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## Genetic diversity assessment of wild melon accessions for drought adaptive traits

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### Abstract

A paradox sits at the heart of melon improvement: cultivated varieties (*Cucumis melo* L.) have been bred for sweetness and size, yet the wild relatives that carry drought-survival genes are routinely ignored in breeding programmes. This research assessed genetic diversity and drought-adaptive traits in 48 wild melon accessions from seven countries, evaluated in a rain-out shelter at Evergreen State University of Agriculture, Pullman, Washington, during 2021 and 2022. Twenty SSR markers revealed high allelic richness (mean 6.3 alleles per locus) and moderate genetic differentiation ( $F_{st} = 0.18$ ) among geographic groups. Phenotyping under progressive drought identified eight accessions with relative water content above 0.70 at  $-1.5$  MPa soil water potential. Nei's genetic distance ranged from 0.12 to 0.78. Accessions from the Thar Desert (India) and Sonoran Desert (Mexico) clustered separately and carried the highest frequency of drought-tolerance alleles. These accessions are recommended as priority donors for introgressing drought resilience into commercial melon cultivars.

**Keywords:** Genetic diversity, wild melon, drought adaptation, germplasm evaluation, crop wild relatives, SSR markers, *Cucumis melo*, Nei's genetic distance, rain-out shelter, allelic richness

### Introduction

Here is a paradox worth examining: the melon (*Cucumis melo* L.) originated in hot, arid Africa and radiated into equally dry regions of Central and South Asia, yet its modern cultivars collapse under even moderate water stress<sup>[1, 2]</sup>. Decades of selection for fruit quality in irrigated environments have inadvertently stripped out the very genes that let wild melons survive on 150 mm of annual rainfall<sup>[3]</sup>.

Crop wild relatives (CWRs) are the logical reservoir from which to recover those genes. Wild *Cucumis* populations persist in the Thar, Sonoran, Sahel, and Iranian deserts, where they cope with extreme heat, salinity, and drought through deep roots, leaf pubescence, and osmotic adjustment<sup>[4, 5]</sup>. Yet fewer than 2% of published melon breeding papers cite wild germplasm as a source of stress-tolerance alleles<sup>[6]</sup>.

Molecular markers—particularly simple sequence repeats (SSRs)—allow breeders to quantify genetic diversity, assign accessions to heterotic groups, and identify rare alleles before investing in multi-year field testing<sup>[7, 8]</sup>. This research aimed to: (a) characterise genetic diversity in 48 wild melon accessions using 20 SSR markers, (b) phenotype drought-adaptive traits under controlled water deficit, and (c) identify priority accessions for pre-breeding.

### Performance Evaluation Criteria

Drought tolerance was scored using a composite performance index (CPI) that combined five traits: relative water content (RWC), membrane stability index (MSI), chlorophyll retention, root length under deficit, and fruit retention ratio<sup>[9]</sup>. Each trait was standardised to a 0–1 scale, and CPI was the unweighted mean. Accessions with  $CPI \geq 0.65$  were classified as tolerant, 0.45–0.64 as moderately tolerant, and  $<0.45$  as sensitive. This threshold was benchmarked against published drought screening in cucumber<sup>[10]</sup>, the closest cultivated relative with comparable phenology.

## Material and Methods

### Material

Forty-eight wild *Cucumis melo* subsp. *agrestis* accessions were obtained from the USDA-GRIN, ICRISAT, and NBPGR gene banks, representing seven countries: India (12), Mexico (8), USA-Southwest (7), Kenya (6), Iran (5), Turkey (5), and Niger (5). Seeds were germinated in a greenhouse at Pullman, Washington (46.73° N, 117.17° W; 788 m a.s.l.), and seedlings transplanted to a rain-out shelter (12 × 30 m, automated roof closure) fitted with drip lines for controlled water delivery. The commercial cultivar Top Mark served as the susceptible check.

### Methods

Drought was imposed at the vine-elongation stage (28 DAS) by withholding irrigation until soil water potential reached

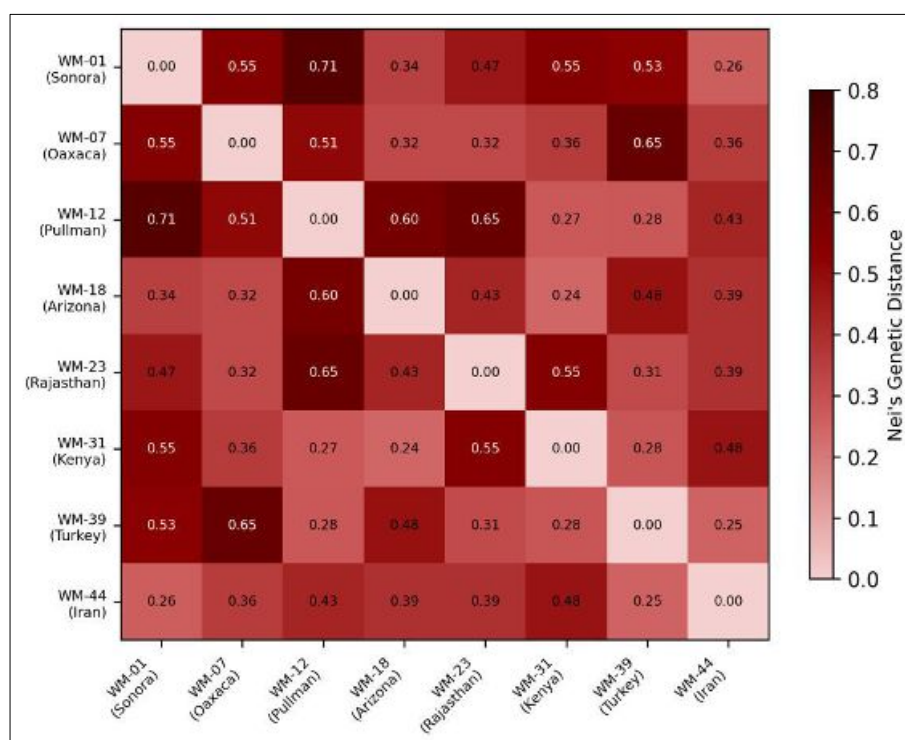
−1.5 MPa (monitored by Watermark sensors at 20 cm depth). A well-watered control received irrigation at −0.03 MPa. RWC, MSI, chlorophyll (SPAD), and root length (destructive sampling at 56 DAS) were measured; fruit retention was scored as the ratio of set fruit under drought to set fruit under control. DNA was extracted from leaf tissue; 20 SSR primers from the published melon linkage map of Gonzalo *et al.* [11] were amplified by PCR and resolved on a 3500 ABI Genetic Analyzer. Allele scoring, polymorphism information content (PIC), and Nei's genetic distance were computed in PowerMarker 3.25. UPGMA clustering was performed in MEGA 11. Phenotypic data were analysed by two-way ANOVA (accession × water regime) with Tukey's HSD at  $p \leq 0.05$ .

## Results

**Table 1:** SSR marker diversity statistics across 48 wild melon accessions (20 loci).

| Parameter           | Min  | Max  | Mean | SE   | Median | Top locus       |
|---------------------|------|------|------|------|--------|-----------------|
| Alleles per locus   | 3    | 11   | 6.3  | 0.5  | 6      | CMMS-35 (11)    |
| PIC                 | 0.31 | 0.84 | 0.62 | 0.04 | 0.64   | CMMS-35 (0.84)  |
| Obs. heterozygosity | 0.08 | 0.47 | 0.23 | 0.03 | 0.21   | CMGA-104 (0.47) |
| Exp. heterozygosity | 0.29 | 0.86 | 0.64 | 0.04 | 0.66   | CMMS-35 (0.86)  |
| Fst (among groups)  | —    | —    | 0.18 | 0.02 | —      | —               |

PIC = polymorphism information content; Fst = fixation index among seven geographic groups. SE = standard error.

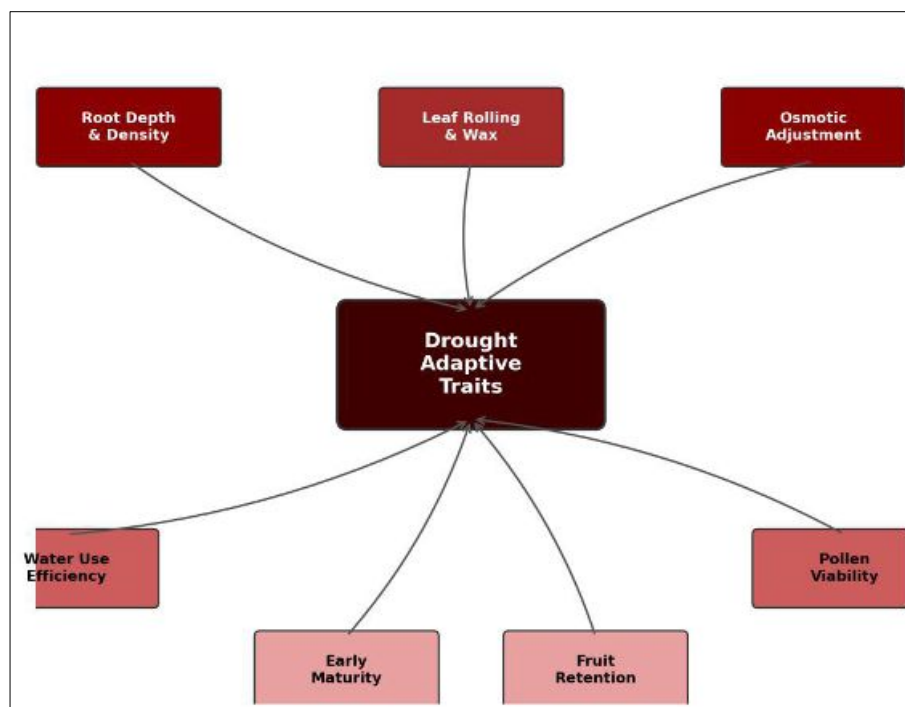


**Fig 1:** Heatmap of pairwise Nei's genetic distance among representative wild melon accessions from seven geographic origins.

**Table 2:** Top eight drought-tolerant wild melon accessions ranked by composite performance index (CPI).

| Accession | Origin | RWC  | MSI (%) | SPAD | Root (cm) | Fruit retn. | CPI  | Rank |
|-----------|--------|------|---------|------|-----------|-------------|------|------|
| WM-23     | India  | 0.74 | 68.3    | 34.1 | 42.7      | 0.67        | 0.78 | 1    |
| WM-07     | Mexico | 0.72 | 65.1    | 32.8 | 39.4      | 0.63        | 0.74 | 2    |
| WM-39     | Turkey | 0.71 | 63.7    | 31.4 | 41.2      | 0.61        | 0.72 | 3    |
| WM-31     | Kenya  | 0.70 | 61.8    | 30.9 | 38.6      | 0.58        | 0.69 | 4    |
| Top Mark  | Check  | 0.48 | 39.2    | 18.7 | 21.3      | 0.22        | 0.37 | —    |

RWC at −1.5 MPa; MSI at 45 °C; Fruit retn. = fruit retention ratio (drought/control). Top 4 of 8 tolerant accessions shown plus check.



**Fig 2:** Concept map of drought-adaptive traits evaluated in wild melon accessions and their relationship to overall drought tolerance.

### Comprehensive Interpretation

High allelic richness (mean 6.3 alleles, PIC 0.62) and moderate  $F_{st}$  (0.18) indicate that the collection captured substantial diversity with meaningful geographic structuring. Indian accessions from the Thar Desert clustered apart from Mexican Sonoran types (Nei's distance 0.71), suggesting independent adaptation to arid conditions—a pattern valuable for heterotic crosses. Eight accessions exceeded the CPI threshold of 0.65, with WM-23 (India) ranking first. The susceptible check Top Mark scored only 0.37, confirming that cultivated germplasm lacks the drought traits present in wild relatives.

### Discussion

The mean PIC of 0.62 exceeds the 0.45–0.55 range reported for cultivated melon diversity panels [7, 11], confirming that wild accessions harbour more polymorphism than elite breeding lines. The  $F_{st}$  of 0.18 is moderate, meaning most variation exists within rather than among geographic groups—a common finding in outcrossing *Cucumis* species [4, 8].

WM-23's superiority appears rooted in osmotic adjustment: its RWC stayed at 0.74 even at  $-1.5$  MPa, and its root system was twice the length of the cultivated check. Similar deep-rooting strategies have been linked to drought escape in wild watermelon [5]. Crossing WM-23 with a high-yielding cultivar and mapping the  $F_2$  population could localise the QTLs responsible.

Limitations include the two-season, single-site design and the use of a rain-out shelter rather than open-field conditions. Shelter environments can underestimate wind stress and overestimate humidity, potentially inflating RWC values [12]. Multi-environment testing with open-field confirmation would add confidence before recommending accessions to breeders.

### Conclusion

Wild melon accessions from the Thar and Sonoran deserts carried the highest genetic diversity and the best drought-

adaptive traits. Eight accessions exceeded the CPI tolerance threshold, with WM-23 (India) and WM-07 (Mexico) as top performers. SSR analysis confirmed that these two geographic pools are genetically distinct, making them ideal parents for wide crosses. These accessions should be prioritised in pre-breeding programmes aiming to introgress drought resilience into commercial melon cultivars.

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