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FULL PAPER

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Biological control of Fusarium wilt of tomato with hypovirulent binucleate *Rhizoctonia* in greenhouse conditions

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Abstract Four isolates of hypovirulent binucleate *Rhizoctonia* (HBNR), G1, L2, W1, and W7 were used for control of Fusarium wilt of tomato (FWT) caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL). HBNR isolates could significantly reduce disease severity (foliar symptoms and discoloration inside the stem) among five experiments under single and double applications. Reduction of disease severity by HBNR isolates, however, differed depending on HBNR isolates and treatments. Of four isolates, isolate HBNR W7 could significantly and consistently reduce ($P = 0.05$) disease severity. Application of HBNR isolates significantly reduced ($P = 0.01$) the number of colony-forming units of FOL in stems and roots of tomato. Among the HBNR isolates, G1, W1, and W7 could significantly increase ($P = 0.05$) fresh weight of the plants (stems and leaves). These results indicate that isolates of HBNR have a greater potential as biocontrol agents against FWT. This is the first report of biocontrol of FWT by HBNR under greenhouse conditions.

Key words Biocontrol · *Fusarium oxysporum* f. sp. *lycopersici* · Hypovirulent binucleate *Rhizoctonia* · Tomato

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Introduction

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL), is one of the most destructive and economically damaging diseases of tomato. It causes stunted seedlings or yellowing and defoliation of older leaves, and the infected plants frequently wilt and die (Jones et al. 1991). Although the use of Fusarium-resistant cultivars of tomato can provide some degree of control against the disease, the emergence of new races of the pathogen that overcome resistance to the cultivars is a continuing problem. Currently there are no highly resistant cultivars of tomato to FOL (Jones and Crill 1974). Chemical control of the disease is not satisfactory, and biological control has shown potential as an alternative disease management strategy of the disease (Lemanceau and Alabouvette 1993; De Cal et al. 1995; Larkin and Fravel 1998).

A variety of soil microorganisms have demonstrated their potential as biocontrol agents against various Fusarium diseases including Fusarium wilt (Ogawa and Komada 1984; Locke et al. 1985; Paulitz et al. 1987; Amemiya et al. 1989; Yamaguchi et al. 1992; Larkin et al. 1996; Larkin and Fravel 1998; Singh et al. 1999; Bapat and Shah 2000). On the other hand, several authors have reported that hypovirulent binucleate *Rhizoctonia* (HBNR) effectively controlled Rhizoctonia diseases in several plants (Burpee and Goulty 1984; Cardoso and Echandi 1987a; Ichielevich-Auster et al. 1985; Villajuan-Abgona et al. 1996; Pascual et al. 2000). HBNR is also reported to control diseases caused by other pathogens such as damping-off disease caused by *Pythium ultimum* (Harris et al. 1993) and black-shank disease caused by *Phytopththora parasitica* var *nicotianae* (Cartwright and Spurr 1998). However, as far as we know, there is no report on the use of HBNR against Fusarium wilt of tomato (FWT) and Fusarium diseases of other host plants, except one that stated that HBNR could reduce Fusarium crown and root rot of tomato caused by *F. oxysporum* f. sp. *radicis-lycopersici* (Louter and Edgington 1990).

Colonization of host tissues is an important factor for crop protection with HBNR (Cardoso and Echandi 1987b) because it triggers production of host defense compounds such as peroxidases, glucanases, and chitinases (Xue et al. 1998). In addition, HBNR can colonize target areas of pathogen attack such as lateral roots, taproots, and basal hypocotyls (Villajuan-Abgona 1995), and survive inside roots and in the soil for a long time (Summer and Bell 1994; Cubeta et al. 1991). Furthermore, some HBNR isolates were reported to stimulate plant growth (Sneh et al. 1986; Harris et al. 1994; Villajuan-Abgona et al. 1996), and such plant growth promotion ability was partly attributed to their disease suppression ability (Hyakumachi 2002). As HBNR has these advantages as biocontrol agent, it is interested to know whether HBNR could also suppress Fusarium wilt diseases, which are known to be difficult to control.

In this study, we evaluated the ability of HBNR to protect tomato plants against Fusarium wilt and their effects in reducing population of pathogen in roots and stems. The efficacy of HBNR isolates was compared with nonpathogenic *F. oxysporum*, which is effective in controlling Fusarium crown and root rot of tomato (Komada et al. 1996). In addition, the effect of HBNR on growth promotion of tomato was examined. Part of the results of suppressing of Fusarium wilt of tomato using HBNR isolates has been reported previously (Muslim et al. 2000).

Materials and methods

Fungi

Isolates of hypovirulent binucleate *Rhizoctonia* (HBNR): G1 (unknown anastomosis group), L1 (AG-Ba), W1, and W7 (AG-A), which were obtained from soil samples collected in Gifu Prefecture, were used as biocontrol agents. An isolate of nonpathogenic *F. oxysporum* (NPF) isolate F13 (provided by Dr. H. Komada) was also used for the purpose of comparison. *F. oxysporum* f. sp. *lycopersici* race 2 (FOL) isolate Ku11 obtained from an infected tomato plant was used as the pathogen.

Plant

Tomato cv. "House Momotaro," a popular cultivar in Japan and susceptible to FWT, was used throughout the experiments. All seeds were surface-sterilized with 1% hydrochloric acid for 15 min and rinsed three times in sterile distilled water before sowing.

Inoculum preparation

HBNR

Each isolate of HBNR was cultured on potato dextrose agar (PDA) in 9-cm Petri dishes for 3 days at 25°C in the dark. Five mycelial disks (5 mm diameter) of the isolates cut from the edges of 3-day-old cultures were added to 100 g moist autoclaved barley grain (1:1, dry barley grain/distilled water, w/v) contained in a 500-ml Erlenmeyer flask. The barley grain was autoclaved at 121°C for 20 min. The cultures were incubated in the dark for 10 days at 25°C and shaken by hand every day to aid even colonization. The colonized barley grain was air-dried at laboratory temperature (23°–25°C) for 7 days and stored at 4°C until use.

Fusarium

The isolates of NPF F13 and FOL Ku11 were grown on PDA for 3 days in the dark at 25°C. Three mycelial disks (5 mm diameter) of the isolates were transferred in 100 ml potato dextrose broth in a 500-ml flask and incubated for 7 days at 25°C with shaking (115 rpm). To obtain microconidial inocula of NPF F13 and FOL Ku11, the fungal cultures were filtered through eight layers of sterile gauze. The fungal suspensions were then diluted with sterile distilled water and used as inocula sources. Barley grain inoculum of NPF F13, prepared as described previously, was also prepared for inoculation in a double application.

Assay of HBNR for control of Fusarium wilt of tomato

Effect of single application of HBNR

The HBNR-colonized grain was pulverized in a blender for approximately 30 s (1–2 mm particle size) and mixed with potting medium (2%, w/w) "Star Bed" (JA Zennoh, Tokyo, Japan), which consists of humus, peat, rock phosphate, and composted plant material. The nitrogen, phosphorus (P_2O_5), and potassim (K_2O) content of the potting medium was 200:1500:200 $mg\,l^{-1}$. Paper pot set (6.5 cm depth × 1.5 cm diameter/pot), manufactured by Nippon Beet Sugar (Kyushu, Japan), was filled with approximately 16 g potting medium. The potting medium was amended with either HBNR (2% w/w, ground barley grain inoculum) or NPF suspension (20 ml, 3×10^7 spores/ml). One disinfested tomato seed was sown in each pot. The seedlings were allowed to grow for 21 days at 25°C in a growth chamber with a 10- and 14-h light (24 000 lux)/dark period.

Three experiments were carried out during the year of 1999 and 2000. Twenty-one-day-old tomato seedlings grown in paper pots with the HBNR- or NPF-amended soils were transferred to plastic pots (7 × 8 × 7 cm) containing 200 g FOL-infested potting medium. The concentration of FOL was adjusted to 10^5 spores/g soil. The plants were kept in a greenhouse for 42–56 days at 25°–30°C. Seedlings not treated with HBNR or NPF F13 and challenged or not challenged with FOL were set up as controls. For each treatment, four replicates were made and each replicate consisted of four plants. Foliar symptoms severity was assessed using a scale of 0 to 4: 0 = healthy; 1 = yellowing; 2 = slight wilting; 3 = severe wilting; 4 = dead. Discoloration severity was assessed as index of ratio of the browning area of vascular tissue, cortex, and xylem at the bottom of the tomato stem to the whole area of the stem using a scale of 0

to 3 : 0 = healthy (no vascular discoloration); 1 = ≤1/3; 2 = >1/3–2/3; 3 = >2/3. Percent reduction was calculated as % reduction = (foliar symptoms or discoloration severities of pathogen treatment − foliar symptoms or discoloration severities of HBNR or NPF treatments)/(foliar symptoms or discoloration severities of pathogen treatment) × 100.

Effect of double application of HBNR

Two experiments were carried out during the year of 2000 and 2001. Twenty-one-day-old tomato seedlings grown in paper pots with the HBNR- or NPF-amended soil (2% w/w ground barley grain inoculum) were transferred to plastic pots (7 × 8 × 7 cm) containing 200 g potting medium. Before transplanting, the potting medium was amended with HBNR or NPF isolates (1% w/w ground barley grain inoculum). Twenty-four hours later, the spore suspension of FOL was inoculated in the potting medium at the concentration of 10^5 spores/g soil. The plants were kept in a greenhouse for 49–63 days at 25°–30°C. Seedlings not treated with HBNR or NPF F13 and challenged or not challenged with FOL were set up as controls. The treatments and the control plants consisted of four replicates with four plants per replicate. Foliar symptoms and discoloration severities were assessed and recorded as described previously.

Pathogen population in stems and roots

Populations of FOL in the stems and roots with different scores of discoloration severity were estimated at the end of experiment 1 in the double application. The stems (0–20 cm above soil surface) and whole roots from the plants with each score of 16 plants were separately washed with running tap water to remove adhering soil and were combined. The stems and roots were added with sterile distilled water (1 : 10 w/v) and homogenized using a blender (Type H; Teraoka Toyo Keisokuki, Osaka, Japan) at 8000 rpm for 5–7 min. The homogenized stems and roots were filtered through two layers of gauze, diluted 10- to 100 fold, and plated on Komada's selective medium (Komada 1975). The number of colony-forming units of FOL per gram of fresh weight of the stems and roots with different scores of discoloration severity were counted. Populations of FOL in soil were also estimated at the end of experiment 1 of double application. The soil was collected from each pot in each treatment and combined. The combined soil was diluted 10- to 1000 fold and plated on Komada's selective medium. The experiments were replicated six times for each sample.

Reisolation of HBNR from stems and roots

Reisolation of HBNR from stems was recorded at the end of experiment 2 in the single application and experiment 2 in double application. All plants treated with HBNR were examined. The stems were washed with running tap water, cut into 2- to 3-mm sections at 1, 5, 10, 15, and 20 cm above soil surface, and then rinsed three times in sterile distilled water. The stem sections (four replicates; each replicate consisted of four stem sections) in each position were placed on acidified water agar (pH 4.5). After 48 h incubation at 25°C, fungal growth on the samples was examined visually and microscopically. The presence of HBNR was confirmed by its characteristic growth and the morphology of its mycelium. Reisolation of HBNR from roots was also recorded at the end of experiment 2 in the single application and experiment 2 in double application. Four plants were randomly sampled from each treatment. The roots were cut off into two parts, inside paper pot and outside paper pot. The roots from each part were washed with running tap water, cut into 1-cm sections, and combined. Combined sections were thoroughly stirred and rinsed three times in sterile distilled water. Ten root sections were randomly selected, blotted dry, and placed on acidified water agar (pH 4.5) for checking the presence of HBNR using the same procedure as described earlier. The experiment was replicated six times; each replication consisted of ten root sections.

Effect of HBNR on the growth promotion

The seedlings (21-day-old) grown in the paper pot containing potting medium and amended with HBNR (2%, w/w), were transferred to pots containing potting medium only and then allowed to grow for another 42–56 days. The seedlings treated with barley grain only were set up as a control. At the end of the experiment, the stems and leaves were harvested and weighed. The treated and the control plants consisted of four replicates with four plants per replicate. The experiment was repeated two times.

Data analysis

The experiments were carried out in randomized block design. Treatment means obtained for severity of foliar symptoms and discoloration inside the stem, population of pathogen, and fresh weight of stems and leaves were compared using Fisher's least significant difference (LSD) test at $P = 0.05$ and $P = 0.01$.

Results

Assay of HBNR for control of Fusarium wilt of tomato

Effect of single application

On the whole, HBNR isolates significantly reduced foliar symptoms severity among the three experiments. Reduction of the foliar symptoms by HBNR isolates, however, differed depending on HBNR isolates and treatments. In all three experiments, HBNR W1 and W7 isolate could significantly and constantly reduce the foliar symptoms (Fig. 1A–C). NPF F13 could not reduce foliar symptom severity in

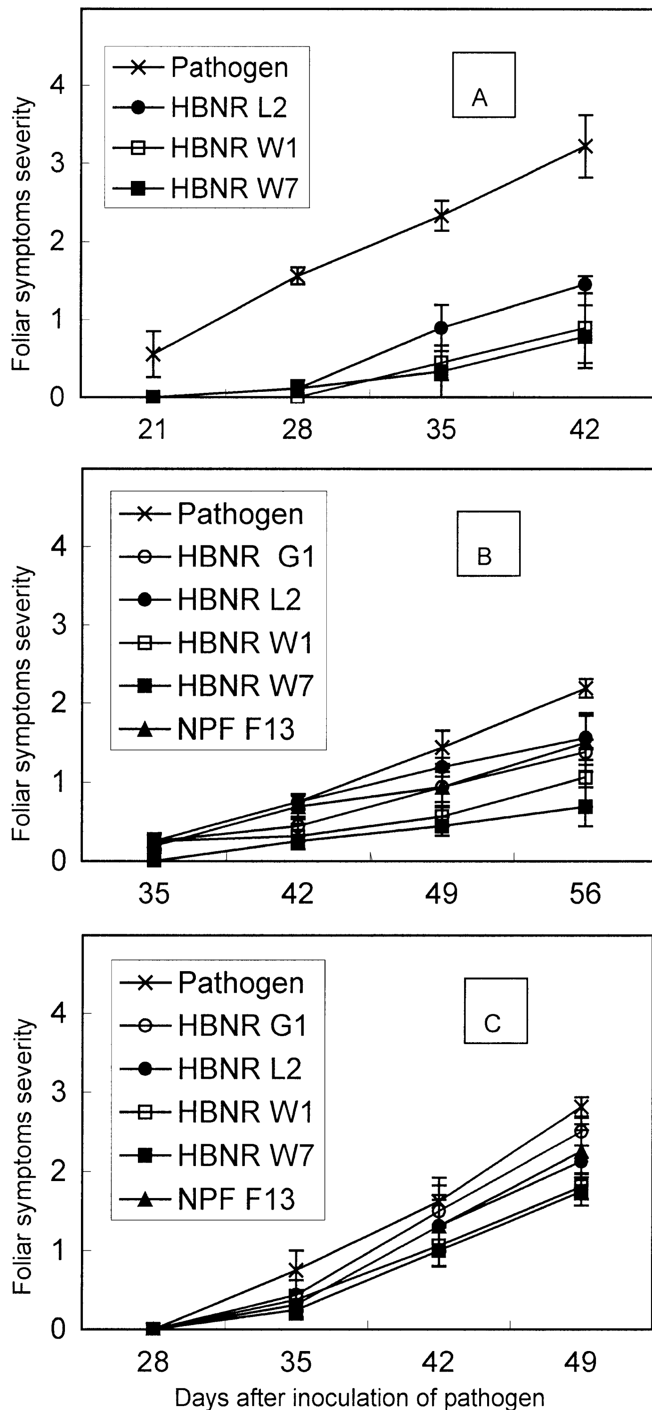

Fig. 1. Progress of foliar symptoms severity by *Fusarium oxysproum* f. sp. *lycopersici* on tomato treated or not treated with hypovirulent binucleate *Rhizoctonia* (HBNR) isolates and nonpathogenic *Fusarium oxysporum* (NPF F13) in experiments 1 (**A**), 2 (**B**), and 3 (**C**) under single application. Data are the means of four replications with four plants per replication. *Bars*, standard error of the mean

experiments 2 and 3 (Fig. 1B,C). The results of reduction in severity of discoloration inside the stem by HBNR isolates were almost the same as those of foliar symptom severity. Reduction in discoloration by HBNR isolates also varied depending on HBNR isolates and treatments. In all three experiments, only HBNR W7 could significantly and consistently reduce the discoloration inside the stem. Significant reduction in the severity of discoloration inside the stem was observed on NPF F13 in experiment 2 but not in experiment 3 (Table 1). In all three experiments of the single application, plants treated with HBNR W7 showed 38%–100% and 31%–70% reduction in the severity of foliar symptom and discoloration inside the stem, respectively.

Table 1. Effect of hypovirulent binucleate *Rhizoctonia* (HBNR) and nonpathogenic *Fusarium oxysporum* (NPF F13) on severity of discoloration inside the stem of Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL)[a]

Treatments	Severity of discoloration inside the stem[b]				
	Single application			Double application	
	Experiments			Experiments	
	1	2	3	1	2
Pathogen	2.44 b[c]	2.38 c	2.44 b	2.56 b	2.06 c
HBNR G1	ND	1.63 ab	2.31 ab	0.81 a	0.94 ab
HBNR L2	1.33 a	1.75 bc	2.19 ab	0.88 a	1.13 b
HBNR W1	0.67 a	1.38 ab	2.00 ab	0.63 a	0.50 ab
HBNR W7	0.67 a	1.00 a	1.69 a	0.75 a	0.88 ab
NPF F13	ND	1.50 ab	1.94 ab	1.06 a	0.38 a

ND, not done

[a] HBNR- or NPF-treated seedlings (21-day-old) in single application of experiments 1, 2, and 3 were transplanted in FOL-infested soil on July 27, 1999, June 24, 2000, and August 17, 2000, respectively, and in double application of experiments 1 and 2 were transplanted on September 15, 2000, and June 11, 2001, respectively; data were taken 42, 56, and 49 days after inoculation of FOL in experiment 1, 2, and 3 of single application, respectively, and were 49 and 63 days in experiment 1 and 2 of double application, respectively

[b] Severity of discoloration inside the stem was assessed as index of ratio of the browning area of vascular tissue, cortex, and xylem at the bottom of the tomato stem to the whole area of the stem using a scale of 0 to 3 : 0 = healthy (no vascular discoloration); 1 = ≤1/3; 2 = >1/3–2/3; 3 = >2/3

[c] Mean of four replications with four plants per replication; values followed by the same letter in each column do not differ significantly ($P = 0.05$) according to Fisher's least significant difference test

Effect of double application of HBNR

When HBNR was applied twice at the sowing and transplanting stages, foliar symptom severity of Fusarium wilt was significantly reduced for all HBNR-treated plants (HBNR G1, L2, W1, and W7) at all sampling dates of experiment 1 (Fig. 2A). The reduction of the foliar symptoms was 81%–100%, 81%–100%, 85%–100%, and 81%–83% for HBNR G1, L2, W1, and W7, respectively. In experiment 2, significant reduction of foliar symptom severity was also obtained for all HBNR isolates tested 49–63 days after inoculation of the pathogen (Fig. 2B). The reduction of the foliar symptoms was 54%–68%, 44%–50%, 69%–72%, and 60%–78% for HBNR G1, L2, W1, and W7, respectively. NPF F13 also significantly reduced the foliar symptoms by 63%–100% and 83%–100% in experiment 1 and 2, respectively (Fig. 2A,B). All HBNR isolates also

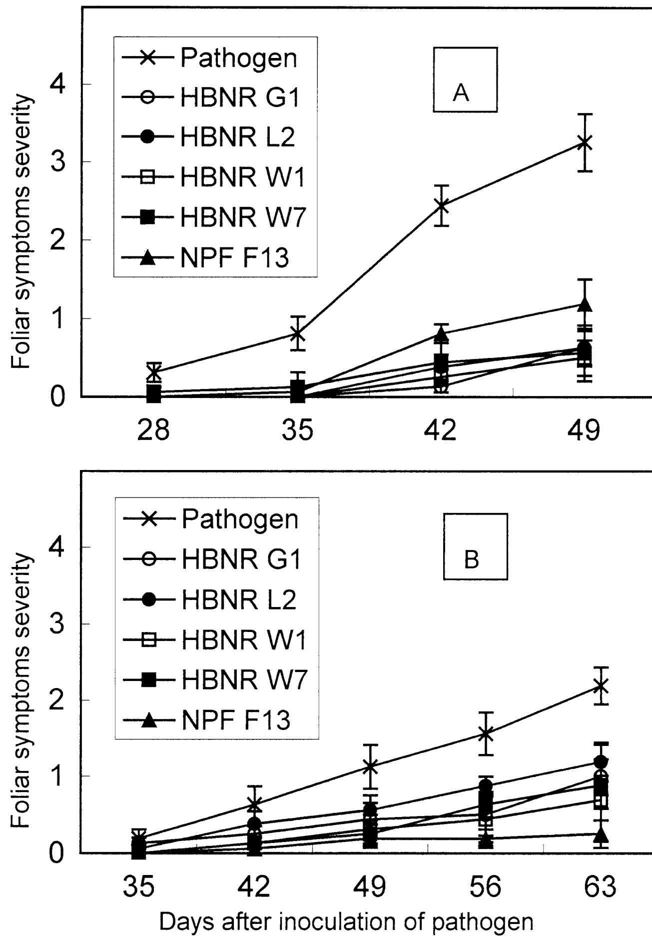

Fig. 2. Progress of foliar symptoms severity by *Fusarium oxysproum* f. sp. *lycopersici* on tomato treated or not treated with hypovirulent binucleate *Rhizoctonia* (HBNR) isolates and nonpathogenic *Fusarium oxysporum* (NPF F13) in experiments 1 (**A**) and 2 (**B**) under double application. Data are the means of four replications with four plants per replication. *Bars*, standard error of the mean

Table 2. Effect of hypovirulent binucleate *Rhizoctonia* (HBNR) and nonpathogenic *Fusarium oxysporum* (NPF F13) treatments on reduction of population density of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) in tomato stems and roots under double application

Treatments	Population of FOL in score ($\times 10^4$ cfu/g fresh weight)[a]				Average[c]
	0[b]	1	2	3	
In stems					
Pathogen	2.98 c[d]	38.18 d	131.60 e	178.80 c	153.87 b
HBNR G1	1.52 b	9.98 b	32.00 c	78.00 a	14.13 a
HBNR L2	0.04 a	19.12 c	61.00 d	82.00 b	22.80 a
HBNR W1	0.26 a	3.94 a	17.80 ab	55.00 a	8.66 a
HBNR W7	0.06 a	6.46 a	20.40 b	61.40 b	11.08 a
NPF F13	2.90 c	20.46 c	12.60 a	64.20 c	20.54 a
In roots					
Pathogen	9.86 d	18.30 d	20.80 c	27.10 e	23.79 c
HBNR G1	2.60 a	8.00 b	12.00 b	10.20 a	6.19 ab
HBNR L2	4.01 bc	11.30 c	6.00 a	23.10 d	9.08 b
HBNR W1	3.34 ab	8.00 b	10.12 b	12.76 ab	5.52 ab
HBNR W7	2.71 a	1.50 a	6.90 a	14.10 b	4.51 a
NPF F13	4.82 c	4.20 a	5.90 a	19.10 c	8.48 ab

cfu, colony-forming units

[a] Data were recorded at the end of the experiment 1 of double application, 49 days after inoculation of pathogen

[b] Scores are based on the severity of discoloration inside the stem, which was assessed as index of ratio of the browning area of vascular tissue, cortex, and xylem at the bottom of the tomato stem to the whole area of the stem using a scale of 0 to 3:0 = healthy (no vascular discoloration); 1 = ≤1/3; 2 = >1/3–2/3; 3 = >2/3

[c] Average of population (cfu/g fresh weight) = $(P_0A + P_1B + P_2C + P_3D)/N \times 100$, where P_0, P_1, P_2, and P_3 = population of pathogen in score 0, 1, 2, and 3, respectively: A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; N = total number of plants; values of average of FOL population are means of four replications with four plants per replication

[d] Data are the means of six replicates; values followed by the same letter in each column do not differ significantly (P = 0.01) according to Fisher's least significant difference test

significantly reduced (P = 0.05) the severity of discoloration inside the stem in both experiments (see Table 1). In experiment 1, the reduction of discoloration caused by HBNR G1, L2, W1, and W7 was 68%, 66%, 75%, and 71%, respectively. In experiment 2, NBNR G1, L2, W1, and W7 reduced the discoloration by 54%, 45%, 76%, and 57%, respectively. Significant reduction of the discoloration was also observed on the plants treated with NPF F13 in both experiment 1 (59%) and experiment 2 (82%) (see Table 1).

Pathogen population in stems and roots

FOL population in stems and roots at all scores of the severity of discoloration inside the stem were significantly reduced (P = 0.01) in the treatments with all HBNR isolates (Table 2). NPF F13 also significantly reduced (P = 0.01) FOL population in stems and roots at all scores except in stems at score 0 (Table 2). FOL populations were consistently reduced in stems treated with HBNR isolates by 49%–99%, 50%–90%, 54%–86%, and 54%–69% at scores 0, 1, 2, and 3, respectively, relative to the pathogen-infested control. Inconsistent reductions were observed in NPF F13-treated plants, which reduced the populations by 3%, 46%, 90%, and 64% at scores 0, 1, 2, and 3, respectively.

In roots, plants treated with HBNR isolates showed 59%–74%, 38%–92%, 42%–71%, and 15%–62% reduction of FOL population at score 0, 1, 2, and 3 respectively, whereas 51%, 77%, 72%, and 30% reductions were recorded in NPF F13 at score 0, 1, 2, and 3, respectively. The average numbers of FOL population in stems and roots were also significantly reduced (P = 0.01) in plants treated with all HBNR isolates (Table 2). The reduction of FOL population was 91%, 85%, 94%, and 93% in plants treated with HBNR G1, L2, W1, and W7, respectively. HBNR G1, L2, W1, and W7 also reduced FOL population in roots by 74%, 62%, 77%, and 81%, respectively. Similarly, NPF also signifcantly reduced FOL population in stems (87%) and in roots (64%).

Application of HBNR isolates could not significantly reduce population densities of FOL in soil (data not shown).

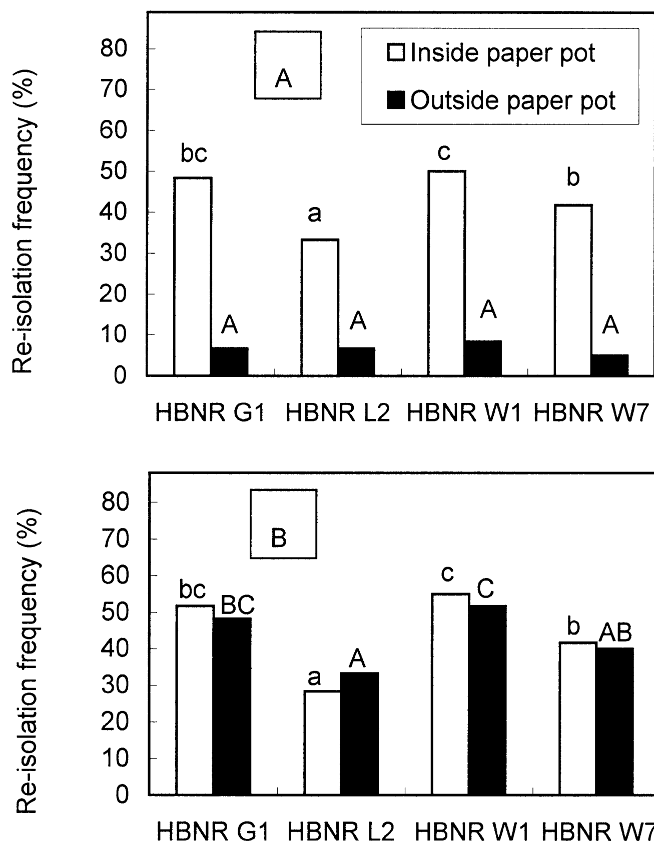

Fig. 3. Isolation of hypovirulent binucleate *Rhizoctonia* (HBNR) isolates from roots inside and outside paper pot in single application (**A**) and double application (**B**). Plants were collected from experiment 2 in single application and experiment 2 in double application, 56 days and 63 days after inoculation of pathogen, respectively. Data are the mean of six replications with ten root sections per replication. *Bars* labeled with the same letter are not significantly different according to Fisher's least significant different test ($P = 0.05$)

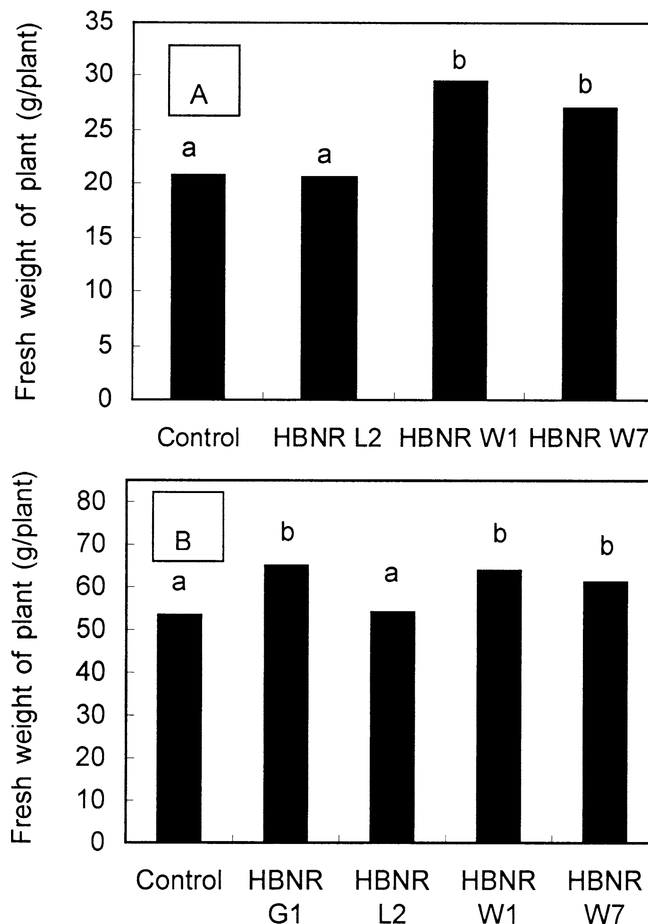

Fig. 4. Effect of hypovirulent binucleate *Rhizoctonia* (HBNR) on the fresh weight of plants (stems and leaves) from experiments 1 (**A**) and 2 (**B**). Data were recorded 63 days and 77 days after sowing in experiments 1 and 2, respectively. Data are the means of four replications with four plants per replication. *Bars* labeled with the same letter are not significantly different according to Fisher's least significant different test ($P = 0.05$)

Reisolation of HBNR from stems and roots

HBNR isolates variously colonized the roots inside and outside the paper pot under single and double applications (Fig. 3A,B). In single application, among four HBNR isolates, the colonization ability of L2 on the roots inside the paper pot was significantly ($P = 0.05$) lower than G1, W1, and W7. HBNR isolates colonized 33%–50% of the roots inside the paper pot. There was no significant difference in colonization ability among the four HBNR isolates on roots outside the paper pot. HBNR isolates could only colonize 5%–8% of the roots outside the paper pot (Fig. 3A). In the double application, HBNR L2 was also significantly lower ($P = 0.05$) than G1, W1, and W7 on roots inside the paper pot. Colonization of L2 outside the paper pot was significantly lower ($P = 0.05$) than G1 and W1 but was not significantly lower than W7. HBNR isolates colonized 28%–55% and 33%–52% of the roots inside the paper pot and outside the paper pot under double application (Fig. 3B). In the experiments of single and double applications, HBNR isolates were not obtained from the stem parts at 1, 5, 10, 15, or 20cm above the soil surface (data not shown).

Effect of HBNR on the growth promotion

Application of HBNR W1 and W7 resulted in significant increase ($P = 0.05$) in the fresh weight of plants (stems and leaves) in both experiments 1 and 2 (Fig. 4A,B). HBNR W1 and W7 increased fresh weight of the plant by 30% and 23% and by 17% and 13% in experiments 1 and 2, respectively. However, no significant increase was observed in HBNR L2. In experiment 2, HBNR G1 also significantly increased the fresh weight of plants by 18%.

Discussion

This study is the first report on the control of Fusarium wilt of tomato (FWT) caused by *F. oxysporum* f. sp. *lycopersici*

with HBNR. Among the five experiments, HBNR W7 could consistently result in significant suppression of FWT. Many researchers have worked on the biological control of Fusarium diseases and most of them have reported that nonpathogenic *Fusarium* (NPF) was effective as a biocontrol agent against Fusarium diseases (Louter and Edginton 1990; Yamaguchi et al. 1992; Larkin et al. 1996; Larkin and Fravel 1998). However, in our study, although we tested only one isolate of NPF F13 for comparison, HBNR isolates reduced FWT comparably with NPF F13. The biocontrol ability of HBNR against FWT obtained in this study holds a great possibility for their use as protective agents against Fusarium diseases.

Two application methods of HBNR, single and double applications, were made in this study. When HBNR was applied only at the seedling stage (single application), some isolates reduced FWT inconsistently. However, when applications of HBNR were made both at seeding and transplanting stages (double application), all the HBNR isolates showed consistent results in significant reduction of FWT. When a single application was made, HBNR was reisolated frequently from roots inside the paper pot where it was inoculated whereas reisolation was limited to roots that extended outside the paper pot. On the other hand, in double application, HBNR could be reisolated frequently from roots both inside and outside the paper pots. These results indicated that occupation of the infection site for FOL by HBNR isolates might contribute to the consistent results in double application. Several studies have shown that colonization of biocontrol agents at the target site is a prerequisite for the suppression of plant pathogens (Bull et al. 1991; Poromarto et al. 1998; Hwang and Benson 2002), whereas in the isolate of HBNR W7 there was no difference in reducing FWT between single and double applications. This isolate might move only slightly on the roots from inside to outside the paper pot in a single application, which suggests that a mechanism other than competition for invasion sites might be involved in the reduction of FWT. Biles and Martyn (1989) and De Cal et al. (1997) suggested that biocontrol agents in contact with plant roots could induce resistance in whole plant parts.

Sneh et al. (1989) reported that, if an antagonist and pathogen are closely related fungal species, it is likely that they compete for the same nutrients and infection sites. However, binucleate *Rhizoctonia* could also control FWT caused by *F. oxysporum* f. sp. *lycopersici*, which is not related to *Rhizoctonia*. Additionally, we did not observe hyperparasitism, hyphal interference, or an antibiosis between HBNR isolates G1, L2, W1, and W7 and FOL in vitro (data not shown). Also, no reduction in FOL population by HBNR in soil at the end of the experiments using a double application was observed. These results suggested that a mechanism other than competition for an ecological niche might be important, as suggested by Cardoso and Echandi (1987b), that competition may not be the only mechanism involved in the protection of bean seedlings treated with HBNR against Rhizoctonia root rot caused by *R. solani*. Further research is needed to determine what mechanisms are involved in our system.

In the present study, preinoculation of tomato seedlings with HBNR then challenged with FOL not only suppressed the disease but also suppressed pathogen population in stems and roots in any disease scores. These results support previous reports that application of nonpathogenic *F. oxysporum* Fo47 and *Pseudomonas putida* WCS 358 decreased the population of pathogenic *F. oxysporum* in roots of flax (Duijff et al. 1999). Similar results, that in stems and roots of the resistant cultivars the population of pathogenic species of *Fusarium* was significantly lower than in susceptible cultivars, have been obtained (Elgersma et al. 1972; Stromberg and Corden 1977).

HBNR G1, W1, and W7 increased fresh weight of tomato (stems and leaves). The association between HBNR and the plant has not yet been fully studied. Further investigations on mechanism(s) in promoting plant growth are needed to evaluate its potential use in future agricultural practices. In this study, of four HBNR isolates, only HBNR L2 failed to promote plant growth. The reason for this might be low colonization ability on the roots by the isolate. HBNR W7 showed a plant growth promotion effect, which may reflected in significant and constant results in suppressing the disease. Such a plant growth promotion effect, in addition to disease suppression effects, indicate that isolates of HBNR may have possible benefits as biocontrol agents.

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