California Seed Association Voluntary Cucurbit Seed Testing Program Standard Operating Procedure (SOP) for 2024 Effective February 1, 2024

I. PURPOSE AND OBJECTIVE

The purpose of the California Seed Association (CSA) Cucurbit Seed Testing Program (Program), is to preserve the usefulness of the Northern California counties for cucurbit seed production and varietal research and development. More specifically, the Program is intended to prevent the introduction and establishment of Cucumber Green Mottle Mosaic Virus (CGMMV) in these counties.

The objective of the 2024 CSA Cucurbit Seed Testing Program is to achieve 100% voluntary participation by the cucurbit seed industry in pre-plant testing of seed lots destined for open field or outdoor net cage isolated seed production or research and development trialing. Individuals and entities agreeing to adhere to this SOP shall be referred to as Participants, each a Participant.

II. SCOPE

A. Notwithstanding exceptions described in IV., seed lots of the species listed in III. shall be tested for the presence of CGMMV if they will be used for open field or outdoor net cage isolated seed production, research and development trials (nurseries, selection trials, breeder seed increases) or product development trials (experimental variety trials) in the counties listed below. Seed and transplants primarily for the production of fruit to be sold as food, feed or as ornamental squash is outside the scope of this SOP.

B. The Program applies to transplants destined for and direct seedings in the California counties of Butte, Colusa, Glenn, Sacramento, San Joaquin, Solano, Sutter, Tehama, Yolo and Yuba.

III. KINDS AND SPECIES

The following crop kinds and species of the Family Cucurbitaceae are covered by the Program:

Bitter gourd, Chinese bitter melon (*Momordica charantia*);

Calabash, bottle gourd, opo squash, long melon (*Lagenaria siceraria*);

Cucumber (Cucumis sativus);

Gherkin (*Cucumis anguria*);

Melon, cantaloupe, oriental melon (*Cucumis melo*);

Watermelon (Citrullus lanatus);

Winter squash (*Cucurbita moschata*, *Cucurbita maxima* and varieties derived from interspecific hybrids of these two species).

Summer squash, pumpkin (Cucurbita pepo)

IV. SEED TESTING REQUIREMENTS AND EXCEPTIONS

- A. Direct Field Planting. Prior to direct field planting, a seed lot shall be sampled and tested per the requirements of this SOP and found negative for CGMMV. Note exceptions to this in D. below.
- B. Greenhouse Transplant Production. Prior to greenhouse sowing for the production of transplants, a seed lot shall be sampled and tested per the requirements of this SOP and found negative for CGMMV. This applies to transplant production inside and outside the ten California counties listed in II. B., when the transplants are expected to be brought to these counties. Participants shall also ensure and document that all seed lots planted and/or grown in the same greenhouse structure were sampled, tested and found free of CGMMV. Note the exception to this in C. below.
- C. R&D Transplant Sow While in Test Allowance. Where a Participant is conducting research and development trials or product development trials at a facility they own or lease, and Participant will raise their own transplants at said facility, a sow while in test program may be utilized prior to taking said plants to the facility's field. All lots must be sampled, tested per the requirements of this SOP and found free of CGMMV prior to transplanting into fields.
- D. Direct Sown Cucurbita pepo Allowance. For *Cucurbita pepo* seed direct sown to an open field or net cage isolated portion of an open field **at a Participant's research and development facility**, a sow while in test program can be utilized so long as testing is completed no later than 60 days after sowing. If CGMMV is detected in a lot that has been sown, as a minimum, removal and destruction of all plants from that lot must be done as soon as reasonably possible after receiving such test results.

In all cases, cucurbit seed to be used for seed production or trialing, in the counties listed in II. B., at any facility not owned or leased by the Participant must be tested prior to sowing.

V. SAMPLING

- A. Samplers. Seed lots sampled and tested prior to the Participant agreeing to this 2024 SOP are exempt from the requirements of parts V. A and B. if they were previously tested and found to be free of CGMMV. Sampling of candidate seed lots shall be carried out by a qualified local, state or federal official, or by a sampler:
 - a. accredited by the International Seed Testing Association (ISTA) or the Accredited Seed Sampling Program (ASSP; of the USDA Agriculture Marketing Service),
 - b. certified by the Association of American Seed Control Officials (AASCO) or
 - c. trained on seed sampling by the Participant through a program based on the ISTA, ASSP or AASCO programs, or the Sampling guidelines of Association of Official Seed Analysts (AOSA) "Rules for Testing Seeds".

Samplers working in research and development roles must be trained to the standards of one of the programs cited above, and this must be documented accordingly. This training can be done by certified company samplers as a part of an overall sampling program.

- B. Procedures¹. Candidate seed lots shall be sampled following the latest guidelines of the Association of American Seed Control Officials' "AASCO Handbook on Seed Sampling" or the International Seed Testing Association's "International Rules for Seed Testing."
- C. Treated Seed². Seed lots that have been treated with a pesticide (e.g., insecticide or fungicide) may be tested prior to treatment or post treatment. Seed lots that have been heat-treated, prior to sampling and testing for CGMMV, to mitigate a CGMMV detection in the seed lot are not eligible for distribution and planting under this agreement until a validated seed testing method is developed and approved for heat-treated seed.
- D. Small Lot Sample Size. For lots containing less than 40,000 seeds, a 5% sample size is sufficient for CGMMV testing. The quantity of the lot should be based on the full quantity of the seed lot, not on the quantity possessed by the Participant.

VI. TESTING

- A. Laboratories⁴. Laboratories authorized through the National Seed Health System (NSHS) or Naktuinbouw Accredited Laboratories (NAL) system, with accreditation for testing the presence of CGMMV, are approved laboratories for the Program.
- B. Method^{3,5}. National Seed Health System, CB 3.1 Cucumber green mottle mosaic virus, Version: Latest. General parameters: 2000 seeds per seed lot; 100 seed subsample size resulting in 20 subsamples, evaluated via an ELISA.
- C. Small Lot Sample³. Samples from small seed lots may be combined and analyzed in a composite sample so long as the size of subsamples does not exceed 100 seeds. Should a subsample test positive for CGMMV, none of the seed lots from which the subsample was generated may be used. Additional individual lot testing or composite sampling may occur to resolve which lot(s) are contaminated and which are free of CGMMV.

VII. RECORDS

- A. Data. Participants are required to maintain the following information regarding CGMMV tested negative seed lots:
 - 1. Crop species;
 - 2. Crop variety;
 - 3. Unique seed lot identification number;
 - 4. Seed origin;
 - 5. Year produced;
 - 6. Testing laboratory location, test number and date;
 - 7. If testing is done at an external laboratory, a testing laboratory contact name, address, phone, and email

Records may be paper-based or paper-less (in an electronic database).

- B. Confidentiality. Records shall remain with and under the control of each participant.
- C. Records of test results should be retained for a minimum of three years post final seed lot use, after the date of test completion or two years after the consumption or disposal of the seed lot, whichever is later which is in alignment with NSHAPP retention.

D. Audit. Upon request by the California Seed Association (CSA) and/or the county agriculture office, at an arranged place, date and time, participants will make testing data available for confidential review by CSA staff and/or the county agriculture staff.

VIII. SOP MAINTENANCE, ANNUAL REVIEW AND AMENDMENT

- A. SOP Maintenance. CSA's Vegetable / Flower Seed / Grower Shipper Liaison & Plant Health Committee will form a subcommittee called "Cucurbit Seed Production Testing Program." The subcommittee meetings will be led and conducted per CSA's By-Laws. Membership on the subcommittee is open to one representative of each program participant.
- B. Annual Review. The subcommittee shall meet at least once a year, preferably prior to CSA's Mid-year Meetings and make a report to the full committee regarding the program and its recommendations for the next year's SOP.
- C. Amendments. If necessary, a participant can make a request to CSA to call a meeting of the subcommittee to consider in-year amendments.

IX. REFERENCES

1. Association of American Seed Control Officials (AASCO) sampling guidance can be found at: http://www.seedcontrol.org/accreditation.html

The International Seed Testing Association sampling guidance can be ordered from ISTA: https://www.seedtest.org/en/productrubric.html

- 2. NSHS Progress Report, Treated Seed Study: (page 4). Published December 10, 2019 via email to the NSHAPP member representatives. Tracy L. Bruns, Ed Podleckis, Gary Munkvold.
- 3. National Seed Health System (https://seedhealth.org/). METHODS / SEED HEALTH TESTING METHODS / CUCURBIT / Cb 3.1 Cucumber Green Mottle Mosaic Virus. https://seedhealth.org/cb3-1/.
- 4. National Seed Health System (https://seedhealth.org/). ACCREDITATION / APHIS ACCREDITED ENTITIES.

https://www.aphis.usda.gov/plant_health/acns/downloads/SeedHealthProgram/Entities/EntitiesAccredited.pdf.

Naktuinbouw Accredited Laboratories list: https://www.naktuinbouw.com/nal-authorized-laboratories

5. <u>ISTA Validated Seed Health Testing Method 7-026:</u> Detection of *squash mosaic virus*, *cucumber green mottle mosaic virus* and *melon necrotic spot virus* in cucurbit seed. https://www.seedhealth.org/wp-content/uploads/2019/04/ISTARules2019SHmethods7-026.pdf

Exhibit A.

Type of	Direct Sowing		Transplant Sowing	
seed sown	Cucurbita pepo	Other spp.	Cucurbita pepo	Other spp.
Parental or Basic Seed Increase, Commercial Seed Increase	Test prior (IV. A.), but 60-day window allowance (IV. E.) at own facility	Test prior (IV. A.)	Test prior (IV. B.)	Test prior (IV. B.)
Research and Development Trialing and Seed Increase, Product Development Trialing	Test prior (IV. A.), but 60-day window allowance (IV. E.) at own facility	Test prior (IV. A.)	Test prior (IV. B.), but sow while in test allowance (IV. C.) at own facility	Test prior (IV. B.), but sow while in test allowance (IV. C.) at own facility