

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
WASHINGTON, D.C.

**NOTICE OF RELEASE OF ROMAINE LETTUCE GERMPLASM WITH
RESISTANCE TO DIEBACK AND IMPROVED SHELF-LIFE**

Executive summary

The Agricultural Research Service, United States Department of Agriculture announces the release of two F8 romaine breeding lines of lettuce (*Lactuca sativa* L.) with resistance to dieback and with good shelf-life. One of the breeding lines was selected from a cross between Darkland and PI491224, while the other one was developed from Green Towers x (Darkland x PI491224). Resistance to the disease in both breeding lines is derived from PI491224, a primitive romaine-type lettuce that is highly perishable when processed for salad. In replicated field trials, the two breeding lines showed complete resistance to dieback. Field observations were confirmed through the analysis of molecular markers closely linked to the dieback resistance gene *Tvr1*. Testing of salad-cut lettuce in modified atmosphere packaging indicated slower decay in the two breeding lines compared to other dieback-resistant romaines.

Introduction

California and Arizona are the two largest lettuce-producing states that together account for over 95 percent of the lettuce grown in the United States. Since the early 1990's, dieback disease of lettuce has been observed throughout the main producing areas of California and Arizona. Symptoms of the disease include mottling, yellowing, and necrosis of older leaves, and the stunting and eventual death of plants (Wisler and Duffus 2000; Obermeier et al. 2001; Grube et al. 2005). The disease affects romaine and leaf-type lettuce, often leading to a crop loss of 60 percent or more (Wisler and Duffus 2000). The disease is caused by two closely related soilborne viruses from the family Tombusviridae -- Tomato bushy stunt virus (TBSV) and Lettuce necrotic stunt virus (LNSV) (Obermeier et al. 2001). Previous studies have provided no evidence that either chemical treatment or rotation with non-host crops can effectively reduce, remove, or destroy the virus in infested soil (Wintermantel and Anchieta 2003); therefore, genetic resistance remains the only option for disease control.

Lettuce resistance to the disease is coded by a single dominant gene (*Tvr1*) that is positioned on molecular linkage group 2 (Grube et al. 2005, Simko et al. 2009). Three romaine-type breeding lines with resistance to the disease were previously released by the USDA-ARS in Salinas, CA (Grube and Ryder 2003). All three breeding lines (01-778M, 01-781M, and 01-789M) were derived from a primitive romaine-type accession PI491224 that has complete resistance to dieback. However, our recent observations show that PI491224 and all three breeding lines derived from this accession have a very short shelf-life after processing into fresh-cut salad. Similarly, feedback from the lettuce industry also indicates that recently developed romaine

cultivars with resistance to dieback quickly decay when processed for salad (Hayes and Liu 2008). Therefore, our breeding program has focused on developing breeding lines with a resistance to dieback as well as having acceptable shelf-life when processed into salad.

SM09A (F8) breeding line was selected from Green Towers x (Darkland x PI491224), while SM09B (F8) originated from the cross between Darkland and PI491224. The dieback resistance in both breeding lines is derived from PI491224, which is a primitive, romaine-type lettuce accession collected in Greece that is not used for commercial production in the United States. Green Towers is a large and tall romaine cultivar with an erected habit and intense dark green color developed by Harris Moran Seed Company (COMPOSITdb 2009). Darkland is a romaine cultivar with a strong bolting tolerance and with smooth midribs (COMPOSITdb 2009). Darkland was developed by Central Valley Seeds, Incorporated. Shelf-life of Green Towers and Darkland is acceptable to the industry; however, both cultivars are highly susceptible to the dieback disease.

Development of breeding lines, assessment of disease resistance and shelf-life

SM09A and SM09B were developed by selecting for resistance to dieback disease and horticultural characteristics in experiments conducted in TBSV and LNSV infested field sites. Using this approach, single plant selections were made through seven generations of self-pollinations. In 2008 and 2009, resistance was evaluated in SM09A, SM09B, Green Towers, Darkland, PI491224, 01-778M, 01-781M, 01-789M, and two recently developed romaine-type cultivars resistant to dieback (Triple Treat and Bandit). The breeding lines were in the F7 and F8 generations, respectively. Both trials were maintained using standard cultural practices for the Salinas Valley lettuce production. Resistance to the disease was assessed weekly in order to discriminate between plants dying due to dieback and those due to unrelated causes. The percentage of plants that showed typical dieback symptoms (or were dead due to dieback) was recorded at harvest maturity. At the same time, two additional trials were performed in a field where dieback disease was not observed. Plants from these experiments were harvested at market maturity, processed for salad, and stored in controlled conditions (Hayes and Liu 2008). The rate of tissue decay was visually evaluated on a scale of 1 to 5, where 1 is no obvious decay while 5 indicates total (or close to total) decay of all tissue.

All plants of SM09A, SM09B, PI491224, 01-778M, 01-781M, 01-789M, Triple Threat, and Bandit were free from dieback symptoms during both years. The average disease incidence for Darkland and Green Towers was 56 percent and 64 percent, respectively. Analysis with molecular markers confirmed the presence of the susceptible allele in cvs. Darkland and Green Towers, while all resistant breeding lines, accessions, and cultivars contained alleles associated with resistance to the disease (Simko et al. 2009). The average values of tissue decay when testing of shelf-life were: 2.7 for Darkland, 2.9 for Green Towers, 3.0 for SM09A, 3.1 for SM09B, 3.9 for 01-778M, 4.0 for Triple Threat, 4.1 for PI491224, 4.2 for 01-781M and 01-789M, and 4.4 for Bandit. Overall, the two breeding lines (SM09A and SM09B) performed significantly ($p < 0.05$) better than all other dieback-resistant accessions and were not significantly worse than dieback-susceptible cvs. Darkland and Green Towers.

Morphological description

Both SM09A and SM09B produce closed heads of good size, with a core length about 15 percent longer than cvs. Darkland and Green Towers. SM09A is light-green, while SM09B has a medium-green color. Limited testing for resistance to biotic (downy mildew, lettuce drop due to Sclerotinia minor, race 1 isolates of Verticillium wilt) and abiotic (tipburn) factors did not show a significant difference from cvs. Darkland and Green Towers.

Availability

Limited samples of seeds are available for distribution to all interested parties for research purposes, including the development and commercialization of new cultivars. Samples will also be deposited in the National Plant Germplasm System. It is requested that appropriate recognition be made if the breeding lines contribute to research or the development of new germplasm, breeding lines, or cultivars. Written requests should be sent to Dr. Ivan Simko, USDA-ARS, 1636 E. Alisal St., Salinas, CA 93905.

Signature:



Deputy Administrator, Crop Production and Protection
Agricultural Research Service, U.S. Department of Agriculture



Date