Supplementary Material

**Optimizing *Amaranthus palmeri* S.Watson (Palmer amaranth) genetic testing of seeds using real-time (quantitative) PCR**

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**Table S1.** *Amaranthus* species used in this study with details including common name, geographic origin, GRIN accession, lot, and plant name (if applicable).

**Table S2.** Resulting Cq values for the primer concentration optimization testing for primer pairs M2 and M3. Forward and reverse primer concentrations were tested at 300, 500, and 800 nM (final reaction concentration). *A. palmeri* DNA was used at 2 ng and 10 ng total concentration. The average quantification cycle (Cq) value from duplicate wells is presented for each set of primer concentrations.

**Table S1.** *Amaranthus* species used in this study

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Species** | **Common name** | **Origin** | **Accession** | **Lot** | **Plant Name** |
| *A. albus* L. | prostrate pigweed | USA, Missouri | PI 608029 | 02ncai01 SD | Ames 25459 |
| *A. arenicola* I.M. Johnst. | sandhill amaranth | USA, Kansas | PI 607459 | 03ncai01 SD | Pop 57 |
| *A. australis* (A. Gray) Sauer | southern amaranth | USA, Florida | PI 553076 | 05ncab01 SD | Giant Amaranth |
| *A. blitoides* S. Watson | mat amaranth | USA, Iowa | PI 553079 | 04ncai01 SD | DB8901 |
| *A. californicus* (Moq.) S. Watson | California amaranth | USA, California | PI 595319 | 02ncai01 SD | DB 955 |
| *A. deflexus* L. | largefruit amaranth | USA, North Carolina | PI 632246 | 04ncai01 SD | DB 200127 |
| *A. dubius* Mart. Ex Thell. | spleen amaranth | USA, Iowa | PI 612850 | 00ncao51 SD | Separation from RRC 109B |
| *A.* hybridIowa1 | amaranth hybrid | USA, Iowa | PI 568179 | 01ncai01 SD | Ames 12991 |
| *A.* hybridPennsylvania | amaranth hybrid | USA, Pennsylvania | Ames 5658 | 11ncai01 SD | RRC 1159 |
| *A.* hybridIowa2 | amaranth hybrid | USA, Iowa | Ames 24805 | 98 ncai01 SD | Hybrid 1 |
| *A.* hybridIllinois | amaranth hybrid | USA, Illinois | PI 603879 | 98ncao51 SD | Pop 32 |
| *A. hybridus* L. | slim amaranth | Guatemala, Escuintla | PI 677074 | 94ncai01 SD | 16360 |
| *A. hypochondriacus* L. | Prince-of-Wales feather | India | Ames 15301 | 05ncai01 SD | RRC 874 |
| *A. palmeri* S. Watson | carelessweed | USA, Kansas | PI 607451 | 99ncao51 SD | Pop 49 |
| *A. palmeri* S. Watson*\** | carelessweed | USA, Minnesota | - |   | DC17 |
| *A. powellii* S. Watsonssp. *bouchonii* (Thell.) Costeau & Carretero | Powell's amaranth | USA, Washington | PI 666332 | 03ncai01 SD | RRC 653 |
| *A. retroflexus* L. | redroot amaranth | USA, Iowa | PI 603845 | 04ncai01 SD | Pop 2 |
| *A. spinosus* L. | spiny amaranth | USA, North Carolina | PI 632248 | 04ncai01 SD | DB200130 |
| *A. tuberculatus* (Moq.) Sauer | roughfruit amaranth | USA, Iowa | PI603850 | 98ncao51 SD | Pop 8 |
| *A. watsonii* Standl. | Watson's amaranth | Mexico, Colima | PI 633593 | 14ncai01 SD | RRC 1192 |
| *A. wrightii* S. Watson | Wright's amaranth | USA, Texas | PI 632243 | 03naci01 SD | 215 |

**Table S2.** Results of reaction primer concentration optimization for the M2 regular, M2 fast, and M3 fast qPCR cycles

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Forward Primer (nM)** | **Reverse Primer (nM)** | **M2 Regular** | **M2 Fast** | **M3 Fast** |
| **2 ng *A. palmeri* DNA** | **10 ng *A. palmeri* DNA** | **2 ng *A. palmeri* DNA** | **10 ng *A. palmeri* DNA** | **2 ng *A. palmeri* DNA** | **10 ng *A. palmeri* DNA** |
| 300 | 300 | 28.3 | 25.5 | 31.4 | 29.4 | 26.3 | 23.6 |
| 500 | 300 | 27.6 | 24.8 | 32.3 | 29.1 | 25.1 | 22.7 |
| 800 | 300 | 27.2 | 24.3 | 31.5 | 26.6 | 25.1 | 22.5 |
| 300 | 500 | 27.9 | 25.4 | 31.8 | 29.0 | 25.7 | 23.4 |
| 500 | 500 | 27.2 | 24.9 | 30.5 | 27.2 | 25.6 | 22.9 |
| 800 | 500 | 26.4 | 23.8 | 28.3 | 25.9 | 25.2 | 22.4 |
| 300 | 800 | 27.7 | 24.9 | 29.9 | 26.1 | 26.0 | 23.1 |
| 500 | 800 | 27.4 | 24.6 | 30.0 | 26.5 | 25.2 | 23.0 |
| 800 | 800 | 26.3 | 23.7 | 28.0 | 26.0 | 25.3 | 22.5 |

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**Figure S1.** A standard qPCR cycle using M2 primers was completed using annealing temperatures ranging from 52-62°C. Three species of *Amaranthus* were tested to determine the optimal quantification cycle (Cq) spread between positive and negative samples including *A. palmeri*, *A. dubius*, and *A. spinosus*.

**Figure S2.** A standard qPCR cycle using M3 primers was completed using annealing temperatures ranging from 52-62°C. Three species of *Amaranthus* were tested to determine the optimal quantification cycle (Cq) spread between positive and negative samples including *A. palmeri*, *A. dubius*, and *A. spinosus*.

**Figure S1.** Annealing temperature results for M2 qPCR cycle optimization

**Figure S2.** Annealing temperature results for M3 qPCR cycle optimization