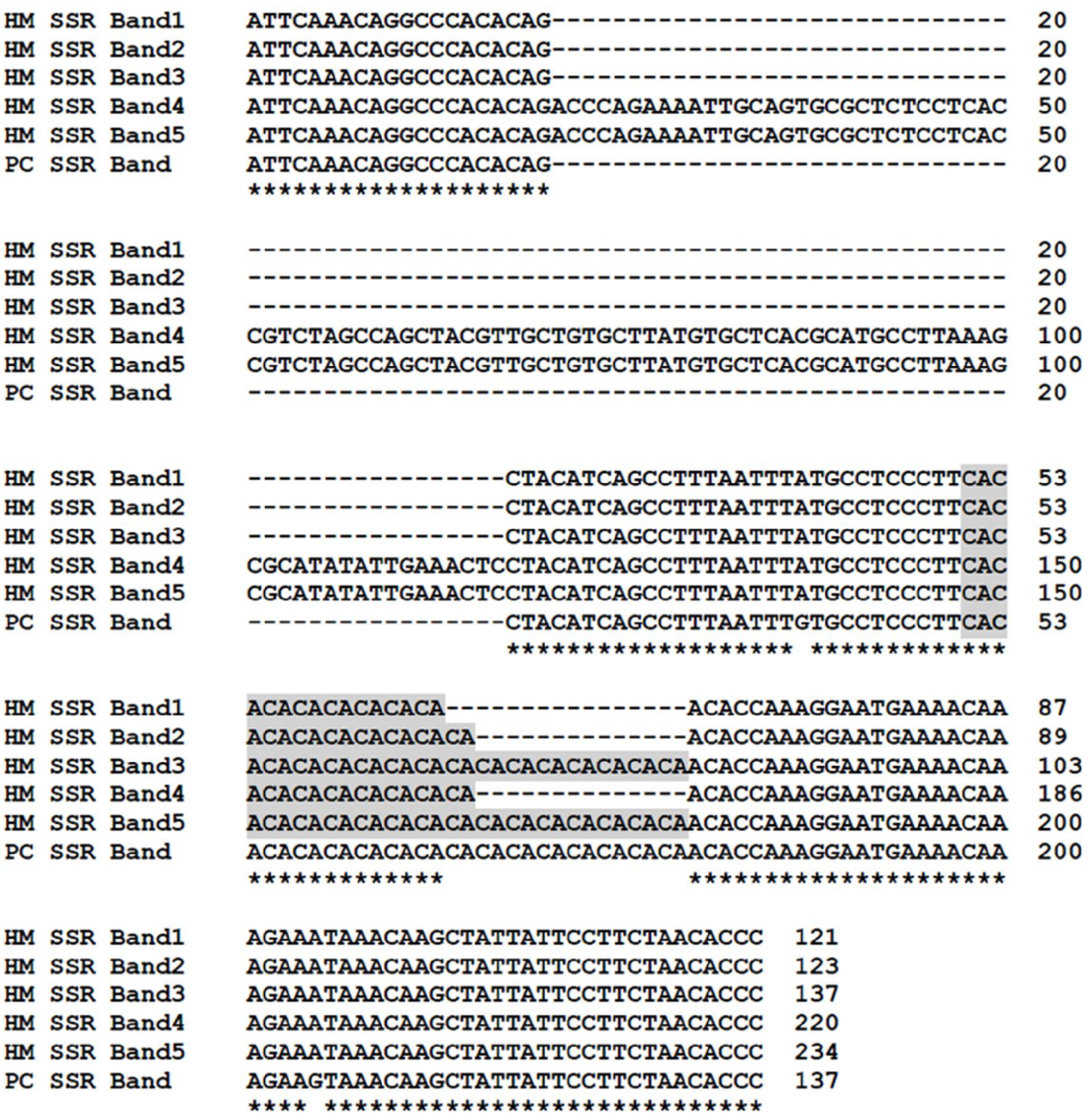
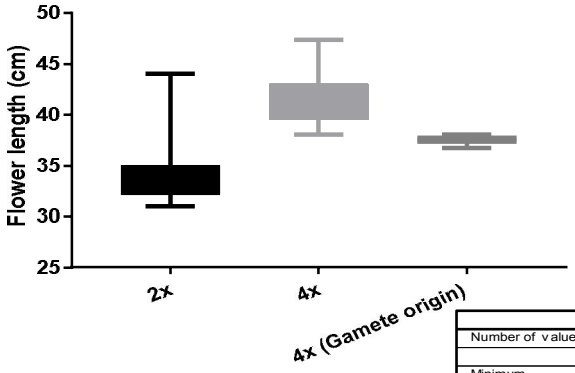



**FIGURE S2** | Multiple sequence comparison of the SSR locus (Pchi44) in *H. megalanthus* (HM) and *P. chichipe* (PC). Sequences were aligned using the multiple alignment procedure of Clustal W provided by the program (http://www.ebi.ac.uk/Tools/msa/muscle/). Asterisks indicate sequence identity. Nucleotides that are marked with a grey background are tandem repeat units.

**FIGURE S3** | Association between DNA ploidy and flower length. All the di-haploid lines were regarded as one group, the tetraploid of gamete origin, as a second group, and all the tetraploid lines of somatic origin, as a third group.

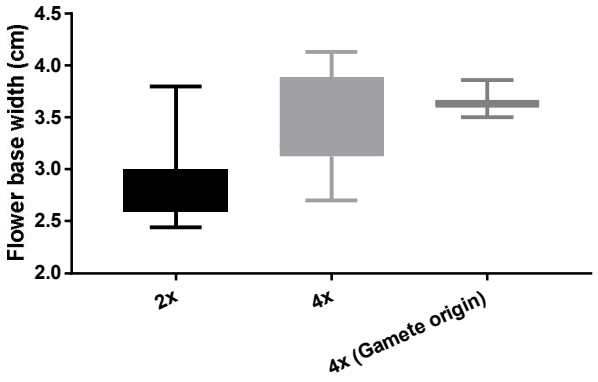



| | 2x | 4x | 4x (Gamete origin) |
|---|---|---|---|
| Number of values | 16 | 13 | 3 |
| | | | |
| Minimum | 31 | 38 | 36.7 |
| 25% Percentile | 32.13 | 39.5 | 36.7 |
| Median | 33.25 | 41 | 37.5 |
| 75% Percentile | 35 | 43 | 38 |
| Maximum | 44 | 47.3 | 38 |
| | | | |
| Mean | 34.16 | 41.58 | 37.4 |
| Std. Deviation | 3.048 | 2.963 | 0.6557 |
| Std. Error of Mean | 0.762 | 0.8217 | 0.3786 |
| | | | |
| Lower 95% CI | 32.53 | 39.78 | 35.77 |
| Upper 95% CI | 35.78 | 43.37 | 39.03 |

| | | | | | |
|---|---|---|---|---|---|
| Table Analyzed | flower length One-way ANOVA data | | | | |
| Data sets analyzed | A : 2x | B : 4x | C : 4x (Gamete origin) | | |
| | | | | | |
| ANOVA summary | | | | | |
| F | 23.31 | | | | |
| P value | <0.0001 | | | | |
| P value summary | **** | | | | |
| Significant diff. among means (P < 0.05)? | Yes | | | | |
| R square | 0.6165 | | | | |
| | | | | | |
| Brown-Forsythe test | | | | | |
| F (DFn, DFd) | 0.8103 (2, 29) | | | | |
| P value | 0.4546 | | | | |
| P value summary | ns | | | | |
| Are SDs significantly different (P < 0.05)? | No | | | | |
| | | | | | |
| Bartlett's test | | | | | |
| Bartlett's statistic (corrected) | | | | | |
| P value | | | | | |
| P value summary | | | | | |
| Are SDs significantly different (P < 0.05)? | | | | | |
| | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Treatment (between columns) | 394.8 | 2 | 197.4 | F (2, 29) = 23.31 | P<0.0001 |
| Residual (within columns) | 245.6 | 29 | 8.468 | | |
| Total | 640.4 | 31 | | | |
| | | | | | |
| Data summary | | | | | |
| Number of treatments (columns) | 3 | | | | |
| Number of values (total) | 32 | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Number of families | 1 | | | | | | | |
| Number of comparisons per family | 3 | | | | | | | |
| Alpha | 0.05 | | | | | | | |
| | | | | | | | | |
| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Summary | Adjusted P Value | | | |
| | | | | | | | | |
| 2x vs. 4x | -7.419 | -10.1 to -4.736 | Yes | **** | <0.0001 | A-B | | |
| 2x vs. 4x (Gamete origin) | -3.244 | -7.765 to 1.278 | No | ns | 0.1968 | A-C | | |
| 4x vs. 4x (Gamete origin) | 4.175 | -0.4276 to 8.778 | No | ns | 0.0811 | B-C | | |
| | | | | | | | | |
| | | | | | | | | |
| Test details | Mean 1 | Mean 2 | Mean Diff. | SE of diff. | n1 | n2 | q | DF |
| | | | | | | | | |
| 2x vs. 4x | 34.16 | 41.58 | -7.419 | 1.087 | 16 | 13 | 9.657 | 29 |
| 2x vs. 4x (Gamete origin) | 34.16 | 37.4 | -3.244 | 1.831 | 16 | 3 | 2.506 | 29 |
| 4x vs. 4x (Gamete origin) | 41.58 | 37.4 | 4.175 | 1.864 | 13 | 3 | 3.168 | 29 |

**FIGURE S4** | Association between DNA ploidy and flower width. All the di-haploid lines were regarded as one group, the tetraploid of gamete origin, as a second group, and all the tetraploid lines of somatic origin, as a third group.

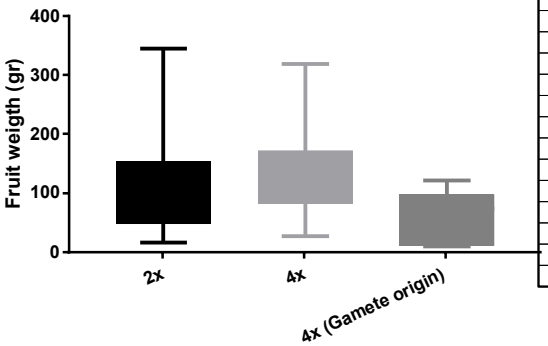



| | 2x | 4x | 4x (Gamete origin) |
|---|---|---|---|
| Number of values | 16 | 13 | 3 |
| | | | |
| Minimum | 2.44 | 2.7 | 3.5 |
| 25% Percentile | 2.595 | 3.125 | 3.5 |
| Median | 2.82 | 3.23 | 3.63 |
| 75% Percentile | 3 | 3.885 | 3.86 |
| Maximum | 3.8 | 4.13 | 3.86 |
| | | | |
| Mean | 2.863 | 3.398 | 3.663 |
| Std. Deviation | 0.3265 | 0.4583 | 0.1823 |
| Std. Error of Mean | 0.08162 | 0.1271 | 0.1053 |
| | | | |
| Lower 95% CI | 2.689 | 3.121 | 3.21 |
| Upper 95% CI | 3.036 | 3.675 | 4.116 |

| | | | | | |
|---|---|---|---|---|---|
| Table Analyzed | flower width | | | | |
| Data sets analyzed | A : 2x | B : 4x | C : 4x (Gamete origin) | | |
| | | | | | |
| ANOVA summary | | | | | |
| F | 10.1 | | | | |
| P value | 0.0005 | | | | |
| P value summary | *** | | | | |
| Significant diff. among means (P < 0.05)? | Yes | | | | |
| R square | 0.4105 | | | | |
| | | | | | |
| Brown-Forsythe test | | | | | |
| F (DFn, DFd) | 1.378 (2, 29) | | | | |
| P value | 0.2681 | | | | |
| P value summary | ns | | | | |
| Are SDs significantly different (P < 0.05)? | No | | | | |
| | | | | | |
| Bartlett's test | | | | | |
| Bartlett's statistic (corrected) | | | | | |
| P value | | | | | |
| P value summary | | | | | |
| Are SDs significantly different (P < 0.05)? | | | | | |
| | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Treatment (between columns) | 2.915 | 2 | 1.457 | F (2, 29) = 10.1 | P=0.0005 |
| Residual (within columns) | 4.186 | 29 | 0.1443 | | |
| Total | 7.101 | 31 | | | |
| | | | | | |
| Data summary | | | | | |
| Number of treatments (columns) | 3 | | | | |
| Number of values (total) | 32 | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Number of families | 1 | | | | | | | |
| Number of comparisons per family | 3 | | | | | | | |
| Alpha | 0.05 | | | | | | | |
| | | | | | | | | |
| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Summary | Adjusted P Value | | | |
| | | | | | | | | |
| 2x vs. 4x | -0.536 | -0.8863 to -0.1856 | Yes | ** | 0.0020 | A-B | | |
| 2x vs. 4x (Gamete origin) | -0.8008 | -1.391 to -0.2105 | Yes | ** | 0.0062 | A-C | | |
| 4x vs. 4x (Gamete origin) | -0.2649 | -0.8659 to 0.3361 | No | ns | 0.5287 | B-C | | |
| | | | | | | | | |
| | | | | | | | | |
| Test details | Mean 1 | Mean 2 | Mean Diff. | SE of diff. | n1 | n2 | q | DF |
| | | | | | | | | |
| 2x vs. 4x | 2.863 | 3.398 | -0.536 | 0.1419 | 16 | 13 | 5.343 | 29 |
| 2x vs. 4x (Gamete origin) | 2.863 | 3.663 | -0.8008 | 0.239 | 16 | 3 | 4.738 | 29 |
| 4x vs. 4x (Gamete origin) | 3.398 | 3.663 | -0.2649 | 0.2434 | 13 | 3 | 1.539 | 29 |

**FIGURE S5** | Association between DNA ploidy and fruit weight. All the di-haploid lines were regarded as one group, the tetraploid of gamete origin, as a second group, and all the tetraploid lines of somatic origin, as a third group.




| | 2x | 4x | 4x (Gamete origin) |
|---|---|---|---|
| Number of values | 90 | 88 | 8 |
| | | | |
| Minimum | 15.92 | 27.2 | 10.15 |
| 25% Percentile | 47.51 | 82.13 | 10.51 |
| Median | 94.45 | 124.6 | 74.03 |
| 75% Percentile | 152.9 | 171.8 | 97.13 |
| Maximum | 344.9 | 319.3 | 121.1 |
| | | | |
| Mean | 107.1 | 136.2 | 61.1 |
| Std. Deviation | 70.54 | 65.76 | 44.5 |
| Std. Error of Mean | 7.436 | 7.01 | 15.73 |
| | | | |
| Lower 95% CI | 92.32 | 122.2 | 23.9 |
| Upper 95% CI | 121.9 | 150.1 | 98.31 |

| | | | | | |
|---|---|---|---|---|---|
| Table Analyzed | Fruit weigth | | | | |
| Data sets analyzed | A : 2x | B : 4x | C : 4x (Gamete origin) | | |
| | | | | | |
| ANOVA summary | | | | | |
| F | 7.2 | | | | |
| P value | 0.0010 | | | | |
| P value summary | *** | | | | |
| Significant diff. among means (P < 0.05)? | Yes | | | | |
| R square | 0.07295 | | | | |
| | | | | | |
| Brown-Forsythe test | | | | | |
| F (DFn, DFd) | 0.9683 (2, 183) | | | | |
| P value | 0.3817 | | | | |
| P value summary | ns | | | | |
| Are SDs significantly different (P < 0.05)? | No | | | | |
| | | | | | |
| Bartlett's test | | | | | |
| Bartlett's statistic (corrected) | 2.287 | | | | |
| P value | 0.3187 | | | | |
| P value summary | ns | | | | |
| Are SDs significantly different (P < 0.05)? | No | | | | |
| | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Treatment (between columns) | 65540 | 2 | 32770 | F (2, 183) = 7.2 | P=0.0010 |
| Residual (within columns) | 832917 | 183 | 4551 | | |
| Total | 898457 | 185 | | | |
| | | | | | |
| Data summary | | | | | |
| Number of treatments (columns) | 3 | | | | |
| Number of values (total) | 186 | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Number of families | 1 | | | | | | | |
| Number of comparisons per family | 3 | | | | | | | |
| Alpha | 0.05 | | | | | | | |
| | | | | | | | | |
| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Summary | Adjusted P Value | | | |
| | | | | | | | | |
| 2x vs. 4x | -29.08 | -52.98 to -5.185 | Yes | * | 0.0125 | A-B | | |
| 2x vs. 4x (Gamete origin) | 45.99 | -12.82 to 104.8 | No | ns | 0.1571 | A-C | | |
| 4x vs. 4x (Gamete origin) | 75.08 | 16.21 to 133.9 | Yes | ** | 0.0083 | B-C | | |
| | | | | | | | | |
| | | | | | | | | |
| Test details | Mean 1 | Mean 2 | Mean Diff. | SE of diff. | n1 | n2 | q | DF |
| | | | | | | | | |
| 2x vs. 4x | 107.1 | 136.2 | -29.08 | 10.11 | 90 | 88 | 4.067 | 183 |
| 2x vs. 4x (Gamete origin) | 107.1 | 61.1 | 45.99 | 24.89 | 90 | 8 | 2.613 | 183 |
| 4x vs. 4x (Gamete origin) | 136.2 | 61.1 | 75.08 | 24.91 | 88 | 8 | 4.262 | 183 |