Improvement of Rust Resistance in Bunching Onion

(Allium fistulosum L.) by Recurrent Selection

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Summary
To improve rust resistance of bunching onion (Allium fistulosum L.), we applied a recurrent selection program by using six cultivars (C1); 'Seito Ippon', 'Iwai 2', 'Choju', 'Senami', 'Fuyuogi Ippon' and 'Toyokawa Futo'. Each cycle of recurrent selection consisted of two steps: selfing and selection among selfed progenies in the first year, and intercrossing and maternal line selection in the second year. A second-cycle improved population (C2) consisting of 10 lines was obtained by two cycles of recurrent selection. Furthermore, we conducted two generations of selfing and progeny selection, and obtained 13 C2S2 lines. To evaluate the effectiveness of this recurrent selection, we conducted two rounds of simultaneous inoculation tests and compared the rust resistance of all generations obtained in the selection program. Under inoculation tests in the spring and autumn, the value of the area under the disease progress curve (AUDPC), an index of disease intensity, definitely decreased with the progress of recurrent selection. Although the resistance gain from C1 to C2 was small, much progress was made in the C2S2 generation; the AUDPC in C2S2 lines was approximately 38% of that in the initial parental cultivars. Our results demonstrate that recurrent selection is effective in improving the rust resistance of bunching onion.

Key Words: Allium fistulosum, bunching onion, disease resistance, recurrent selection, rust.

Introduction
Bunching onion (Allium fistulosum L.) is one of the most important vegetable crops in east Asian countries, especially in Japan, Korea, and China (Inden and Ashira, 1990; Kumazawa and Katsumata, 1965). In 2002, the annual output of bunching onion in Japan was worth 133 billion yen, which was twice that of bulb onion (A. cepa L.). Rust caused by Puccinia allii Rudolphi is a serious foliar disease of bunching onion. This disease occurs frequently in the autumn and spring, and causes substantial deterioration of quality and yield of bunching onion. Since no existing commercial cultivar possesses sufficient resistance to rust disease, large amounts of fungicides are used every year to prevent and exterminate this disease. Breeding for rust resistance will provide an effective, economical, and ecological method to prevent epidemics of this disease.

In a previous study, we observed differences in susceptibility among 133 cultivars of bunching onion under artificial inoculation in the experimental field (Wako et al., 1999). Just as in leek (A. ampeloprasum L.) (Smith et al., 2000), no completely resistant cultivar was found in bunching onion. However, considerable variations were observed in the degree of disease severity, namely the area under the disease progress curve (AUDPC). Cultivars of 'Senju Aigara', 'Kaga', and 'Kujo' types exhibited high susceptibility to the disease, whereas cultivars of 'Senju Aiguro' and 'Senju Kurogara' types were found to be more resistant than the others. This demonstrated that the rust resistance present in bunching onion was a quantitative trait. Among the 133 cultivars tested, 'Seito Ippon', 'Iwai 2', 'Choju', 'Senami', 'Fuyuogi Ippon', and 'Toyokawa Futo' showed the lowest values of AUDPC. Using these relatively resistant cultivars, a recurrent selection program was initiated to achieve a high level of field resistance to rust, because this system has been successful in improving quantitatively inherited traits in other outcrossing crops. For example, recurrent selection improved resistance to larvae of the European corn borer (Ostrinia nubilalis Hubner) and fall armyworm (Spodoptera frugiperda J. E. Smith) in maize (Penny et al. 1967, Widstrom et al. 1992), resistance to common rust (Puccinia sorghi Schw.) in sweet corn (Abedon and Tracy, 1998), and resistance to Sclerotinia crown and stem rot (Sclerotinia trifoliorum Eriks.) in alfalfa (Kanbe et al. 1997).

In this work, we conducted two cycles of recurrent
selection for rust resistance, and two to four generations of continuous selfed–line selection following each cycle of recurrent selection. By comparing the AUDPCs among the generations obtained, we evaluated the effectiveness of recurrent selection for rust resistance in bunching onion.

Materials and Methods

Recurrent selection

Following the procedure of recurrent selection (Fig. 1), we initiated recurrent selection in 1997. We evaluated 133 bunching onion cultivars for rust resistance by artificial inoculation, as described below, in the experimental field of the National Institute of Vegetable and Tea Science (NIVTS). The resulting six slow-rusting cultivars selected were (C5): ‘Seito Ippon’, ‘Iwai 2’, ‘Choju’, ‘Senami’, ‘Fuyagi Ippon’, and ‘Toyokawa Futo’, coded as numbers 32, 48, 58, 62, 74, and 165, respectively. In the spring of 1998, 60 C5S1 lines were produced by selfing the C5 plants and they were evaluated for rust resistance in the autumn of 1998. A total of 69 plants of 15 C5S1 lines were selected. The first intercross was then conducted among the selected C5S1 plants to produce the first-cycle improved population (Cycle 1 or C1). C1 seed lots, harvested in the spring of 1999 from each of the selected C5S1 plants, were not bulked but sown separately as maternal lines. The resulting 59 C1 maternal lines were evaluated for rust resistance, and 16 lines, 4 to 12 plants per line, were selected. These C1 plants were selfed in 2000 and 83 C1S1 lines were produced, from which 10 lines, one plant per line, were selected for rust resistance. These C1S1 plants were intercrossed in 2001 to produce the second-cycle improved population (Cycle 2 or C2), which was composed of 10 C2 maternal lines. After these C2 maternal lines were evaluated for rust resistance, two lines, 10 plants per line, were selected. A total of 20 selected C2 plants were selfed in 2002, producing 20 C2S1 lines, from which seven of relatively rust resistant lines, one or two plants per line, were selected in this first round of simultaneous inoculation tests. The selfing was repeated with 13 C2S1 plants selected to produce 13 C2S2 lines in 2003. Selection and selfing were also repeated from C2S1 and C1S1 for two and three subsequent generations, respectively.

Simultaneous inoculation test

To evaluate the effectiveness of the recurrent selection program, we conducted two rounds of simultaneous inoculation tests and compared the levels of rust resistance of all the generations obtained. Because of the practical limitations of our experimental design, it was difficult to test simultaneously all lines produced during the recurrent selection. Therefore, from each generation, we extracted two to six lines demonstrated to have the highest resistance in the year when selection was performed; lines used in the inoculation test are described below in “Results”. ‘Yoshikura’, ‘Kincho’, ‘Ishikura Nebuka Fuyu Futo’, and ‘Togoku’ were used as susceptible controls. The simultaneous inoculation test for rust resistance was conducted in the spring and autumn of 2003.

In the first round of simultaneous inoculation tests, seeds were sown on 200-cell plug trays on July 4, 2002. Seedlings of each cultivar or line were transplanted to plots replicated three times in a plastic greenhouse of the NIVTS on September 9. Inoculum ofuredospores of P. allii was isolated at a field in Seiro, Niigata prefecture, and multiplied with bunching onion plants in NIVTS fields. Uredospores were collected by using a brush from fresh rust pustules on bunching onion leaves and transferred into 10 mL plastic tubes, and then stored in the refrigerator at -30°C for over eight months until just before being used. Before inoculation, we sprayed the plants with 0.006% polyoxyethylene nonylphenyl ether, Kumiten (Kumiai Chemical Industry Co., Ltd.) to stimulate the germination of the inoculated spores. We then sprayed the plants with a uredospore suspension (5 x 10^7/mL) in 0.01% polyoxyethylene sorbitan monolaurate (Tween 20, Nacalai Tesque, Inc.) on March 24, 2003. The severity of the rust symptoms was scored on four fully-grown leaves of each plant (on April 14, 28, and May 7). The results were ranked as follows: grade 0, no pustule; grade 1, 1-3 pustules per leaf; grade 2, 4-10 pustules per leaf; grade 3, more than 11 pustules per leaf; grade 4, pustules distributed over the whole leaf; and grade 5, pustules distributed densely over the whole leaf. To evaluate disease intensity, the AUDPC was calculated according to Shaner and Finney (1977), with the following equation,
Fig. 2. The disease intensity AUDPC in six generations during recurrent selection for rust resistance. The AUDPC was scored in the year when selection was performed. Mean AUDPCs of lines tested and selected, and AUDPCs used in simultaneous inoculation test are represented separately. Vertical bars represent SE.

\[
\text{AUDPC} = \sum_{i=1}^{n} (t_i - t_{i-1}) (DS_{i-1} + DS_i) / 2
\]

where \(n\) = number of observations; \(t_i\) = days at the \(i\)th observation (\(t_0 = 0\)); and DS = rust severity at the \(i\)th observation (DS = 0).

In the second round of simultaneous inoculation tests, seeds were sown on June 23, 2003, and seedlings of each cultivar or line were transplanted to plots replicated five times in the plastic greenhouse on August 13. Uredospores of \(P. allii\) were inoculated on October 14 as above. Rust severity was scored on October 29, November 6, 12, and 17, and the AUDPC was calculated.

Results

Figure 2 summarizes the AUDPC distributions in the six generations obtained annually in the course of the recurrent selection. Selection intensity (percentage of the number of lines selected in the number of lines tested) was in the range of 12% (C_5S_i) to 27% (C_0) (Figs 1 and 2). Although six cultivars were selected for \(C_0\) in 1997 (Fig. 2A), ‘Senami’ and ‘Fuyuogi Ippon’ were not used in the simultaneous inoculation tests in 2003, because their seeds were not available. The top 6 of 15 lines selected from the \(C_5S_i\) generation (Fig. 2B) were the \(S_i\) progenies of ‘Seito Ippon’, ‘Iwai 2’, ‘Chouji’, ‘Fuyuogi Ippon’ and ‘Toyokawa Futo’. In the trial of 1998, 48 - 10s exhibited a remarkably high level of rust resistance. From the \(C_1\) generation, maternal lines 32 - 9s - 2ic and 48 - 10s - 7ic were used (Fig. 2C), because they were the progenitors of the selected \(C_5\) maternal lines, 32 - 9s - 2ic - 5s - 3ic and 48 - 10s - 7ic - 3s - 2ic (Fig. 2F), respectively. We also used four \(C_5S_i\) and five \(C_5S_2\) lines derived from these two \(C_5\) lines (Fig. 2D, E). For comparison with the two lines, 20 \(C_5S_i\) and 13 \(C_5S_2\) lines derived from these two \(C_5\) maternal lines were tested. Additionally, three \(C_5S_i\) and three \(C_5S_2\) lines derived from the \(C_5S_i\) line 48 - 10s, and five \(C_5S_i\) and six \(C_5S_2\) lines derived from the two \(C_5S_i\) lines, 32 - 9s - 2ic - 5s and 48 - 10s - 7ic - 3s, were tested.

In the first round of simultaneous inoculation tests, the rust disease developed slowly; AUDPCs of the tested
Table 1. AUDPCs scored in two rounds of simultaneous inoculation tests of two cultivars and their progenies produced in the course of recurrent selection.

<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Cultivar or line</th>
<th>1st inoculation test AUDPC</th>
<th>1st inoculation test P-value</th>
<th>2nd inoculation test AUDPC</th>
<th>2nd inoculation test P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C0</td>
<td>Seito Ippon</td>
<td>36.3 ± 8.2 *</td>
<td>-</td>
<td>89.2 ± 4.3</td>
<td>-</td>
</tr>
<tr>
<td>C0S1</td>
<td>32 - 9s</td>
<td>21.3 ± 3.8</td>
<td>0.171</td>
<td>66.5 ± 4.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C1</td>
<td>32 - 9s - 2ic</td>
<td>18.7 ± 3.8</td>
<td>0.122</td>
<td>48.4 ± 7.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C1S1</td>
<td>32 - 9s - 2ic - 5s</td>
<td>11.3 ± 6.6</td>
<td>0.076</td>
<td>54.4 ± 9.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C2</td>
<td>32 - 9s - 2ic - 5s - 3ic</td>
<td>6.0 ± 2.7</td>
<td>0.024</td>
<td>47.4 ± 11.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C2S1</td>
<td>32 - 9s - 2ic - 5s - 3ic - *s'</td>
<td>2.3 ± 1.8</td>
<td>0.015</td>
<td>36.3 ± 3.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C2S2</td>
<td>32 - 9s - 2ic - 5s - 3ic - 10s - *s'</td>
<td>nt</td>
<td>nt</td>
<td>41.1 ± 5.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C2S2</td>
<td>32 - 9s - 2ic - 5s - *s'</td>
<td>8.0 ± 2.1</td>
<td>0.015</td>
<td>61.3 ± 5.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C2S2</td>
<td>32 - 9s - 2ic - 5s - *s - *s'</td>
<td>4.7 ± 1.7</td>
<td>0.031</td>
<td>60.0 ± 4.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C2S2</td>
<td>32 - 9s - 2ic - 5s - 10s - 1s - *s'</td>
<td>nt</td>
<td>nt</td>
<td>60.0 ± 3.4</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Pedigree 2

| C0       | Iwai 2         | 18.0 ± 9.1                | -                           | 73.7 ± 9.6                | -                           |
| C0S1     | 48 - 10s       | 13.3 ± 6.6                | 0.698                       | 34.4 ± 6.4                |   <0.01                     |
| C1       | 48 - 10s - 7ic - 3s | 13.3 ± 5.3                | 0.680                       | 54.2 ± 6.5                |   0.134                     |
| C1S1     | 48 - 10s - 7ic - 3s | 6.7 ± 1.7                | 0.287                       | 27.7 ± 5.7                |   <0.01                     |
| C2       | 48 - 10s - 7ic - 3s - 2ic | 16.7 ± 14.2               | 0.941                       | 48.0 ± 7.6                |   0.070                     |
| C2S1     | 48 - 10s - 7ic - 3s - 2ic - *s' | 6.4 ± 2.1                | 0.350                       | 27.5 ± 2.4                |   <0.01                     |
| C2S2     | 48 - 10s - 7ic - 3s - 2ic - *s - *s' | nt                     | nt                          | 30.2 ± 2.7                |   <0.01                     |
| C2S2     | 48 - 10s - 7ic - 3s - *s' | 8.9 ± 2.1                | 0.423                       | 48.1 ± 4.9                |   0.058                     |
| C2S2     | 48 - 10s - 7ic - 3s - *s - *s' | 12.5 ± 2.9               | 0.309                       | 55.8 ± 4.3                |   0.150                     |
| C2S2     | 48 - 10s - 7ic - 3s - *s' | 5.2 ± 0.5                | 0.248                       | 38.0 ± 5.2                |   0.050                     |
| C2S2     | 48 - 10s - 7ic - 3s - *s - *s' | 2.5 ± 1.1                | 0.161                       | 51.6 ± 5.7                |   0.187                     |
| C2S2     | 48 - 10s - 7ic - 3s - 10s - 1s - *s' | nt                     | nt                          | 45.5 ± 6.2                |   0.063                     |

* Significance of difference from C0.
* Significance of difference from C0.
* 2 to 10 lines were tested.
* Mean ± SE (n = 3 - 18 in 1st inoculation test and n = 5 - 50 in 2nd inoculation test).
* Not tested.

lines, including susceptible controls, were generally small (Fig. 3A). No significant differences were detected at the 5% level by analysis of variance between the C0 and the other generations in the first inoculation test. Based on the results of the first inoculation test, three lines from the C1S1 generation (32 - 9s - 2ic - 5s - 10s - 1s, 48 - 10s - 7ic - 3s - 3s - 1s, and 48 - 10s - 10s - 7ic - 3s - 10s - 1s) and seven lines from the C2S1 generation (32 - 9s - 2ic - 5s - 3ic - 10s, 48 - 10s - 7ic - 3s - 2ic - 4s, 48 - 10s - 7ic - 3s - 2ic - 4s, 48 - 10s - 7ic - 3s - 2ic - 4s, 48 - 10s - 7ic - 3s - 2ic - 4s, 48 - 10s - 7ic - 3s - 2ic - 4s, and 48 - 10s - 7ic - 3s - 2ic - 4s) were selected as parental lines to produce six C1S1 and 13 C2S2 lines (Table 1). In the second inoculation test, a rapid epidemic development was observed, so that the disease intensity was consequently much higher than in the first inoculation test (Fig. 3B). Severe rust symptoms were observed in susceptible and C0 cultivars. However, the mean of the AUDPCs among the five replicated plots in each generation decreased with progress of recurrent selection (Fig. 3B). Significant differences were detected between the C0 and the other generations at the 1% level by analysis of variance. The most prominent effects of recurrent selection appeared at the C1 and C2S1 generations in contrast to the small genetic gain detected in the C1S1 and C2 generations. The mean of the AUDPCs in the C2S2 lines (31.9) was 38% of that in C0 cultivars (84.4), which demonstrates the high efficiency of the recurrent selection method for rust resistance. The efficiency of the recurrent selection differed between the component pedigrees of the improved population, derived from 'Seito Ippon' and 'Iwai 2'. In the pedigree from 'Seito Ippon', the AUDPC apparently decreased with progress of recurrent selection in the first inoculation test, although the difference was not significant between 'Seito Ippon' and any progeny generation based on the analysis of variance at the 1% level (Table 1). In the second inoculation test, the AUDPCs of progenies were significantly lower than that of 'Seito Ippon'. The AUDPC of the C1S1 line, 32 - 9s - 2ic - 5s, was higher than that of the C1 maternal line, 32 - 9s - 2ic. No difference was detected between the AUDPCs of the C1 and C2 lines (48.4 and 47.4). The AUDPCs of the selfed lines (C1S1 to C1S1) derived from 32 - 9s - 2ic (C1) were higher than that of C1, whereas those of the C1S1 and C2S2 lines were much smaller than that of the C2.
line, 32-9s-21c-5s-3ic, and less than 46 % of that in ‘Seito Ippon’.

The AUDPCs of the C1 and C2 lines in the pedigree from ‘Iwai 2’ were not smaller than those of C0S1 and C1S1, respectively, in either of the tests. The level of resistance of 48–10s was much higher than that of 32–9s, corresponding with the result of the 1998 trial (Fig. 2B). The high level of resistance observed in 32–9s-21c-5s was inconsistent with the result of the trial in 2000 (Fig. 2D). The levels of resistance in the C1S1 and C2S1 generations in the pedigree from ‘Iwai 2’ were the highest in the second inoculation test. The high levels of resistance in C0S1, 48–10s, and in C1S1, 48–10s-7ic-3s, had not been fixed in their selfed progenies, C0S2, C0
S3, C1S2, C1S3, or C2S2. However, the mean of AUDPC for the non-s selected C2S2 lines was as small as 41 % of that for ‘Iwai 2’, which was comparable to that for the selected C2S1 lines.

Discussion

Recurrent selection can be used successfully in many outcrossing crops to improve quantitative traits with low heritability. In vegetable crops, for example, the fruit yield of cucumber has been improved by 10 cycles of recurrent selection (Wehner and Cramer, 1996). Improved populations for resistance to Verticillium dahliae were developed by two cycles of recurrent selection in pepper (Palloix et al., 1990). In this study, all the bunching onion generations produced in the course of the recurrent selection program were simultaneously evaluated for rust resistance under inoculation tests in the spring and autumn. It is desirable to assay rust resistance of whole populations of each generation to evaluate the efficiency of recurrent selection. But because so many lines were produced in each generation, it was not practical to conduct the inoculation test in all the populations at the same time. We, therefore, compared rust resistance among C0 cultivars and elite lines selected from each generation. Although no significant AUDPC difference was observed between susceptible and C0 cultivars in the two tests, the rust resistance of intercrossed generations derived from C0 cultivars was improved steadily with progress of the recurrent selection. Our results demonstrate that this method is effective in improving rust resistance of bunching onion, even if cultivars with significantly high rust resistance do not exist within this species. In the continuously selfed generations (C0S1 to C0S10, C1S1 to C1S10), distinct reductions in the AUDPC were not observed in the second inoculation test. These results indicate that genes related to rust resistance had still not sufficiently accumulated in the C1 generation or in the material cultivars. It would be desirable to accumulate more rust resistance genes in further intercross generations. In maize, Weyhrich et al. (1998) reported that S1–progeny and S2–progeny selection methods had greater responses for grain yield in comparison with five other methods of recurrent selection. In general, the effect of selection is low in the early generations of recurrent crossing when the frequency of desirable genotypes in the initial population is low (Kanbe et al., 1997), but increases as selection proceeds. In our breeding work, rust resistance significantly increased in the early intercross generations. We consider that the efficiency of the S1–progeny selection used in this study was high, and that rust resistance in bunching onion might be governed by a relatively small number of genes with high heritability compared with the resistance to larvae of European corn borer or fall armyworm in maize (Penny et al., 1967; Widstrom et al., 1992).

Rust disease in leek occurs frequently in European countries (Maude, 1990). Although intraspecific variation in rust resistance has been observed among leek cultivars (Dixon, 1976; Smith et al., 2000; Uma and Taylor, 1991), commercial cultivars with sufficient resistance to rust have not yet been developed. Information on the genetics of rust resistance has accumulated in other major crops, such as wheat (Dedryver et al., 1996; Huang and Gill, 2001; Mago et al., 2002; Raupp et al., 1983; Stein et al., 2000) and barley (Dreisiel and Steffenson, 2000; Feusterstein et al., 1990; Jagathriya et al., 2003). Recently, the wheat leaf rust resistance genes, Lr10 (Feuillet et al., 2003) and Lr21 (Huang et al., 2003), and the barley stem rust resistance gene, Rpg1 (Brueggeman et al., 2002), were successfully cloned by map-based isolation strategies. In bunching onion, however, rust resistance genes have not yet been identified.

The rust resistance developed in this study is not true resistance but rather that known as field resistance. This type of resistance is thought to be controlled by polygenes, and is therefore more effective against a broad range of pathogenic races and more durable than monogenic resistance, which may break down under severe epiphytotics (Brewbaker, 1983). The lines selected from the C0S2 generation of this study will be a good source of inbreds with high levels of field resistance to rust.

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循環選抜によるネギ(Allium fistulosum L.)のさび病抵抗性の改良

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摘 要

ネギのさび病抵抗性を改良するため、「温冬一本」、「岩井2号」、「長寿」、「せなみ」、「冬霞一本」、「豊川大」の6品種を育種素材(C1)として循環選抜を行った。循環選抜の1サイクルは2段階となり、最初の年に自殖および自殖系統選抜を行い、2年目に相互交配および母系系統選抜を行った。2サイクルの循環選抜により、10系統系統からなる改良集団(C2)を得た。さらに、2世代の自殖と自殖系統選抜を行い、13系統システムを得た。実施した循環選抜の効果を評価するために、2回の親種検定により上記の選抜で得られた世代のさび病抵抗性の程度を比較した。春季および秋季の接種検定において、発病程度の指標であるarea under the disease progress curve (AUDPC)の値は循環選抜が進むにともない明らかに減少し、抵抗性の向上が認められた。C1からC2世代にかけて抵抗性的変化は小さかったものの、C2S2世代では大幅な向上が認められ、C2S2系統のAUDPCは素材品種の約38%となった。以上の結果、ネギのさび病抵抗性の改良に循環選抜は有効であることが実証された。