Selection for Resistance of Onions to Botrytis allii by Scale Inoculation Method

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Botrytis neck rot, caused by Botrytis allii Munn, is one of the most serious diseases of onion (Allium cepa L.) cultivars during storage. Uniform maturity of F1 hybrids has provided tolerance to natural infection in the United States. Cultivar differences in resistance to this disease have been detected by artificial inoculation in Europe. However, gain from selection for resistance by systematic artificial inoculation has not been reported (1, 2, 4, 5).

A drop of spore suspension (102 spores/ml) was placed on the inner side of rectangular pieces (4 cm x 3 cm) cut from the 2nd and 3rd outer fleshy scales which had been removed from mature bulbs. After incubation at room temperature (about 27°C) for 7 days, each inoculated piece of scale was evaluated and a disease index developed as follows:

0 = Very little or no mycelial growth.
1 = Slight light colored, mycelial growth, no sporulation.
2 = Moderate mycelial growth, sporulation not obvious.
3 = Heavy mycelial growth, little sporulation, some tissue breakdown.
4 = Heavy mycelial growth and sporulation, tissue breakdown occurring under mycelial pad.

Bulbs of open-pollinated cultivars Sapporo Ki (17 bulbs), Kitami Ki (61 bulbs), HI No. 2 (20 bulbs), and HI No. 3 (58 bulbs) were inoculated and evaluated in March of 1980. From this test, 11 bulbs which showed a low disease index (resistant?) and 8 bulbs with a high disease index (susceptible?) were each self pollinated and their progeny grown in the field of the Kitami Agr. Expt. Sta., Hokkaido, Japan, in 1981. After harvest and storage, 12-71 bulbs of each progeny were inoculated (March of 1982) using the same method as in 1980 in order to compare parent and offspring performances.

An additional evaluation for resistance was made on 156 bulbs of the open-pollinated cultivar Kitami Ki in March of 1980. Two subgroups consisting of 24 resistant bulbs and 14 susceptible bulbs were each mass pollinated in separate cages in 1980. These 2 subgroups were identified as BASOR and BA8OS. Our objective was to evaluate the effectiveness of our method selection for either resistance or susceptibility. Bulbs of ‘Kitami Ki’, BASOR, and BA8OS were grown at the Kitami Agricultural Experiment Station. After harvest and storage, 50 bulbs of each pop-

Table 1. The distribution of disease index of each self pollinated progeny.

<table>
<thead>
<tr>
<th>Progeny</th>
<th>Average disease index* of 2nd and 3rd scales</th>
<th>Total no. bulbs</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAS8026</td>
<td>0 1 1 0 0 17 0 2 12 2.75 a</td>
<td>62 2.62 a</td>
<td></td>
</tr>
<tr>
<td>BAS8020</td>
<td>0 1 1 1 3 8 0 1 21 2.48 ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAS8044</td>
<td>0 0 6 8 4 12 8 2 46 2.39 ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAS8045</td>
<td>0 0 6 8 4 12 8 2 46 2.39 ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAS8017</td>
<td>0 0 6 8 4 12 8 2 46 2.39 ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAS8015</td>
<td>4 2 10 11 10 6 2 1 0 46 1.57 cd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAS8050</td>
<td>3 9 14 12 11 4 1 0 54 1.32 cd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAS8049</td>
<td>1 9 3 5 1 2 0 1 1 23 1.28 cd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAS8016</td>
<td>6 5 3 2 4 1 1 0 22 1.00 cde</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14 27 39 41 37 26 10 8 239 1.90</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

*Disease index, 0 = no symptom, 4.0 = sporulation over entire surface.
The mean disease indices of the self-pollinated progenies derived from resistant bulbs tended to be lower than the mean indices for all self-pollinated progenies derived from susceptible bulbs. BAS8001 and BAS8016 were exceptions (Table 1). The percentage of bulbs of the self-pollinated progenies selected for resistance and susceptibility, respectively, was computed for each disease index (Table 1). Indices lower than 1.0 were obtained for 37.3% of bulbs derived from resistant parents and for 17.2% of bulbs derived from susceptible parents, suggesting that heritable differences in resistance to this disease were obtained by a single selection using artificial scale inoculation.

Two open-pollinated progenies derived from resistant and susceptible bulbs, respectively, were compared with 'Kitami Kii', the original seed source, using simultaneous scale inoculation (Fig. 1). Progeny from bulbs selected for resistance in 1980 (BA80R) had a reduced average index in 1982. However, the bulked progeny from the susceptible selections did not differ from 'Kitami Kii'. By the Kolmogorov-Smirnov test for the maximum vertical distance between cumulative distribution functions, the distribution of the resistant open-pollinated population (BA80R) in 1982 differed from both the susceptible population (BA80S, P = 0.001) and the original cultivar ('Kitami Kii, P = 0.007), but the distributions of BA80S and 'Kitami Kii' are not significantly different (P > 0.9). Selection for resistance was more effective than selection for susceptibility.

Commercial onion cultivars generally are more resistant to B. allii than most Allium species (5). It may be difficult to develop resistant inbreds, except by introducing resistant genes from other species. However, recognizing the difficulty of interspecific hybridization in Alliums, our study was carried out to estimate the level of resistance to B. allii obtainable by cumulative selection within A. cepa.

It was concluded that this method of inoculation has the following advantages: 1) in comparison with field inoculation, the environment is more uniform; 2) selection for resistance can be conducted during storage, thereby identifying superior parent bulbs prior to planting the breeding nursery.

Infection by this laboratory method is different from natural infection. At harvest, bulbs with green tops are infected naturally when tops are cut, and they may rot during the storage period (4, 6). Also, it has been reported that seed inoculum is correlated with disease development during the storage period (3). With field infection, hyphae probably penetrate fleshy scales from the bulb neck. In our laboratory method, spore germination and hyphae development occur on the surface of the fleshy scale, and hyphae penetrate parenchyma cells through epidermal tissues on the inner side of the fleshy scales.

The correlation of resistance selected by this method to field resistance has not been completed.

Literature Cited


Aseptic Multiplication of Banana from Excised Floral Apices

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Most economically important bananas and plantains are large triploid seedless herbs that must be propagated vegetatively by removing small side shoots or “suckers” from the parent plant or by planting seed pieces of larger corns. Consequently, multiplication of stock material is time consuming. Recently, the rapid production of young banana plantlets suitable for use as “seed” material has been described (1, 3). Vegetative shoot apices were isolated and multiplied using aseptic tissue culture techniques. Although these multiplication systems, once established, can produce thousands of plants in a relatively short period of time, their establishment necessitates the initial sacrifice of an individual specimen, which may not always be desirable or prudent should a limited parent stock be available. We describe here the production and multiplication of rooted banana plantlets from the isolation and culture of terminal floral apices.

Plants of the dessert banana (Musa acuminata Colla) clone “Dwarf Cavendish” [designated AAA by Simmonds and Shepard (5)] were grown in the greenhouse to fruiting size in 54 x 50 cm pots. After the flower bud had emerged from the pseudostem, growth of the fruit bunch was allowed to proceed until all female hands of fruit had appeared. The large terminal floral bud then was removed, and the male flowers with their subtending bracts were removed in a stepwise fashion. The final minute flower clusters and bracts were removed with forceps and scalpel under a disecting microscope to reveal the elevated apical dome. The distal 0.2 mm of the floral apex was excised by making 2 angled cuts into the subtending tissue. It then was surface sterilized by soaking in 50 ml of a 0.105% aqueous solution of NaOCl (2% v/v Clorox) with 2 drops of Tween 20 for 5 min and rinsed 4 times with sterile distilled water. Disinfested apices were cultured in liquid medium composed of the mineral salts of Murashige and Skoog (4) supplemented with 5.55 mM inositol, 2.97 mM thiamine HCI, 0.12 mM sucrose, 10% v/v coconut water, and 22.0 μM BA. The pH of the culture medium was adjusted to 5.6 with KOH, and 20 ml were poured into 125 ml Erlenmeyer flasks and autoclaved for 20 mm at 127°C and 1.1 kg cm². Cultures were maintained on rotary shakers at 100 rpm in growth chambers at 30°C in a 16:8 hr L:D cycle at 10.2 Klux (Sylvania Grolux wide spectrum).

Isolated apices in a liquid medium grew slowly for 2 months into tiny green shoots. Shoot multiplication was stimulated by transferring tissue pieces alternately between liquid and semisolid medium of the same composition at 2 week intervals as previously described (1). The medium (50 ml in 100 ml screw cap jars) was solidified with 0.7% Difco Bacto agar. Thereafter, shoot cultures were maintained by subdividing multiplying shoot clusters with a few scalpel incisions and transferring them to fresh semisolid medium at 3 week intervals (1). Plantlets were rooted by placing individual shoots on semisolid medium containing 5.5 μM NAA and 0.025% activated charcoal. The first roots appeared within 5 days, and plantlets could be potted in Pro...