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

NOTICE OF RELEASE OF BABY LEAF ROMAINE BREEDING LINES OF LETTUCE WITH RESISTANCE TO BACTERIAL LEAF SPOT CAUSED BY *Xanthomonas campestris* pv. *vitians*.

Executive Summary

The Agricultural Research Service, United States Department of Agriculture announces the release of two F2:4 breeding populations of lettuce (Lactuca sativa L.) with resistance to bacterial leaf spot (BLS) caused by Xanthomonas campestris pv. vitians. The breeding populations are designated RH12-3370 and RH12-3371 and were selected from the cross Batavia Reine des Glaces × Eruption. In replicated testing, the level of resistance to BLS in these lines was significantly greater than 'Eruption' and equivalent to 'Batavia Reine des Glaces'. Their shelf-life of salad-cut lettuce is commercially acceptable. In limited testing they exhibited susceptibility to leafminers and downy mildew. RH12-3370 and RH12-3371 are romaine type populations with variable leaf morphology. These should be used to select different types of lettuce cultivars possessing BLS resistance and good salad shelf-life for use in baby leaf lettuce production.

Introduction

The popularity of commercially produced and packaged spring-mix salads has increased in the US, and California is the top producer of this product. Baby leaf lettuce (Lactuca sativa L) is the primary component of spring-mix, which typically includes a mixture of a number of different leafy green vegetable species. Baby leaf lettuce is grown at extremely high densities, up to 7.4 million seeds per hectare. The crop is mechanically harvested when the first four true leaves are about 5 to 13 cm (2 to 5 in.) long, typically 20 to 30 days after planting. Approximately 15 to 20 different types of lettuce with unique combinations of leaf shape, margin serration, lobbing, undulation, pliability, savoy and color have been used for baby leaf lettuce production, although most commercially prepared salads contain less than 10 types of lettuce. The diversity of types used in baby leaf production creates a challenge for public plant breeders attempting to enhance the cultivar genepool with new traits such as disease resistance. Introgressing a new trait into a new cultivar of each type is too expensive to be feasible for public breeding programs. Surveys of existing baby leaf cultivars of each type can be executed to determine which types have the greatest need for resistance breeding. This information can be used to prioritize breeding efforts, and screening of 36 commercially used baby leaf cultivars indicated that improved resistance is needed most in red leaf and red romaine type cultivars. Additionally, breeders could select and publicly release early generation (F2 to F4) populations genetically fixed for disease resistance, but with sufficient leaf variability to encompass two or more lettuce types used in baby leaf production. Private seed companies could then select multiple lettuce types from a single resistant population.

Bacterial leaf spot (BLS) of lettuce, caused by Xanthomonas campestris pv. vitians (Xcv), is an economically damaging disease in California during spring and fall production. The pathogen causes small angular leaf spots, which are initially water-soaked, later become necrotic (brown to black) and papery, and eventually coalesce into large necrotic patches. The high planting densities of baby leaf lettuce exacerbate the severity of many lettuce pests, including BLS. Furthermore, the small size and mechanical harvest of the crop reduce the feasibility of culling infected leaves. Consequently, baby leaf crops with bacterial leaf spot disease may be abandoned, which results in a complete yield loss.

Host resistance is an efficient and cost-effective tool to manage BLS of lettuce. Diverse sources of resistance are known including the Latin type cultivar Little Gem, the Batavia type cultivars Batavia Reine des Glaces (synonym Reine des Glaces) and Iceberg, and seven resistant iceberg type breeding lines from the cross (Salad Crisp × Iceberg) × Salinas 88 that were developed by the USDA. 'Little Gem' and its numerous derivatives are used in baby leaf production, while the remaining known sources of resistance are not suitable for baby leaf production. We are reporting the release of two F2:4 (F2 derived F4) breeding populations designated RH12-3370 and RH12-3371 from the cross Batavia Reine des Glaces × Eruption. These populations are genetically uniform for BLS resistance, but genetically variable for leaf morphology. 'Batavia Reine des Glaces' (PI634668, http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1594749; CGN05864, http://applicaties.wageningenur.nl/applications/cgngenis/AccessionDetails.aspx?ID=uh22ve45&acnumber=CGN05864) is an heirloom cultivar with crisp, highly serrate, medium green leaves. The cultivar typically exhibits a degree of leaf cupping and head closure, and may in some cases be considered an iceberg type rather than a Batavia type. 'Batavia Reine des Glaces' has black colored seeds and is highly susceptible to lettuce drop caused by Sclerotinia minor and S. sclerotiorum. The shelf-life of packaged salad prepared from Batavia Reine des Glaces in USDA experiments is sufficient to be considered commercially acceptable, although Batavia Reine des Glaces is not widely used for commercial lettuce production. 'Eruption' (PI 613577, http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1600187) is a dark red colored cultivar developed by the seed company Enza Zaden B.V. for use in commercial baby leaf production. 'Eruption' carries the short leaf 1 (sl1) gene, which confers a diminutive stature to 'Eruption' that is architecturally similar to many Latin type cultivars. Progeny from intertype crosses with 'Eruption' that do not inherit sl1 can have a romaine type architecture. 'Eruption' has white colored seeds. 'Eruption' is resistant to race 1 isolates of V. dahliae and to lettuce drop caused by Sclerotinia minor and S. sclerotiorum, but is susceptible to BLS. The shelf-life of packaged salad prepared from 'Eruption' is inferior to many romaine cultivars, but the cultivar has been used in commercially prepared spring-mix salads.

Development and BLS Resistance of RH12-3370 and RH12-3371

Lettuce is a diploid (2n=2x=18), naturally self-pollinating species, and cultivars are inbred lines. Artificial cross-pollinations were made between 'Batavia Reine des Glaces' and 'Eruption' to produce the F1 generation. The F2:3 and F3:4 generation were produced by growing plants in the greenhouse and allowing each plant to naturally self-pollinate. Seed from each plant was kept separate unless otherwise noted.

Experiments to select for and to evaluate bacterial leaf spot resistance were conducted using greenhouse grown seedlings in plug trays with plant populations of approximately 2,380 plants·m-2. Three diverse Xcv strains (BS339, BS340 and BS347) isolated from diseased plants in California were used as inoculum. Suspensions of bacteria were adjusted to approximately 1 × 108 colony forming units·ml-1 and sprayed on four-week old seedlings until run-off. The seedlings were incubated at near 100 percent humidity for one week prior to disease evaluation. Previous results using this greenhouse assay with 'Batavia Reine des Glaces' and other diverse populations were highly correlated with results from field experiments. Seedlings of F2 Batavia Reine des Glaces × Eruption were grown and evaluated for BLS disease severity, leaf shape, and leaf color. Up to 486 F2 seedlings along with 18 seedlings of 'Eruption' and 'Batavia Reine des Glaces' were grown, and 38 seedlings were selected for low disease severity, romaine type leaf shape and red leaf color. These seedlings were transplanted into larger pots to produce seed. The resulting 38 F2:3 families were subsequently evaluated for BLS resistance and eight potentially resistant families and one susceptible family were selected for retesting. The remaining 29 families were discarded.

The nine selected F2:3 families, their parents and 'Vista Verde' (susceptible control) were evaluated for resistance to BLS in two experiments. 'Batavia Reine des Glaces' had significantly less disease than 'Eruption' and 'Vista Verde' in both experiments (Table 1). Among the F2:3 families, the susceptible selection RH11-1931 had the greatest amount of disease. Three F2:3 families (RH11-1906, RH11-1922 and RH11-1927) had disease levels that were significantly less than 'Eruption', and not significantly different from 'Batavia Reine des Glaces' in both experiments. In addition, the variability between seedlings in RH11-1906, RH11-1922 and RH11-1927 was similar to Batavia Reine des Glaces and not significantly different than the estimated environmental variance, indicating the potential of homozygosity and homogeneity of resistance genes in these families. Families RH11-1922 and RH11-1927 segregated for plants with dark red and green colored romaine shaped leaves. These families were selected for additional testing while the remaining F2:3 families were discarded. Every plant from RH11-1922 and RH11-1927 evaluated for BLS resistance was transplanted into larger pots to produce F3:4 seed. This resulted in 47 F3:4 families from RH11-1922 and 79 F3:4 families from RH11-1927.

Thirty-eight F3:4 families from RH11-1922 and 72 F3:4 families from RH11-1927 were randomly selected and tested for BLS resistance along with 'Batavia Reine des Glaces', 'Eruption' and 'Vista Verde'. 'Batavia Reine des Glaces' and the two populations of F3:4 families had equal amounts of disease, which were significantly less than 'Eruption' and 'Vista Verde' (Table 2). Analysis of disease levels of each F3:4 family within the RH11-1922 and RH11-1927 populations indicate that these populations do not segregate for BLS resistance. The variance between F3:4 families was not significantly different from the estimated environmental variance. Additionally, 100 percent of RH11-1922 F3:4 and 94 percent of RH11-1927 F3:4 families were significantly better than 'Eruption' for disease severity. Equal aliquots of the remaining seed of all F3:4 families from RH11-1922 and RH11-1927 were massed together to produce populations RH12-3370 and RH12-3371.

Salad Shelf-life and Susceptibility to Leafminer and Downy Mildew

Two field experiments were conducted using F2:3 RH11-1922, F2:3 RH11-1927, 'Eruption', 'Batavia Reine des Glaces', and standard check cultivars to assess salad shelf-life and susceptibility to leafminers (Liriomyza langei) and downy mildew (Bremia lactucae) due to natural infestations. These experiments indicate that the levels of susceptibility to leafminers and downy mildew in RH11-1922 and RH11-1927 are similar to their parents. Salad shelf-life was evaluated by harvesting more than 50 fully-grown mature heads of each population, parent, and the baby leaf check cultivars Annapolis (red romaine) and Parris Island Cos (green romaine) and making one bag of salad from each head. Each bag was 22.8 × 15-cm and contained 170 g of 2.5 cm2 lettuce pieces. The bags were triple flushed with N2 prior to sealing. These experiments demonstrated that RH11-1922, RH11-1927 possess shelf-life that is equivalent or improved compared to 'Eruption' and 'Annapolis', similar to 'Batavia Reine des Glaces', but inferior to 'Parris Island Cos'.

Morphological Description

RH12-3370 and RH12-3371 are romaine type populations that segregate for leaf morphology (Fig. 1). RH12-3370 segregates for black and white seed color. When grown to whole head maturity, the plants are generally romaine type with open-tops (rather than closed-top hearting type romaine). Variability for days to flower and seed set as well as resistance or susceptibility to other diseases or physiological defects not discussed in this report are unknown.

Availability and Use of RH12-3370 and RH12-3371

Limited samples of seed are available for distribution to all interested parties for the development and commercialization of new cultivars. These families are resistant to BLS and have commercially acceptable shelf-life when used in packaged salads. They should be used to select inbred lines of lettuce suitable for baby leaf lettuce production. Based on limited field tests, selection of red or green romaine type cultivars is feasible. It is requested that appropriate recognition be made if these breeding lines contribute to research or the development of new germplasm, breeding lines, or cultivars. Written requests should be sent to Dr. Ryan Hayes, USDA-ARS, 1636 E. Alisal St., Salinas, CA 93905, or Ryan.Hayes@ars.usda.gov.

Ryan J. Hayes, Mark Trent, Beiquan Mou, Ivan Simko, and Carolee T. Bull
Agricultural Research Service, United States Department of Agriculture, Salinas, CA 93905.

Signature:

K Simmons

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

7/18/13

Date