Identification and Molecular Mapping of a Wheat Gene for Resistance to an Unadapted Isolate of *Colletotrichum cereale*

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ABSTRACT

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To elucidate genetic mechanisms of host species specificity between graminicolous anthracnose fungi and gramineous plants, infection assays were performed with a *Sorghum* isolate (*Colletotrichum sublineolum*), a *Digitaria* isolate (*C. hanaui*), a *Polypogon* isolate (*C. cereale*), and an *Avena* isolate (*C. cereale*). They were specifically virulent on the plants from which they were isolated. When 72 wheat lines were inoculated with an unadapted isolate from Asia Minor bluegrass (Cgp29), however, some exceptional cultivars were recognized. Although most cultivars were resistant to Cgp29, ‘Hope’ was susceptible. In F₂ populations de-

rived from crosses between three resistant cultivars—‘Norin 4’ (N4), ‘Chinese Spring’ (CS), and ‘Shin-chunaga’ (Sch)—and the susceptible Hope, resistant and susceptible seedlings segregated in a 3:1 ratio, suggesting that a major gene is involved in the resistance of each cultivar to Cgp29. In F₂ populations derived from crosses between the three resistant cultivars, all seedlings were resistant, suggesting that these three cultivars carry the same gene. This resistance gene was designated as “resistance to *Colletotrichum cereale* 1” (*Rcc1*). Analysis with the CS–Hope chromosome substitution lines and molecular mapping revealed that *Rcc1* was located on the long arm of chromosome 5A. Cytologically, *Rcc1* was mainly associated with hypersensitive reaction. These results suggest that major genes similar to those controlling cultivar specificity are involved in the resistance of wheat against the unadapted isolate of *C. cereale*.

Plants are exposed to various types of potentially invasive organisms but only a few of these actually damage the plants. The host–parasite specificity between plants and microbes can be divided into two types: that is, plant (host) species specificity determining host species range and cultivar specificity determining cultivar range within a given host species (10). Cultivar specificity has been known to be controlled by gene-for-gene interactions, whereas host species specificity has been considered to be genetically complex. Elucidation of host species specificity determinants will help to employ nonhost resistance to the breeding of resistant crops. This will also help to understand mechanisms of host specialization and host jump; namely, emergence of new pathogens that gained an ability to colonize a plant species which was formerly resistant.

Molecular analyses of plant–microbe interactions over the last two decades revealed that plants have evolved at least two lines of active defense (8): pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) (2,12). The first line (PTI) is basal defense through recognition of conserved PAMPs. The second line (ETI) is a defense system that relies on perception of specific pathogen effectors by plant resistance proteins. According to this definition, PTI is multigenically controlled while ETI is controlled by gene-for-gene interactions. With regard to the relationship between PTI and ETI and nonhost or host resistance, Jones and Dangl (12) described that nonhost resistance could be mediated by at least

two mechanisms, little or no suppression of PTI due to ineffectiveness of effectors, and induction of ETI caused by the recognition of one or more of the effector complement by the nucleotide-binding site leucine-rich repeat repertoire (i.e., gene-for-gene interactions). This implies that some types of nonhost resistance involve gene-for-gene interactions.

Colletotrichum spp. are causal agents of anthracnose diseases in a wide range of crops and ornamental plants, including at least 42 genera in the family Poaceae (3). Recently, graminicolous *Colletotrichum* isolates were systematically characterized by molecular phylogeny and have been divided into two major subgroups (3). One subgroup is associated with cool-season hosts and is composed of a single species, *Colletotrichum cereale* (5,7). The other subgroup is associated with warm-season hosts and is subdivided into 13 species (4,6,15). Host ranges of these species have been a matter for debate. Crouch and Beirn (3) described that host range has some utility for the diagnosis of species, especially for *C. cereale* (on Pooid grasses), *C. eleusine* (on *Eleusine* sp.), *C. graminicola* (on corn), *C. hanaui* (on crabgrass), *C. miscanthi* (on *Miscanthus sinensis*), and *C. navitas* (on switchgrass), but that definition of other species using host range criteria may be less reliable due to shared hosts for multiple species. With regard to specialization within the wide-host-range species *C. cereale*, Crouch et al. (7) inferred that this species was divided into 10 highly specialized populations corresponding to ecosystem or host plant. However, this inference was based on molecular phylogenetic analysis, and has not been confirmed by systematic infection assays.

In the present study, we first examined whether host species specificity exists between graminicolous *Colletotrichum* spp. and gramineous plants through infection assays with some representative isolates. Second, we analyzed genetic mechanisms of the specificity using wheat and a *C. cereale* isolate from Asia

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Minor bluegrass. We successfully identified a major resistance gene, “resistance to *Colletotrichum cereale* 1” (*Rcc1*), in wheat that is effective against this unadapted isolate. Its characteristics and the ecological or evolutionary implications of this finding are reported here.

MATERIALS AND METHODS

Fungal materials. Fungal materials used were *C. sublineolum* Cgs61 (MAFF305361) isolated from sorghum (*Sorghum bicolor*), *C. hanaui* Cgd14 (MAFF511014) isolated from crabgrass (*Digitaria ciliaris*), *C. cereale* Cgp29 (MAFF305429) isolated from Asia Minor bluegrass (*Polypogon fugax*), and *C. cereale* Cga71 (MAFF305371) isolated from oat (*Avena sativa*) (Table 1). The code names (e.g., Cgp29) of the isolates are composed of the initials of their genus name (*Colletotrichum*), traditional species name in the Microorganisms Section of the NIAS Genebank, National Institute of Agrobiological Sciences, Tsukuba, Japan (MAFF) collection (*graminicola*), and host genus (*Polypogon*). Current species names of these isolates have been described by Crouch et al. (6,7). They were maintained on filter papers at -80°C, then transferred to a potato dextrose agar slant just before use, and grown at room temperature.

Plant materials. To determine host ranges of the four *Colletotrichum* isolates, six lines belonging to their original host species

Fig. 1. Typical infection types on wheat leaves, 7 days after inoculation with *Colletotrichum* isolates.

were employed: sorghum ‘Sweet’ and ‘Yukijirushi Hybrid’ (YH), crabgrass strain DS136-102, Asia Minor bluegrass strain 10360S, and oat ‘Shokan1’ (SK) and ‘CW-491-1’ (CW). As possible nonhost plants, 38 hexaploid wheat lines ($2n = 6X = 42$, genome AABBDD), 34 tetraploid wheat lines ($2n = 4X = 28$, genome AABB), and 23 barley lines (*Hordeum vulgare*, $2n = 2X = 14$, genome HH) were also employed. These wheat and barley lines were provided by K. Tsunewaki and S. Sakamoto at Kyoto University, and U. Hiura at Okayama University. Representative wheat cultivars used for identification of *Rcc1* were *Triticum aestivum* ‘Norin 4’ (N4), ‘Shin-chunaga’ (Sch), ‘Chinese Spring’ (CS), and ‘Hope’. To identify a wheat chromosome carrying *Rcc1*, 21 chromosome substitution lines were used, each of which carried one chromosome from Hope and the other 20 chromosomes from CS. These lines (KT729 through KT749) were provided by Gene Bank in the Kihara Institute for Biological Research, Yokohama City University.

Infection assay. Wheat, barley, and oat were grown in plastic pots (5.5 by 15 by 10 cm) filled with vermiculite in a controlled-environment room with a 12-h photoperiod of fluorescent lighting for 7 to 9 days at 22°C. Crabgrass and Asia Minor bluegrass were grown in plastic pots filled with Sakata Supermix soil (Sakata, Yokohama, Japan) in a growth chamber with a 12-h photoperiod of fluorescent lighting for 20 days (crabgrass) or 40 days (Asia Minor bluegrass) at 26°C. Sorghum was grown in plastic pots filled with Sakata Primemix soil (Sakata) in a growth chamber with a 12-h photoperiod of fluorescent lighting for 10 days at 26°C.

Mycelial plugs of fresh slant cultures (3 weeks to 3 months old) were transferred to oatmeal agar plates (oatmeal, 20 g; sucrose,

Fig. 2. Symptoms on various gramineous plants, 7 days after inoculation with *Colletotrichum* isolates. Cgs61, *Sorghum* isolate; Cgd14, *Digitaria* isolate; Cgp29, *Polypogon* isolate; Cga71, *Avena* isolate.

TABLE 1. *Colletotrichum* isolates used in this study

Species	Isolate ^a	Original host	Geographic origin	Year	Collector
<i>Colletotrichum sublineolum</i>	Cgs61 (MAFF 305361)	<i>Sorghum bicolor</i>	Chiba, Japan	1957	N. Nishihara
<i>C. hanaui</i>	Cgd14 (MAFF 511014)	<i>Digitaria ciliaris</i>	Tochigi, Japan	1975	N. Nishihara
<i>C. cereale</i>	Cgp29 (MAFF 305429)	<i>Polypogon fugax</i>	Saga, Japan	1977	N. Nishihara
<i>C. cereale</i>	Cga71 (MAFF 305371)	<i>Avena sativa</i>	Saga, Japan	1966	N. Nishihara

^a Provided by Microorganisms Section of the NIAS Genebank, National Institute of Agrobiological Sciences, Tsukuba, Japan (MAFF). Accession numbers in MAFF are shown in parentheses.

10 g; and agar, 10 to 15 g in 500 ml of distilled water) and incubated at 22°C for 10 days. Aerial mycelia on the 10-day-old cultures were washed off by rubbing mycelial surfaces with cotton balls. The colonies were exposed to near-ultraviolet light (360 nm, 40 W) at 22°C for 5 days to induce sporulation. The conidia produced were suspended in water and adjusted to a concentration of 1 to 2 × 10⁶ spores/ml. Conidial suspensions with 0.01% Tween 20 were sprayed on the seedlings mentioned above with an air compressor. The inoculated plants were incubated in a dark, humid box at 26°C for 24 h. Then, they were transferred to a growth chamber controlled at 26°C with a 12-h photoperiod. Seven days after inoculation, symptoms were evaluated based on affected area on leaves. The affected area was rated using six progressive grades from 0 (no visible lesion) to 5 (affected area >80% of the whole leaf) (Fig. 1). Each infection assay was repeated at least twice.

Cytological analysis. The conidial suspensions were sprayed on the adaxial surface of 7- to 9-day-old wheat primary leaves. The inoculated leaves were harvested 48 h after inoculation, then

Fig. 3. Segregation in an F₂ population derived from resistant 'Shin-chunaga' (Sch) × susceptible 'Hope', 7 days after inoculation with *Colletotrichum cereale* Cgp29. Red and white arrowheads indicate susceptible (type 4 or 5) and intermediate (type 3) seedlings, respectively.

fixed by boiling in alcoholic lactophenol (lactic acid/phenol/glycerol/distilled water/ethanol, 1:1:1:1:8, vol/vol/vol/vol/vol) for 2 min. The specimens were mounted on a slide glass in 50% (vol/vol) glycerol and observed with an Olympus BX51 microscope (Olympus, Tokyo) with an exciter filter B. In all, ≈300 cells with appressoria on three leaves were observed in each replication. Infection efficiency was evaluated on the basis of penetration ratios [(cells with infection hyphae/cells with appressoria) × 100] and hypersensitive reaction (HR) ratios [(cells with HR/cells with infection hyphae) × 100].

H₂O₂ accumulation was visualized with 3,3-diaminobenzidine (DAB) staining (16). Inoculated primary leaves were harvested 48 h after inoculation, dipped in DAB (Nacalai tesque, Kyoto, Japan) solution (1 mg/ml), and vacuum-infiltrated for 5 min three times. They were incubated further for 8 h at room temperature in

Fig. 5. Linkage map of the 5A chromosome constructed using F₃ lines derived from resistant 'Norin 4' (N4) × susceptible 'Hope' and F₂ populations derived from resistant 'Chinese Spring' (CS) × Hope and resistant 'Shin-chunaga' (Sch) × Hope.

Fig. 4. Responses of the 'Chinese Spring' (CS)-'Hope' chromosome substitution lines to *Colletotrichum cereale* Cgp29, 7 days after inoculation. Each line is represented by two leaves. Names of substituted chromosomes are shown above the leaf pairs.

TABLE 2. Reactions of wheat lines against *Colletotrichum* isolates

Code	Species ^a	Cultivar or accession	Genomes	Infection type with ^b			
				Cgs61	Cgd14	Cgp29	Cga71
1502	<i>Triticum aestivum</i>	Strain P168	ABD	0	0	0	1
1503	<i>T. aestivum</i>	Chinese Spring	ABD	0	0	0	0
1504	<i>T. aestivum</i>	Norin 26	ABD	0	0	0	1
1505	<i>T. aestivum</i>	Strain Salmon	ABD	0	0	0	0
1506	<i>T. aestivum</i>	Jones Fife	ABD	0	0	0	0
1507	<i>T. aestivum</i>	Selkirk	ABD	0	0	1	0
1508	<i>T. aestivum</i>	S615	ABD	0	0	0	0
1513	<i>T. aestivum</i>	Norin 29	ABD	0	0	0	0
1515	<i>T. aestivum</i>	Shin-chunaga	ABD	0	0	0	0
1520	<i>T. aestivum</i>	Norin 10	ABD	0	0	0	0
1521	<i>T. aestivum</i>	Norin 4	ABD	0	0	0	0
1601	<i>T. aestivum</i>	Hope	ABD	0	0	5	0
1602	<i>T. aestivum</i>	Timstein	ABD	0	0	0	1
1603	<i>T. aestivum</i>	Cheyenne	ABD	0	0	0	0
1835	<i>T. aestivum</i>	Red Egyptian	ABD	0	0	0	0
1857	<i>T. aestivum</i>	Thatcher	ABD	0	0	0	0
H-1	<i>T. aestivum</i>	Chancellor	ABD	0	0	0	0
H-11	<i>T. aestivum</i>	Renown	ABD	0	0	0	0
H-13	<i>T. aestivum</i>	Spica	ABD	0	0	0	0
H-14	<i>T. aestivum</i>	Mendos	ABD	0	0	0	1
H-15	<i>T. aestivum</i>	Timvera	ABD	0	0	0	0
H-16	<i>T. aestivum</i>	Transec	ABD	0	0	0	0
H-17	<i>T. aestivum</i>	Transfed	ABD	0	0	0	0
H-18	<i>T. aestivum</i>	Kavkaz	ABD	0	0	0	0
H-20	<i>T. aestivum</i>	Normandie	ABD	0	0	0	0
H-21	<i>T. aestivum</i>	Little Club	ABD	0	0	0	0
H-22	<i>T. aestivum</i>	Hard Federation	ABD	0	0	0	1
H-23	<i>T. aestivum</i>	Ramona 50	ABD	0	0	0	0
H-24	<i>T. aestivum</i>	Mayo 64	ABD	0	0	0	0
Tat-17	<i>T. aestivum</i>	KU163	ABD	0	0	0	0
1509	<i>T. sphaerococcum</i>	KU161	ABD	0	0	0	0
1510	<i>T. compactum</i>	KU151	ABD	0	0	0	0

(continued on next page)

^a *T. aestivum* L. (*T. aestivum* L. subsp. *aestivum*), *T. sphaerococcum* Perc. (*T. aestivum* L. subsp. *sphaerococcum* (Perc.) MacKey), *T. compactum* Host (*T. aestivum* L. subsp. *compactum* (Host) MacKey), *T. spelta* L. (*T. aestivum* L. subsp. *spelta* (L.) Thell.), *T. macha* Dek. & Men. (*T. aestivum* L. subsp. *macha* (Dek. & Men.) MacKey), *T. carthlicum* Nevski (*T. turgidum* L. subsp. *carthlicum* (Nevski in Kom.) Á. Löve & D. Löve.), *T. dicoccoides* Körn (*T. turgidum* L. subsp. *dicoccoides* (Körn. ex Asch. & Graebner) Thell.), *T. dicoccum* Schübl. (*T. turgidum* L. subsp. *dicoccum* (Schränk ex Schübler) Thell.), *T. paleocolchicum* Men. (*T. turgidum* L. subsp. *georgicum* (Dekapr. & Menabde) MacKey), *T. durum* Desf. (*T. turgidum* L. subsp. *turgidum* conv. *durum* (Desf.) MacKey), *T. orientale* Perc. (*T. turgidum* L. subsp. *turgidum* conv. *turancium* (Jakubz.) MacKey), *T. polonicum* L. (*T. turgidum* L. subsp. *turgidum* conv. *polonicum* (L.) MacKey), *T. turgidum* L. (*T. turgidum* L. subsp. *turgidum* conv. *turgidum*).

^b Cgs61, *Sorghum* isolate; Cgd14, *Digitaria* isolate; Cgp29, *Polypogon* isolate; Cga71, *Avena* isolate.

the dark, then fixed in a mixture of ethanol and acetic acid (96:4, vol/vol) overnight. The specimens were mounted on a slide glass in 50% glycerol and observed with the microscope.

Cell death was visualized with trypan blue staining (24). Inoculated primary leaves were harvested 48 h after inoculation and incubated in alcoholic lactophenol at room temperature overnight. The specimens were boiled in lactophenol solution containing trypan blue (Nacalai tesque) at 250 µg/ml for 2 min, allowed to cool for 1 h, and mounted on a slide glass in 50% (vol/vol) glycerol for microscopy.

Different leaves were employed for the fluorescence microscopy, DAB staining, and trypan blue staining. These experiments were repeated three times.

Molecular mapping. To map *Rcc1*, 85 F₂ plants derived from N4 × Hope were grown in a field and selfed. From each of the resulting 85 F₃ lines, a portion (20 seeds) was retrieved and used for infection assay. Based on these F₃ reactions, genotypes of the F₂ plants at the *Rcc1* locus were determined. Another portion (20 seeds) was retrieved from each of the 85 F₃ lines, grown at 22°C for 7 days, and pooled for DNA extraction. Genomic DNA was extracted from the pooled primary leaves following the procedure of Appels and Moran (1), with the modification by Koebner et al. (13), and used as a sample equivalent to the genomic DNA of the parental F₂ plant.

F₂ populations derived from CS × Hope and Sch × Hope were also employed for mapping. From each population, 93 F₂ seeds

were retrieved and grown at 22°C for 7 to 9 days. Conidial suspensions were sprayed onto the upper portion of the 93 primary leaves. The inoculated seedling were grown for an additional 7 days and scored with the 0 to 5 infection types. Simultaneously, secondary leaves were harvested and used for DNA extraction individually.

Simple sequence repeat (SSR) loci on wheat 5A chromosome were chosen from those reported by Somers et al. (17) and Torada et al. (19), and amplified from genomic DNA of the F₃ lines or F₂ individuals. Polymerase chain reaction products were fractionated by electrophoresis through 2% agarose gels, 13% nondenaturing polyacrylamide gels, or 6% denaturing polyacrylamide gels and visualized by ethidium bromide staining or silver staining. For polyacrylamide gel electrophoresis, the High-Efficiency Genome Scanning system (Nippon Eido, Tokyo) (11) was used. Segregation data were analyzed using MAPMAKER Macintosh V2.0 with the F₂ intercross program. Thresholds of linkage for map construction were a minimum log of the likelihood ratio of 4.0 and a maximum recombination fraction of 0.4. The Kosambi mapping function was employed to compute map distances in centimorgans.

RESULTS

Pathogenicity of *Colletotrichum* isolates. Pathogenicity of the four *Colletotrichum* isolates (Table 1) was checked on four

TABLE 2. (continued from preceding page)

Code	Species ^a	Cultivar or accession	Genomes	Infection type with ^b			
				Cgs61	Cgd14	Cgp29	Cga71
Tat-12	<i>T. compactum</i>	KU150	ABD	0	0	0	1
1511	<i>T. spelta</i>	KU157	ABD	0	0	0	0
1512	<i>T. macha</i>	KU154	ABD	0	0	0	0
Tat-13	<i>T. macha</i>	KU155	ABD	0	0	0	0
Tat-19	<i>T. vavilovii</i>	KU192	ABD	0	0	0	0
St-55	<i>T. carthlicum</i>	KU140	ABD	0	0	0	0
St-11	<i>T. dicoccoides</i>	KU108-1	AB	0	0	0	1
St-12	<i>T. dicoccoides</i>	KU109	AB	0	0	0	3
St-13	<i>T. dicoccoides</i>	KU110	AB	0	0	0	1
St-14	<i>T. dicoccoides</i>	KU195	AB	0	0	0	1
St-15	<i>T. dicoccoides</i>	KU198	AB	0	0	0	0
St-16	<i>T. dicoccum</i>	KU111	AB	0	0	0	3
St-17	<i>T. dicoccum</i>	KU112	AB	0	0	0	4
St-18	<i>T. dicoccum</i>	KU113	AB	0	0	0	2
St-19	<i>T. dicoccum</i>	KU114	AB	0	0	0	0
St-24	<i>T. dicoccum</i>	KU120	AB	0	0	0	3
St-25	<i>T. dicoccum</i>	KU122	AB	0	0	0	3
St-27	<i>T. dicoccum</i>	KU124	AB	0	0	0	0
St-28	<i>T. dicoccum</i>	KU189	AB	0	0	4	0
Tat-14	<i>T. paleocolchicum</i>	KU156	AB	0	0	0	5
St-29	<i>T. paleocolchicum</i>	KU190-1	AB	0	0	0	4
St-30	<i>T. paleocolchicum</i>	KU191	AB	0	0	0	5
Tat-6	<i>T. durum</i>	KU125	AB	0	0	0	0
St-31	<i>T. durum</i>	KU126	AB	0	0	0	0
St-35	<i>T. durum</i>	KU130	AB	0	0	0	0
St-36	<i>T. durum</i>	KU132	AB	0	0	0	2
St-38	<i>T. durum</i>	KU136	AB	0	0	0	1
St-39	<i>T. durum</i>	KU301	AB	0	0	0	0
St-41	<i>T. durum</i>	KU329	AB	0	0	0	0
St-42	<i>T. durum</i>	KU409	AB	0	0	0	0
St-43	<i>T. durum</i>	KU459	AB	0	0	0	0
3020	<i>T. durum</i>	Langdon	AB	0	0	0	0
Tat-7	<i>T. orientale</i>	KU137	AB	0	0	0	1
Tat-9	<i>T. polonicum</i>	KU141	AB	0	0	0	1
St-49	<i>T. polonicum</i>	KU143	AB	0	0	0	0
St-50	<i>T. polonicum</i>	KU144	AB	0	0	0	0
Tat-18	<i>T. abyssinicum</i>	KU185	AB	0	0	0	0
St-57	<i>T. abyssinicum</i>	KU186	AB	0	0	0	1
Tat-10	<i>T. pyramidale</i>	KU146	AB	0	0	0	5
Tat-11	<i>T. turgidum</i>	KU147	AB	0	0	0	2

TABLE 3. Distribution of infection types with *Colletotrichum cereale* Cgp29 in F₂ populations derived from crosses between wheat cultivars

Cross ^a	Number of F ₂ seedlings per infection type (0 to 5)							Segregation analysis 1 ^b				Segregation analysis 2 ^c			
	0	1	2	3	4	5	Total	0–3	4–5	χ^2	<i>P</i>	0–2	3–5	χ^2	<i>P</i>
N4 × Hope	15	73	32	6	14	30	170	126	44	0.07	0.79	120	50	1.77	0.18
CS × Hope	5	42	7	8	6	12	80	62	18	0.27	0.61	54	26	2.40	0.12
Sch × Hope	25	41	13	7	8	20	114	86	28	0.01	0.91	79	35	1.98	0.16
N4 × CS	242	0	0	0	0	0	242	242	0	...	...	242	0	...	...
N4 × Sch	199	0	0	0	0	0	199	199	0	...	...	199	0	...	...
CS × Sch	192	7	2	0	0	0	201	201	0	...	...	201	0	...	...

^a ‘Norin 4’ (N4), ‘Hope’, ‘Chinese Spring’ (CS), and ‘Shin-chunaga’ (Sch) wheat.^b Resistant, infection type 0 to 3; susceptible, infection type 4 to 5; χ^2 (3:1).^c Resistant, infection type 0 to 2; susceptible, infection type 3 to 5; χ^2 (3:1).

gramineous plants. *Sorghum* isolate Cgs61, *Digitaria* isolate Cgd14, *Polypogon* isolate Cgp29, and *Avena* isolate Cga71 were highly virulent on sorghum, crabgrass, Asia Minor bluegrass, and oat, respectively (i.e., the plants from which they were isolated) (Fig. 2), but were avirulent on the other plant species. Several small lesions were observed on sorghum leaves inoculated with Cgd14 and crabgrass leaves inoculated with Cgs61 but did not expand further, suggesting that fungal development is restricted by resistance responses in these interactions.

Reactions of wheat and barley to *Colletotrichum* isolates. To examine interactions between these *Colletotrichum* isolates and staple crops, seedlings of 23 barley lines and 72 wheat lines were inoculated with the four *Colletotrichum* isolates. All barley lines were highly resistant (infection type 0 or 1) to the four isolates

(Supplemental Table 1), suggesting that barley is a nonhost for these isolates.

On the other hand, interactions between wheat and the four *Colletotrichum* isolates were not uniform. Against Cgs61 and Cgd14, all wheat lines were highly resistant (Table 2). Against Cgp29, most of the wheat lines were highly resistant, while two lines, hexaploid wheat Hope and tetraploid wheat accession St-28, were highly susceptible (infection type 4 or 5) (Table 2). Against Cga71, all the hexaploid wheat lines were highly resistant, while the reactions of the tetraploid wheat lines varied from highly resistant through highly susceptible (Table 2).

Segregation analysis with F₂ seedlings. To clarify mechanisms controlling the resistance of wheat cultivars against Cgp29, we performed genetic analysis using three resistant hexaploid

wheat cultivars (N4, CS, and Sch) and the susceptible Hope. When F_2 seedlings derived from N4 $\times$ Hope, CS $\times$ Hope, and Sch $\times$ Hope were inoculated with Cgp29, infection types showed distributions with two peaks at types 1 and 5 (Table 3). Among these populations, seedlings with types 4 and 5 were easily discriminated from others, including those with the intermediate type 3 (Fig. 3). When type 3 was grouped as resistant, resistant and susceptible seedlings segregated in 3:1 ratios (Table 3). Even when type 3 was grouped as susceptible, the segregation was not

significantly different from 3:1 ratios (Table 3). These results suggest that the resistance of each cultivar to Cgp29 is controlled by a major gene. Crosses between the three resistant cultivars yielded no susceptible seedlings (Table 3), suggesting that the resistance of these three cultivars is controlled by the same gene. This gene was designated as *Rcc1*.

Molecular mapping of *Rcc1*. To identify the chromosome carrying *Rcc1*, CS–Hope chromosome substitution lines were inoculated with Cgp29. The 5AH line, which carries the 5A

Fig. 6. Cytological responses of resistant 'Norin 4' (N4) and susceptible 'Hope' to *Colletotrichum cereale* Cgp29, 48 h after inoculation. **A**, Infection hyphae observed under the blight field. **B**, Fluorescence in epidermal cells detected under the dark field. **C**, H₂O₂ accumulation detected with 3,3-diaminobenzidine staining. **D**, Cell death detected with trypan blue staining. Ap, Appressorium; Ih, Infection hypha; Bars = 10 μ m. **E**, Penetration ratio [(cells with infection hyphae/cells with appressoria) $\times$ 100]. **F**, Hypersensitive reaction (HR) ratio [(cells with HR/cells with infection hyphae) $\times$ 100]. **E and F**, Averages and standard errors from three replications are shown. Double asterisks (**) indicate significant difference at $P < 0.01$ (Student's t test).

chromosome of Hope and the other 20 chromosomes of CS, was susceptible, whereas the other 20 lines were resistant (Fig. 4). This result indicates that *Rcc1* is located on the 5A chromosome.

To map the *Rcc1* locus, 85 F₃ lines derived from N4 × Hope were inoculated with Cgp29. All resistant, segregating, and all susceptible F₃ lines were 21, 43, and 21, fitting a 1:2:1 ratio ($\chi^2 = 0.01$, $P = 0.99$) and confirming that the resistance of N4 is conditioned by a single gene. When molecular data were combined with the inoculation data, the *Rcc1* locus was mapped on the long arm of chromosome 5A (Fig. 5). *Rcc1* was also mapped at the same position in the F₂ populations derived from CS × Hope and Sch × Hope (Fig. 5), as expected.

Cytological responses associated with *Rcc1*. To characterize cytological responses associated with *Rcc1*, primary leaves of N4 and Hope were inoculated with Cgp29, fixed at 48 h after inoculation, and observed under a fluorescence microscope. In resistant N4, hyphal growth was restricted (Fig. 6A) by HR of epidermal cells. These cells were accompanied by yellow fluorescence of cell walls and cytoplasm (Fig. 6B). They were positively stained with DAB and trypan blue, suggesting that they are dying as a result of H₂O₂ accumulation (Fig. 6C and D).

The penetration ratio (percentage of epidermal cells penetrated by appressoria against those associated with appressoria) was not significantly different between the resistant and susceptible cultivars (Fig. 6E). By contrast, the HR ratio in the penetrated epidermal cells (percentage of epidermal cells with HR against those penetrated by appressoria) was significantly higher in N4 (41.3%) compared with Hope (5.8%) (Fig. 6F).

DISCUSSION

In the present study, host species specificity was recognized between graminicolous *Colletotrichum* spp. and gramineous plants; that is, the four *Colletotrichum* isolates were exclusively virulent on their original hosts (Fig. 1). It should be noted that the specific parasitism was recognized even within a species, *C. cereale*. The *Polypogon* isolate of *C. cereale* (Cgp29) was virulent on Asia Minor bluegrass but avirulent on oat while the *Avena* isolate (Cga71) was virulent on oat but avirulent on Asia Minor bluegrass. Given this specificity, wheat was expected to be a nonhost to these isolates. However, the interactions between wheat and these isolates contained some exceptions (Table 2); all wheat lines were highly resistant to Cgs61 (*C. sublineolum*) and Cgd14 (*C. hanaui*), whereas some susceptible wheat lines were observed against Cgp29 and Cga71. This seems reasonable because Cgp29 and Cga71 belong to *C. cereale*, the same species as wheat-infecting *Colletotrichum* isolates. Cgp29 and Cga71 seem to retain basic compatibility with wheat because they are virulent on at least one wheat line.

The susceptible Hope made it possible to identify a major resistance gene, *Rcc1*, which controls the resistance of common wheat against Cgp29, a *Polypogon* isolate of *C. cereale*. Three common wheat cultivars subjected to genetic analysis (N4, CS, and Sch) shared the same resistance gene, *Rcc1*, against Cgp29 (Table 3). The frequency distributions of infection types in the F₂ populations derived from the resistant cultivars and Hope suggest that *Rcc1* is incompletely dominant (Table 3). However, homozygous *Rcc1* (*Rcc1/Rcc1*) appears to be almost sufficient to confer complete resistance to Cgp29 because most of the F₂ seedlings derived from crosses among the resistant cultivars were completely resistant (Table 3). When cytologically analyzed, the HR ratio of penetrated epidermal cells was significantly higher in the resistant cultivar compared with that in the susceptible cultivar (Fig. 6F). This indicates that *Rcc1* is mainly associated with HR. Taken together, we suggest that the resistance of wheat to Cgp29 is mainly conditioned by a major gene or genes similar to those involved in cultivar specificity. This may be one example of host

species specificity conditioned by major genes, as exemplified in *Blumeria graminis* (20,22) and *Magnaporthe oryzae* (18) systems.

At present, it is not possible to examine whether *Rcc1* is effective against another *C. cereale* isolate, Cga71, because all common wheat lines tested were resistant to Cga71. Further screening is needed to find an exceptional common wheat line that is susceptible to Cga71. On the other hand, it seems possible to analyze such interrelationships by using tetraploid wheat. An tetraploid accession (St-28) is susceptible to Cgp29 and resistant to Cga71 whereas some other accessions (St-17, St-29, St-30, Tat-10, and Tat-14) are susceptible to Cga71 and resistant to Cgp29 (Table 2). These patterns look similar to cultivar specificity conditioned by various different resistance genes. Genetic crosses among these accessions are underway for analyzing their F₂ and F₃ populations.

New pathogens are still evolving in some fungal species. In the 1980s, severe blast disease occurred on wheat in Brazil and then spread to other countries in South America (23). Its causal agent was proved to be a new subgroup of *M. oryzae*. In the 1990s, a severe blast disease (designated gray leaf spot) occurred on perennial ryegrass in the United States (9,14) and Japan (21). Its causal agents were proved to contain another new subgroup of *M. oryzae* (21). Recently, wheat blast was found in Kentucky. Its causal agent was very similar to the annual ryegrass pathogen and different from the wheat pathogen prevailing in South America, suggesting a host jump of *M. oryzae* from annual ryegrass to wheat (<http://news.ca.uky.edu/article/uk-researchers-find-important-new-disease>). The present study showed that the resistance of some wheat cultivars to a *Polypogon* isolate of *C. cereale* is conditioned by a rather simple genetic mechanism. If the resistance gene is under the control of gene-for-gene interactions, it may not be impossible for the weed isolate of *C. cereale* to jump hosts to wheat.

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