Plant Pathology

Pathogenetic roles of beet necrotic yellow vein virus RNA5 in the exacerbation of symptoms and yield reduction, development of scab-like symptoms, and *Rz1*-resistance breaking in sugar beet

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Abstract

Beet necrotic yellow vein virus (BNYVV) generally has a four-segmented positive-sense RNA genome (RNAs 1–4), but some European and most Asian strains have an additional segment, RNA5. This study examined the effect of RNA5 and RNA3 on different sugar beet cultivars using a *Polymyxa*-mediated inoculation system under field and laboratory conditions. In field tests, the degree of sugar yield served as an index for assessing the virulence of BNYVV strains. Japanese A-II type isolates without RNA5 caused mostly 15%–90% sugar yield reductions, depending on the susceptibility of sugar beet cultivars, whereas the isolates with RNA5 induced more than 90% yield losses in the seven susceptible cultivars, but small yield losses in one *Rz1*-resistant and Rizor cultivars. However, a lab-produced isolate containing RNA5 but lacking RNA3 caused higher yield losses in Rizor than in susceptible plants, and induced scab-like symptoms on the root surface of both susceptible and resistant plants. In laboratory tests, A-II type isolates without RNA5 had low viral RNA accumulation levels in roots of Rizor and *Rz1*-resistant plants at early stages of infection but in the presence of RNA5, viral RNA3 accumulation levels remarkably increased. This increased RNA3 accumulation was not observed in roots of the WB 42 accession with the *Rz2* gene. In contrast, the presence of RNA3 did not affect RNA5 accumulation levels. Collectively, this study demonstrated that RNA5 is involved in the development of scab-like symptoms and the enhancement of RNA3 accumulation and suggests these characteristics of RNA5 are associated with *Rz1*-resistance breaking.

Keywords: BNYVV, RNA5, scab-like symptom, sugar beet, *Rz1* gene, resistance breaking.

1 Introduction

Beet necrotic yellow vein virus (BNYVV) is a causal agent of rhizomania disease in sugar beet (*Beta vulgaris* subsp. *vulgaris*) and is transmitted by the soil-inhabiting plasmodiophorid protist *Polymyxa betae* (Tamada, 2016). Since the first record of the disease in Italy in the 1950s, rhizomania had been noted worldwide, where it can cause a drastic reduction in sugar yield where present (Biancardi and Tamada, 2016). The virus is usually confined to the roots, and infected roots are stunted and constricted (e.g. wineglass-shaped) and develop a massive proliferation of the lateral rootlets. Foliar symptoms are generally pale yellowing and wilting, and rarely vein yellowing.

BNYVV is the type member of the genus *Benyviru*s in the family *Benyviridae* in the realm *Riboviria* (Gilmer and Ratti, 2017) and is a multipartite, single-stranded, positive-sense RNA virus, comprising either four or five segments (RNA1–5) (Richards and Tamada, 1992; Tamada, 2016). The essential genomic components RNA1 and RNA2 control RNA replication, assembly, cell-to-cell movement and suppression of antiviral RNA silencing. The extra-genomic components RNA3, RNA4 and RNA5 play an important but different role in pathogenicity and vector transmission: RNA3 encodes a 25-kDa protein (p25) controlling rhizomania symptoms in sugar beet roots, RNA4 a 31-kDa protein (p31) involved in efficient vector transmission, and RNA5 a 26-kDa protein (p26) associated with symptom severity but dispensable for BNYVV survival (Richards and Tamada, 1992; Tamada, 2016).

Previously, BNYVV isolates were classified into two types, A and B, based on the RNA2-encoded *CP* (coat protein) gene (Kruse *et al*., 1994). A further virus group, P type, which additionally contains the RNA5 segment was isolated from France, Germany and the United Kingdom (Koenig *et al*., 1997; Ward *et al*., 2007) but is otherwise closely related to the A-type virus (Miyanishi *et al*., 1999). The A-type BNYVV is more widely distributed throughout the world (Schirmer *et al*., 2005), while the B-type virus is found in limited areas in Europe. Subsequently, phylogenetic analyses based on the *CP, p25* and *p31* genes showed that worldwide, BNYVV isolates consist of eight strains derived from at least four original lineages (A-I, A-II, A-III and B types) (Chiba *et al*., 2011). The strains belonging to each type are clearly separated

geographically. The Italy strain (A-III type) was detected in Europe, the Middle East and the United States, and the Germany strain (B type) was found in limited areas of Germany and France. These two strains do not contain RNA5. Six other strains (belonging to the A-II or A-I types), i.e. Japan D, Japan O, France P (=P type), China B, China H and China X, originally isolated from Japan, France and China, were found in limited areas with a few exceptions. Most isolates of these six strains contain RNA5 (Koenig and Lennefors, 2000; Chiba *et al*., 2011; Zhuo *et al*., 2015; Tamada *et al*., 2016). Recently, RNA5-containing BNYVV, which was phylogenetically closer to the Japanese isolates than the P-type isolates, was reported to be prevalent in Turkey (Yilmaz *et al*., 2016, 2018).

The most practical, effective control measure for rhizomania disease is thought to be the use of resistant cultivars (Biancardi and Tamada, 2016). Screening tests for resistance started in northern Italy in the mid-1960s (Biancardi *et al*., 2002), and the first resistant cultivar, Rizor, has been grown in infested areas in many countries since the mid-1980s (Panella and Biancardi, 2016). Subsequently, the Holly resistance source (containing the *Rz1* resistance gene), with a higher level of resistance than Rizor, was discovered in sugar beets in the United States in the mid-1980s. Since then *Rz1*-resistant germplasm has been widely exploited in most sugar beet cultivars (Lewellen *et al*., 1987; Panella and Biancardi, 2016). The resistances of Rizor and Holly source with the *Rz1* gene are thought to derive from the same common parent (Stevanato *et al*., 2015). A second resistance gene *Rz2*, with a higher level of resistance than *Rz1,* was identified from *Beta vulgaris* subsp. *maritima* populations in Denmark (Lewellen *et al*., 1987). *Rz1* and *Rz2* were mapped at a distance of about 20–35 cM on chromosome 3 of the sugar beet (Scholten *et al*., 1999; Panella and Biancardi, 2016). In addition to the *Rz1* and *Rz2* genes, other resistance genes such as *Rz3, Rz4* and *Rz5* have been identified (Panella and Biancardi, 2016). Those resistance genes have shown to be involved in restriction of virus multiplication and translocation in taproots rather than in rootlets (Scholten *et al*., 1994; Tamada *et al*., 1999, 2016).

However, severe symptoms in resistant cultivars have been found in several areas of the United States, Europe and the Middle East (Liu *et al*., 2005; Pferdmenges *et al*., 2009; Bornemann and Varrelmann, 2011; Yilmaz *et al*., 2018; Weiland *et al*., 2019). In areas

where A-type BNYVV (A-III type strain reported by Chiba *et al*, 2011) is prevalent, so-called "resistance-breaking (RB) variants" were generated by single amino acid changes in the p25 protein (Schirmer *et al*., 2005; Acosta-Leal *et al*., 2008; Chiba *et al*., 2008). Chiba *et al*. (2011) found that many isolates of the Italy A-III strain were able to overcome *Rz1* resistance with various degrees of RB, whereas other virus strains (such as Germany (=B type), A-I and A-II type strains) could not overcome *Rz1*-mediated resistance. Furthermore, RNA5-containing BNYVV (P-type strain) has been shown to have the ability to overcome *Rz1* resistance (Pferdmenges *et al*., 2009, Chiba *et al*., 2011; Bornemann and Varrelmann, 2011; Galein *et al*., 2018). However, the pathogenic role of RNA5 in RB virus incidence, as well as non-RB virus incidence, remains unclear.

This study investigated the effect of BNYVV RNA5 along with that of the well-characterized RNA3 on symptom development, sugar yield and viral RNA accumulation in different sugar beet cultivars under field and laboratory conditions. The results showed that BNYVV RNA5 and RNA3 are involved in the development of different types of symptoms and that the presence of RNA5 increases viral RNA accumulation levels in roots of sugar beet plants, along with enhancement of symptom severity and *Rz1*-resistance breaking.

2 Materials and methods

2.1 Virus isolates and preparation of inoculum sources

BNYVV isolates used in this study are listed in Table 1. All field isolates were obtained from soil using bait plants (sugar beet seedlings). All sugar beet roots that were infected with BNYVV-carrying *Polymyxa betae* were simultaneously infected with various soil-inhabiting microorganisms, including nematodes, fungi and bacteria. Pure inoculum sources of each virus isolate were produced by the following procedures (Fig. S1a): (i) sap or total nucleic acid extract of bait plant rootlets was inoculated onto leaves of *Tetragonia expansa,* which is an indicator plant of BNYVV, (ii) sap of virus-infected *T. expansa* leaves that displayed various types of yellow spots (Tamada *et al*., 1989) were inoculated onto leaves of *Beta macrocarpa* plants, a systemic host for BNYVV,

and at the same time, virus-free *P. betae* was inoculated to roots of those plants, (iii) after one month, *P. betae* zoospore suspensions obtained from virus-infected roots of *B. macrocarpa* plants were added to roots of healthy sugar beet seedlings. Alternatively, crude homogenate of *B. macrocarpa* roots was inoculated by adding into the seedling roots, and (iv) after grown for at least two months, sugar beet roots were collected, dried and used as inoculum sources. Under this inoculation condition, a large number of *P. betae* resting spore clusters in root epidermal cells were observed in all inoculated plants. Bait sugar beet and *B. macrocarpa* plants were all grown in test tubes (24 mm wide and 120 mm long with a drainage hole, filled with quartz sand) in a growth cabinet at 24 ˚C with a 16 h light/8 h dark cycle. *T. expansa* plants were grown in a greenhouse.

The laboratory BNYVV isolates S-34, S-45, S-5d, S-4, D104-5 and O11-4 (Table 1) were obtained from the original field isolates S, D104 and O11, respectively (Saito *et al*., 1996; Miyanish *et al*., 1999; Chiba *et al*., 2008) by single-lesion transfer in *T. expansa* leaves, as described by Tamada *et al*. (1989). The S-5d isolate contains a RNA5 deletion mutation (Tamada *et al*., 1989; referred to as RNA5a/isolate D6 therein), in which the p26 open reading frame had undergone an internal deletion of 303 nucleotides (Kiguchi *et al*., 1996). *P. betae* cultures carrying each of these single-lesion virus isolates were produced by the procedures mentioned above.

2.2 Field tests

Sugar beet cultivars used in this study are listed in Table S1. In mid-April, sugar beet seeds were sown in paper pots containing sterilized soil, and the seedlings grown in the glasshouse. After three weeks, 30–50 sugar beet seedlings per one plot were inoculated by pouring zoospore suspensions of individual virus-carrying *P. betae* that had been grown in test tubes (Fig. S1b). A zoospore suspension was obtained by collecting water drained from the bottom of test tubes into which nutrient solution had been poured. Each experiment included a control treatment of seedlings in which virus-free *P. betae* was inoculated at the same time. At the end of May, virus infection was checked by ELISA (enzyme-linked immunosorbent assay), and then virus-inoculated and control seedlings were transplanted to the field and were cultivated in accordance with the standard commercial practice of sugar beet planting in Hokkaido.

Experiments were arranged in randomized plots with two replicates for 48-seedling plots, and one replicate for 24-seedling plots. To reduce the effect of infection moving between plots, healthy plants were planted between test plots. During the growing period, from the middle August to the early September, relative chlorophyll content (intensity of yellowing) was assessed using a chlorophyll meter (soil plant analysis development [SPAD], model 502 Plus; Konica Minolta Sensing, Inc., Osaka, Japan) (Uchino and Kanzawa, 1995). Plants were harvested at the end of October, and groups of 40 or 20 taproots per plot were tested for yield parameters (root weight, sugar content and sugar yield), and quality parameters (Na, K and α-amino N content in mEq/100 g of beet). In some experiments, virus concentration in taproots was assessed by ELISA. Statistically significant differences among the mean values of the replicates on the respective experiments were evaluated by analysis of variance (ANOVA). Virus inoculation in the laboratory and greenhouse was conducted at Naganuma (Hokkaido Central Agricultural Experiment Station) unless otherwise stated, and field tests were performed in non-infested fields that had been previously fumigated by 3,5-dimethyl-1,3,5-thiadiazine-2-thione (Dazomet) at Obihiro (Research Center, Nippon Beet Sugar Mfg. Co., Ltd.).

2.3 Laboratory tests

BNYVV isolates and sugar beet plants used in this study are listed in Tables 1 and S1, respectively. Sugar beet or wild beet seedlings were grown in sand culture, and five seedlings per plot were used for inoculation. The dried rootlets used for inoculum were ground in distilled water, and the crude homogenates were added to test tubes in which sugar beet seedlings were grown. After three weeks, the accumulation levels of virus and viral RNA segments in rootlets of inoculated plants were measured by ELISA and Northern blot analysis, respectively. The laboratory tests were conducted at Kurashiki (Institute of Plant Science and Resources, Okayama University).

2.4 ELISA

BNYVV infection and virus content in sugar beet roots were determined by ELISA (Tamada *et al*., 1999). For virus detection from rootlets of sugar beet plants, fresh tissues (0.04 g) were triturated with 2 ml of the extraction buffer using a pestle and mortar. For virus detection from taproots, the extracts were obtained by squeezing fresh

tissues (0.5–1.0 g) from the tip (sized about 1 cm in diameter) of taproots and were prepared with 2–4 ml of the extraction buffer. In the case of quantitative detection of the virus, all further 10-fold dilutions of each extract were prepared. The BNYVV content of root extracts was calculated by interpolating their absorbance values on standard curves produced by plotting the absorbance against concentrations of purified virus.

2.5 Northern blot analysis

Northern blot analysis from the inoculated roots of the plants was conducted as described previously (Chiba *et al*., 2008). Total RNA was extracted from 300–500 mg of root tissue. The blot was hybridized with digoxigenin-labelled cDNA probes specific for the BNYVV RNA1 (nt 5815–6531), RNA2 (nt 144–711), RNA3 (nt 442–1104) and RNA5 (nt 308–1193) (Kiguchi *et al*., 1996; Chiba *et al*. 2008). Equal loading was verified by visualization of ethidium bromide-stained 28S rRNA.

3 Results

3.1 Pathogenicity of BNYVV isolates with RNA3 and/or RNA5

A total of nine sugar beet cultivars, including seven susceptible and two resistant (Rizor and Schwert) cultivars, were used in this study (Table S1). BNYVV isolates belonging to Japan O, Japan D or China H (Japan T) strains were used (Table 1). Sugar beet plants were inoculated with respective BNYVV-carrying *Polymyxa betae* cultures in the greenhouse, and then they were transplanted and grown in the field. Inoculation by BNYVV-free *P. betae* was included as a control plot. Five sets of experiments with different viral and plant combinations were conducted for five consecutive years. Fig. S2 shows an example of the experimental plots at harvest time, showing shoot and root symptoms. Yield and quality parameters were examined on each plot of a group of roots, and consequently, the degree of sugar yield reduction was provided as an index for assessing the virulence (aggressiveness) of BNYVV isolates; therefore, only sugar yields are shown in Fig. 1.

The results obtained from the five sets of experiments are as follows: (i) The degrees of sugar yield reduction caused by infections of BNYVV isolates S-34, O11 and M87

(Japan O), K80 (Japan D) and H45 (China H) (RNA-1+2+3+4) were in accord with the susceptibility of these sugar beet cultivars (Fig. 1). The relative, decreasing order of disease susceptibility was as follows (except Alba P): Monomidori > Mono-ace S > =Monohikari = Monohomare = Dihill > Ema > Rizor = Schwert. (ii) Infections with RNA5-containing isolates S, SH1 and S44 (Japan O), D104 and T101 (Japan D) (RNA-1+2+3+4+5) caused excessive yield losses (more than 90% reduction with a few exceptions) in the susceptible cultivars but were not as large (17%–59% reduction with a few exceptions) in the resistant cultivars (Fig. 1b, c, d, e). (iii) Infection with an RNA5-containing isolate, S-45, lacking RNA3 (RNA-1+2+4+5) caused much more severe damage in the Rizor (and Alba P) cultivar than in the susceptible cultivars (Fig. 1a, b, c). (iv) Infection with isolate S-5d (RNA-1+2+4+5d), which contains an RNA5 internal deletion mutant, showed similar levels of sugar yield to that of the noninfected control (Fig. 1b), suggesting that a full-length RNA5 (probably specifically the p26 protein) is associated with sugar yield reduction. (v) Infection with isolate S-4 (RNA-1+2+4) lacking both RNA3 and RNA5 showed almost the same levels of sugar yield as the control (Fig. 1a), indicating that either RNA3 or RNA5 was associated with sugar yield reduction, which is consistent with a previous report (Tamada *et al*., 1999). (vi) Resistant cultivar Schwert, which possesses the *Rz1* gene, showed almost a similar level of yield loss to Rizor (Fig. 1e). (vii) There were differences among Japanese field isolates without RNA5 in the degree of sugar yield reduction; i.e. the Japan T (H45) strain was more aggressive than the Japan O (M87) or Japan D (K80) strain (Fig. 1d). (viii) There was some variation in symptom severity among experiments (Fig. 1), probably due to different levels of infection; i.e. experiments 3 and 4 had relatively higher levels of infection, compared with experiments 1, 2 and 5. Taken together, it is concluded that RNA3 and RNA5 have different pathogenetic roles in different sugar beet cultivars.

Next, to further investigate the roles of RNA3 and RNA5 as pathogenetic factors, symptom development in shoots and roots, virus content, yield parameters and quality parameters were analysed for each virus group in the susceptible cultivars (except Alba P) and the Rizor-resistant cultivar. The results are described in the following sections, and an integrated infection of sugar beet by each group of BNYVV isolates containing RNA3, RNA5 or both RNAs 3 and 5 is called BN-RNA3, BN-RNA5 or BN-RNA3+5

infection, respectively, hereafter. BN-ΔRNA3/5 means infection with a BNYVV isolate without both RNA3 and RNA5.

3.1.1 Effects of RNAs 3 and 5 on the development of symptoms in roots

BN-RNA3 infection caused abnormal rootlet proliferation (typical rhizomania symptoms) in roots of the susceptible cultivars (Fig. 2a; Fig. S2a, S-34) but did not induce such typical and severe symptoms in the Rizor cultivar (Fig. S2b). BN-RNA3+5 infections induced much more severe symptoms in roots, in which the roots reduced in size and many plants died. In contrast, BN-RNA5 infection did not induce rootlet proliferation but caused "scab-like" symptoms on the surface of taproots (Fig. 2a, S-45). Such scab-like symptoms were observed partially or entirely as a rough area on the surface of the taproots, later followed by dark brown areas that spread (Fig. 2b). Especially in Rizor roots, scabby parts on the roots sometimes developed to necrosis and finally rotted away, and the roots having a wineglass-like shape (one of the typical symptoms of rhizomania) were occasionally observed (Fig. 2b).

In further experiments, the development of scab-like symptoms on taproots of sugar beet plants was confirmed in a greenhouse test. Seedlings of susceptible cultivar Monomidori and Rizor were inoculated with each of the three isolates S, S-34 and S-45, and then were grown in pots containing sterilized soils in the greenhouse for three months. The "rough surface" symptoms were observed entirely on the surface of the taproots infected with the RNA5-containing isolates S and S-45 but were not observed on those inoculated with the RNA5-lacking isolate S-34 (Fig. 2c). No difference in the development of rough surface symptoms was found between the two cultivars (Fig. 2c and data not shown). Under greenhouse conditions, scabby symptoms with dark brown lesions were not observed on both cultivars. Taken together, the results indicate that RNA5 but not RNA3 was involved at least with the development of rough surface symptoms, which is termed here mild scab-like symptoms to distinguish them from scabby symptoms with dark brown lesions.

3.1.2 Effects of RNAs 3 and 5 on the development of symptoms on shoots

In general, the above-ground symptoms of rhizomania are characterized by pale yellowing on the leaves of sugar beet plants (Tamada, 2016). Thus, chlorophyll content

(SPAD values) was expected to correlate well with the sugar yield, and this was shown to be the case in experiments 1 and 2 (Fig. S3a). In the susceptible plants, SPAD values were much lower for either BN-RNA3 or BN-RNA3+5 infection than BN-RNA5 infection (Fig. S3b). However, in the resistant plants, SPAD values were nearly similar between all virus infection plots and the control plots (Fig. S3b). These results indicate that BNYVV RNA3 links to the development of pale yellowing symptoms on shoots, but the effect of RNA5 is small. In this study, BN-RNA5 infection produced no obvious foliar symptoms, but BN-RNA3+5 infection caused slight stunting and yellowing of older leaves, with older leaves of plants withered. Such symptoms are slightly different from those caused by BN-RNA3 infection, which are characterized by pale yellow leaves, with an elongated petiole and more upright growth of shoots (Tamada, 2016).

Another type of above-ground symptom of rhizomania is characterized by "yellow veins" on the leaves of sugar beet plants, which is caused by the systemic movement of BNYVV to shoots, although it is seldom observed in natural field conditions (Tamada, 2016). In this study, yellow vein symptoms were observed frequently in the susceptible plants in experiments 3 and 4, where a higher level of infection occurred (see Fig. 1c, d). Intriguingly, this symptom appeared much more frequently in plots with a BN-RNA3 infection than in those of BN-RNA3+5 infection (Fig. 3), although the latter virus infection caused much more severe damage than the former virus infection. This result suggests that the presence of RNA5 may inhibit the systemic movement of BNYVV to shoots.

3.1.3 Effects of RNAs 3 and 5 on the virus content in roots

Data on virus content in taproots were obtained from experiments 1 and 3 (see Fig. 1a and c). In the susceptible cultivars, BN-RNA3 infection was much higher (approximately 4 times) than that on BN-RNA5 infection, but those results in the resistant cultivar were the opposite; i.e. BN-RNA3 infection was lower (more than twice) than BN-RNA5 infection (Fig. 4a, b). BN-RNA3+5 infection had slightly higher virus content than BN-RNA3 infection in the susceptible plants, but it was lower than BN-RNA5 infection in the resistant plants (Fig. 4b). Furthermore, BN-RNA5 infection had higher levels of virus content than BN-ΔRNA3/5 infection (without RNA3 and RNA5) in both susceptible and resistant plants (Fig. 4a). Thus, the results indicate that

BNYVV RNA5 facilitates virus content in roots, although the effect of RNA3 is much greater in susceptible plants. This result also suggests that RNA3 but not RNA5 is associated with Rizor resistance to BNYVV.

3.1.4 Effects of RNAs 3 and 5 on yield and quality parameters

The sugar beet yield and quality parameters were assessed in experiments 1, 2, 3 and 5 (see Fig. 1). In the susceptible cultivars, compared to the virus-uninfected control BN-RNA3+5, BN-RNA3 and BN-RNA5 infections caused 88%, 68% and 23% reductions in root weight, respectively, while the reductions were 32%, 21% and 45% in the resistant cultivars, respectively (Fig. 5a). Similar trends were observed in sugar content (Fig. 5b): in the susceptible cultivars, BN-RNA3+5, BN-RNA3 and BN-RNA5 infections caused 39%, 25% and 8% reductions relative to the sugar content of the control, respectively, while reductions in the resistant cultivars were 13%, 3% and 12%, respectively. The degrees of sugar yield reduction directly reflected those of root weight and sugar content (Fig. 5c). Thus, in susceptible cultivars, yield parameters were affected by RNA3 more than by RNA5, whereas in resistant cultivars, these were much more affected by RNA5 than by RNA3.

In the case of quality parameters (Fig. 5d, e, f), in the susceptible cultivars BN-RNA3 and BN-RNA3+5 infections greatly decreased α-amino N content and increased K and Na contents. BN-RNA5 infection also increased K content but did not affect α-amino N and Na contents. In the resistant cultivars, BN-RNA3, BN-RNA3+5 as well as BN-RNA5 infections slightly increased K content, but they did not affect α-amino N and Na contents. Thus, BNYVV RNA3 and RNA5 affected quality parameters to different degrees.

3.2 The presence of RNA5 increases RNA3 accumulation levels in *Rz1*-resistant plants

To elucidate the roles of RNA3 and RNA5 as pathogenetic factors in susceptible and resistant plants, levels of viral RNA and virus accumulations in roots of sugar beet plants were examined. Two susceptible cultivars (Monomidori and Monohikari), three resistant cultivars (Rizor, Schwert and Yukihinode), Holly 1-4 accession (original source of the *Rz1* gene) and WB42 accession (*Beta vulgaris* subsp. *maritima*; original

source of the *Rz2* gene) were used (Table S1). Plants were grown in sand culture in a growth cabinet and inoculated with *P. betae* cultures carrying each of D104, O11, D104-5 and O11-4 (see Table 1 for RNA components). Root samples (five plants per plot) were taken at 18 or 21 days post-inoculation in experiments 1 and 2, respectively. All five independent plant roots or a mixture of two plants per plot were examined by ELISA or Northern blot, respectively.

The results of two sets of experiments showed a similar viral RNA accumulation pattern (Fig. 6a, c). In the susceptible plants, infections by the isolate O11 or D104 showed high levels of viral RNA accumulation (RNAs 1, 2 and 3), whereas in Rizor and *Rz1*-resistant plants, infection with the O11 isolate had lower levels of viral RNA accumulation, whereas infection with the D104 isolate had much higher levels of viral RNA accumulation (Fig. 6a, c). It is particularly notable that levels of RNA3 accumulation were markedly higher in D104-infected roots than in O11-infected ones. In roots of WB42 plants, such a marked difference in RNA3 accumulation was not observed (Fig. 6a). This trend was further confirmed in an independent set of inoculation tests (Fig. S4). Additionally, the viral RNA accumulation levels appeared to correlate with the virus content in roots, although the values of individual plants and plots varied (Fig. 6b, d). Taken together, the results indicate that the presence of RNA5 remarkably increased the level of RNA3 accumulation in roots of Rizor and *Rz1*-resistant plants but did not increase the levels of RNA3 in roots of *Rz2*-resistant plants. Moreover, the presence of RNA3 tended to increase the level of RNA5 accumulation to some extent, suggesting crosstalk between the two segments in particular conditions.

3.3 Effects of RB and non-RB isolates on the viral RNA accumulation

A previous study (Chiba *et al*., 2011) showed that A-III type isolates (=A type in Europe and the United States) were able to overcome *Rz1* resistance to different degrees, whereas Japanese A-II type isolates without RNA5 could not overcome *Rz1*-mediated resistance. In this experiment, the effect of RB and non-BR isolates on viral RNA accumulation in susceptible and resistant plants was investigated. Japanese (O11 and T41, A-II and A-I type, respectively), the United States (USTH, A-III type) and European (SLP2, SLN1 and SPC, A-III type; GW, B type) isolates (RNA5 free isolates)

were used (see Table 1). Among them, USTH, SLN1 and SPC are RB isolates (Chiba *et al*., 2011). The isolate O11-4, lacking RNA3, was used as a control.

First, it is notable that in the *Rz1*-resistant plants (Schwert and Holly 1-4), O11-4-infected roots showed higher levels of viral RNA accumulation than in O11-infected ones (Fig. 7a, b), while in the susceptible plants, levels of viral RNA accumulation were similar in the two virus isolates (Fig. 7c). This indicates that the presence of O11 RNA3 reduced genomic RNA replication in the roots of the *Rz1*-resistant plants. Second, compared to the viral RNA accumulation level in O11-infected roots, relatively higher levels of viral RNA accumulation were detected in roots infected with the other virus isolates, in which SLN1, T41 and SPC isolates showed the highest levels of RNA3 accumulation (Fig. 7a, b). Considering that the isolates SLN1, USTH and SPC are RB type (Table 1), these differences in viral RNA accumulation levels roughly reflect differences of the RB ability, although the isolates USTH and T41 are BR type and non-RB type, respectively (Chiba *et al*., 2011). Together, the results suggest that RB-type isolates enhance viral RNA3 accumulation levels in *Rz1*-resistant plants, which also supports the notion that RNA3 mutants are associated with RB ability.

4 Discussion

RNA5-containing BNYVV has been prevalent for a long time in Japan and China (Chiba *et al*., 2011; Zhuo *et al*., 2015; Tamada *et al.*, 2016), and recently has been found to have spread widely from an originally limited area in Europe (Galein *et al*., 2018) and also in the Middle East (Yilmaz *et al*., 2016). RNA5 is known to be associated with symptom severity of the virus (Tamada *et al*., 1996; Heijbroek *et al*., 1999), but little is known about its function. In the field tests using a *Polymyxa*-mediated inoculation system, the symptom severity of BNYVV isolates in sugar beets was shown to strongly correlate with sugar yield reduction, which provides for an accurate indicator of the aggressiveness of BNYVV strains (isolates) on sugar beet cultivars. Thus, BN-RNA3+5 infection causes much greater yield losses than BN-RNA3 infection, reconfirming that the presence of RNA5 increases the aggressiveness of RNA3-containing BNYVV

(Tamada *et al*., 1996; Heijbroek *et al*., 1999; Bornemann and Varrelmann, 2011). Moreover, this study provides the evidence that BNYVV RNA5 is associated with the development of scab-like symptoms on the surface of taproots but is not associated with the production of massive rootlet proliferation that is a hallmark of typical rhizomania.

Scab-like symptoms on sugar beet roots as well as other root crops such as potato, carrot, radish, turnip and parsnips are generally caused by bacterial pathogens; i.e. *Streptomyces* spp. (Tashiro *et al*., 1990; Goyer and Beaulieu, 1997). Therefore, a preliminary experiment was tried to detect bacteria as well as fungi from the BNYVV-infected roots showing scab-like symptoms. Some bacteria and fungi such as *Streptomyces* sp. *Trichoderma* sp. and *Fusarium* spp. were detected from symptomatic roots and from healthy roots as well (data not shown). Considering that severe scab-like symptoms did not appear on roots of sugar beet plants grown in sterilized soils, it could be speculated that RNA5 is associated with the induction of mild scab-like symptom on the surface of the roots, followed by a secondary infection with saprobic or pathogenetic bacteria and/or fungi that consequently cause the severe scab-like symptoms to develop (Fig. 2b). Further study will uncover potential co-relation of BNYVV RNA5 and soil-inhabiting microorganism on the etiology of the scab-like symptoms.

It is notable that Rizor and Alba P cultivars were particularly susceptible to BN-RNA5 infection more than the other susceptible cultivars used in this study, although, unlike Rizor plants, Alba P plants were susceptible to BN-RNA3 infection. The reports that the two cultivars are from the same linage (Stevanato *et al*., 2015; Panella and Biancardi, 2016) suggest that this characteristic (response to BN-RNA5 infection) is controlled genetically. In the case of the Rizor cultivar, BN-RNA5 infection caused similar or slightly greater yield losses than BN-RNA3+5 infection (Fig. 1b). Likewise, BN-RNA5 infection had a similar or somewhat higher level of the virus content in the taproots than BN-RNA3+5 infection (Fig. 4b) Thus, it is possible that in the Rizor cultivar, the presence of RNA3 probably inhibits RNA5 replication (accumulation), which to a certain degree, mitigates the yield loss caused by BN-RNA5 infection.

In the case of susceptible cultivars, BN-RNA3+5 infection produces a massive rootlet proliferation with scab-like symptoms on taproots, which leads to a much more severe

sugar yield reduction. This note may be applicable for resistant cultivars infected by the RB viruses. BNYVV RNA3 has two functions: the development of rootlet proliferation and the facilitation of virus content in roots (Richards and Tamada, 1992; Tamada, 2016). The massive proliferation of fine roots has shown to be due to the interaction of RNA3-encoding p25 protein with auxin-regulated pathways (Fernando *et al*., 2018), but it seems unlikely that RNA5 (or p26 protein) is involved in such a function. The virus content in BN-RNA3 and BN-RNA5 infections were 10 and 3 times higher, respectively, than in BN-ΔRNA3/5 infections (Fig. 4a). Nevertheless, BN-RNA3+5 infections had almost similar or slightly higher levels of the virus content than BN-RNA3 infection (Fig. 4b), suggesting that the presence of RNA5 does not contribute significantly to the enhancement of virus content. Together, it is considered that severe yield losses by BN-RNA3+5 infection in susceptible cultivars is due to synergistic effects of RNA3 and RNA5, each of which has a different pathogenetic effect, rather than the enhancement of virus content.

In field conditions, BNYVV is usually confined to the root system of sugar beets, but if the virus can move from roots to shoots, “yellow vein” symptom appears on leaves (Tamada, 2016). In this study, interestingly, the yellow vein symptom was observed more frequently for BN-RNA3 infection than BN-RNA3+5 infection (Fig. 3), suggesting that the presence of RNA5 reduced the systemic movement of the virus in shoots. It seems likely that the presence of RNA5 may inhibit RNA3 accumulation so that vascular movement of the virus was prevented, because systemic virus infection is dependent on the presence of the RNA3 sequence (Tamada *et al*., 1989; Lauber *et al*., 1998). Alternatively, the presence of RNA5 may physically prevent the vascular movement of the virus at the cellular or tissue level; e.g. RNA5 may induce the formation of barriers that prevent systemic movement.

As regards the RNA3-encoded *p25* gene, a specific amino acid motif “tetrad” at positions 67–70 in the p25 protein is highly variable and has been shown to be involved in the ability of BNYVV to overcome *Rz1*-mediated resistance (see Table 1) (Liu *et al*., 2005; Schirmer *et al*., 2005; Acosta-Leal *et al*., 2008; Chiba *et al*., 2011; Tamada *et al*., 2016). Indeed, Japanese A-II type virus isolates (S, O11 and D104) used in this study (Chiba *et al*., 2011) contain non-RB type RNA3 (AY or AF variant), whereas RNA3

present in Italy A-III type isolates (generally called A-type virus) comprises different levels of RB ability, in which a VC variant (such as Spanish isolate SPC) has the highest level of RB ability (Pferdmenges *et al*., 2009; Acosta-Leal *et al*., 2010; Chiba *et al*., 2011; Bornemann and Varrelmann, 2011). In laboratory tests at the early stage of infection, BN-RNA3 infection (with non-RB type RNA3) had lower levels of viral RNA accumulation in rootlets of the Rizor and *Rz1*-resistant plants, whereas BN-RNA3 infection (with RB type RNA3) had higher levels of viral RNA accumulation in those plants, although there were a few exceptions. Moreover, this study revealed that BN-RNA3+5 infection (with non-BR type RNA3) remarkably increased viral RNA3 accumulation levels in rootlets of the Rizor and *Rz1* plants but did not increase those in the WB42 accession, which has the *Rz2* gene (Fig. 6). Thus, the results indicate that Rizor and *Rz1*-resistances are controlled by a similar mechanism, with which RNA3 is strongly associated, whereas the *Rz2* resistance is governed by different mechanism(s) from that of the *Rz1*.

The observation that the presence of RNA5 increased the level of viral RNA accumulation suggests that RNA5-containing A-II type virus can overcome *Rz1*-resistance. Indeed, in experiments 3 and 4 (Fig. 1c, d), conditioned at a high level of infection, the Rizor cultivar was less resistant to BN-RNA3+5 infection than BN-RNA3 infection, while in experiments 2 and 5 (Fig. 1b, e), conditioned at a low level of infection, Rizor and Schwert (*Rz1*) plants showed almost similar degrees of resistance to both BN-RNA3 and BN-RNA3+5 infections. These results suggest that the effect of RNA5 on virus aggressiveness depends on the level of virus infection, which is influenced by inoculum doses and environmental conditions (Biancardi and Tamada, 2016). Artificial inoculation in this study was conducted using high levels of inoculum doses, and thus infection levels were much higher than under natural field conditions, which are thought to be much lower. In field conditions, regardless of the presence of RNA5, as the roots grow the presence of non-RB type RNA3 preferentially may inhibit viral spread from rootlets to the taproot as pointed out by Tamada *et al*. (1999). Therefore, it is considered that in the case of Japanese strains (A-II type), RNA5 is not so involved in conferring *Rz1*-RB (Table 1).

On the other hand, French P-type strain (with RNA5), which is classified as A-II type (Chiba *et al*., 2011), was shown to have an ability to overcome *Rz1* resistance in greenhouse conditions (Pferdmenges *et al*., 2009; Bornemann and Varrelmann, 2011; Chiba *et al*., 2011). Galein *et al*. (2018) examined the variability and pathogenicity of BNYVV isolates from an area containing A-, B- and P-types, and found that P-type virus (with RNA5) showed the highest aggressiveness, although there is the complexity of genome reassortments between different types of BNYVV strains. Furthermore, Liebe et al. (2020) showed that the presence of P-type RNA5 in the background of the A-type virus clone increased virus accumulation in the resistant plants, suggesting that this P-type RNA5 is involved in *Rz1*-RB. The present study here has provided more definite evidence on the effect of RNA3 and RNA5 on virus accumulation levels in *Rz1*-RB. Thus, in Rizor and *Rz1*-resistant plants, BN-RNA3 infection (with non-RB type RNA3) had low levels of virus accumulation, whereas BN-RNA3+5 infection (with non-RB type RNA3) had high virus accumulation (Fig. 6 b. d). In contrast, it is notable that there were no obvious differences in virus accumulation levels between BN-RNA5 and BN-ΔRNA3/5 infections and between susceptible and resistant plants (Fig. 6 b. d). These results indicate that in the presence of RNA3 (with non-RB type RNA3), RNA5 remarkably increased levels of virus accumulation in roots of Rizor and *Rz1*-resistant plants, but in the absence of RNA3, the presence of RNA5 did not so influence the virus accumulation.

Additionally, it was shown that another Japanese isolate H45, which belongs to the China H (=Japan T) strain in A-I type (Chiba *et al*., 2011), caused more severe yield losses in the Rizor plants, compared with those of A-II type isolates K80 (Japan D strain) and M87 (Japan O strain) in experiment 4 (Fig. 1d). Interestingly, in the laboratory tests, as compared with the O11 isolate (Japan O), the isolate T41 (Japan T) showed higher levels of viral RNA3 accumulation in *Rz1*-resistant plants, which is similar to that of A-III RB type isolates (SLN1 and SPC) (Fig. 7). These results suggest that the Japan T strain is more aggressive to resistant plants than the Japan O or Japan D strain, which also corresponds to those of the incompatible virus-host interaction in rub inoculation tests (Chiba *et al*., 2008, 2011). Thus, there are some variations in virulence among Japanese strains. Although this study did not investigate the aggressiveness of Japan T strain containing RNA5 in resistant cultivars, it would be presumed that the

presence of RNA5 enhances even more virus aggressiveness with increasing degree of the RB ability of RNA3 (Chiba *et al*., 2011; Tamada *et al*., 2016; Galein *et al*., 2018). Further study is needed to clarify how RNA5 contributes to the generation of RB strains.

Acknowledgments

The authors thank O. E. Scholten for kindly providing seeds of WB42 and Holly 1-4 and T. Taguchi for seeds of Schwert and Yukihinode. They also thank M. Fattori (German isolate), R. Subikova (Slovakian isolates), M. E. De Bruyne (Spanish isolate), and C. M. Rush (US isolate) for kindly providing BNYVV samples. This work was supported in part by the Hokkaido Sugar Beet Association.

Conflict of interest

The authors declare that they have no conflict of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supporting Information

Additional supporting information may be found online in the Supporting Information section.

Figure S1. (a) A procedure of virus isolation using bait plants from soil and of preparation of *Polymyxa betae* strains carrying BNYVV isolates. (b) A procedure of *P. betae* zoospore inoculation for field tests. See the text in detail.

Figure S2. An example of field inoculation plots showing symptoms on shoots and roots of sugar beet plants in susceptible cultivar Monomidori (a) and resistant cultivar

Rizor (b) in experiment 1 (Fig. 1a). Plants infected with isolates S-34 (RNA-1+2+3+4) and S-45 (RNA-1+2+4+5) are shown. C shows plots inoculated by BNYVV-free *Polymyxa betae* culture. Photos were taken at harvest time.

Figure S3. Effect of BNYVV RNA3 and RNA5 on the yellowing intensity (measured using SPAD, see text) in leaves of sugar beet plants grown in the field. (a) Correlation between sugar yields and SPAD values. Data were obtained from experiment 1 (triangle) and experiment 2 (circle). (b) SPAD values in leaves of susceptible and resistant plants. Susceptible plants were cultivars Monomidori, Monohikari, Ema (experiments 1 and 5), Mono-ace S, Monohomare (experiments 2, 3 and 5) and Dihill (experiment 3), and resistant plants were cultivars Rizor (experiments 1, 2, 3 and 5) and Schwert (experiment 5) (see Fig. 1). SPAD values were measured on 18 August (experiment 1), 14 September (experiment 2), 24 August (experiment 3) and 9 September (experiment 5). BN-RNA3+5= isolates S and D104, BN-RNA3= isolates S-34 and O11 and BN-RNA5= isolate S-45. Each bar is the average value for replicates, in which the numbers are indicated in parentheses, and includes standard errors. The different letters indicate significant differences ($P \leq 0.05$) between the plots of each of susceptible and resistant plants.

Figure S4. Effect of BNYVV isolates with or without RNA5 on viral RNA accumulation in roots of different sugar beet plants grown in sand culture. Susceptible cultivar Monomidori and resistant cultivar Schwert (*Rz1*) and accession WB42 (*Rz2*) were used. BNYVV isolates used: D104 (RNA-1+2+3+4+5), O11 (RNA-1+2+3+4), D104-5 (RNA-1+2+4+5) and O-11-4 (RNA-1+2+4). See Table 1 for data about these isolates. Root samples of seedlings that had been inoculated with *Polymyxa betae* cultures carrying each of different BNYVV isolates were collected at 18 days post-inoculation. The rows show detection of BNYVV RNA1/2 and RNA3 and a loading control of ethidium bromide-stained 28S rRNA.

Table S1. List of sugar beet cultivars and wild beet accessions used in this study.

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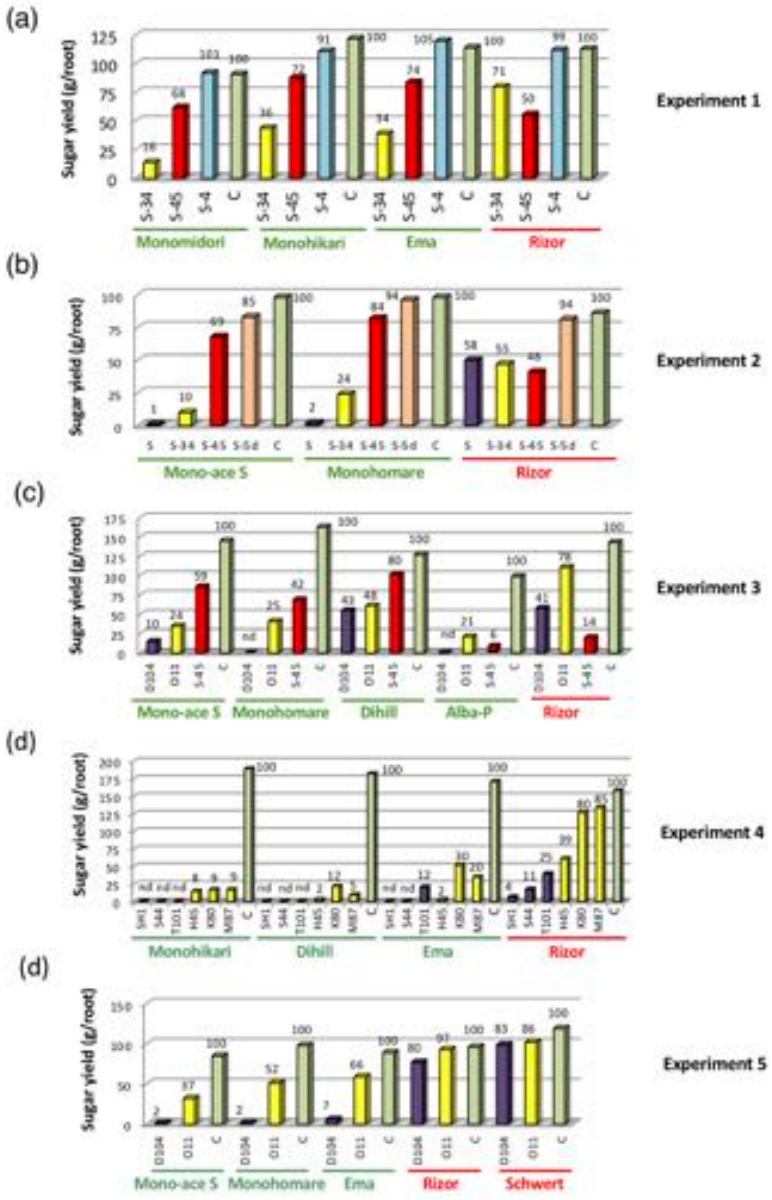

Figure 1. Effects of BNYVV isolates containing RNA3 and/or RNA5 on sugar yields in roots of sugar beet plants grown in the field. (a), (b), (c), (d) and (e) correspond to experiments 1, 2, 3, 4 and 5, respectively. BNYVV isolates used were as follows: isolates S, D104, H45, K80 and T101 (RNA-1+2+3+4+5), S-34, O11, H45, K80 and M87 (RNA-1+2+3+4), S-45 (RNA-1+2+4+5), S-5d (RNA-1+2+4+5d, RNA5 having an internal deletion), and S-4 (RNA-1+2+4). See Table 1 for data of those isolates. C shows plots inoculated by BNYVV-free *Polymyxa betae* culture (control). Sugar beet cultivars used these experiments are listed in Table S1; seven susceptible cultivars and two resistant cultivars Rizor and Schwert. Sugar beet roots were harvested at the end of October, and groups of 40 roots (experiment 1) or 20 roots (experiments 2, 3, 4 and 5) per plot were tested for yield parameters and quality parameters. Only sugar yields are shown by a bar graph. Figures on the bar indicate the relative values against each control plot (%). nd=not determined, because of no sufficient yield to evaluate.

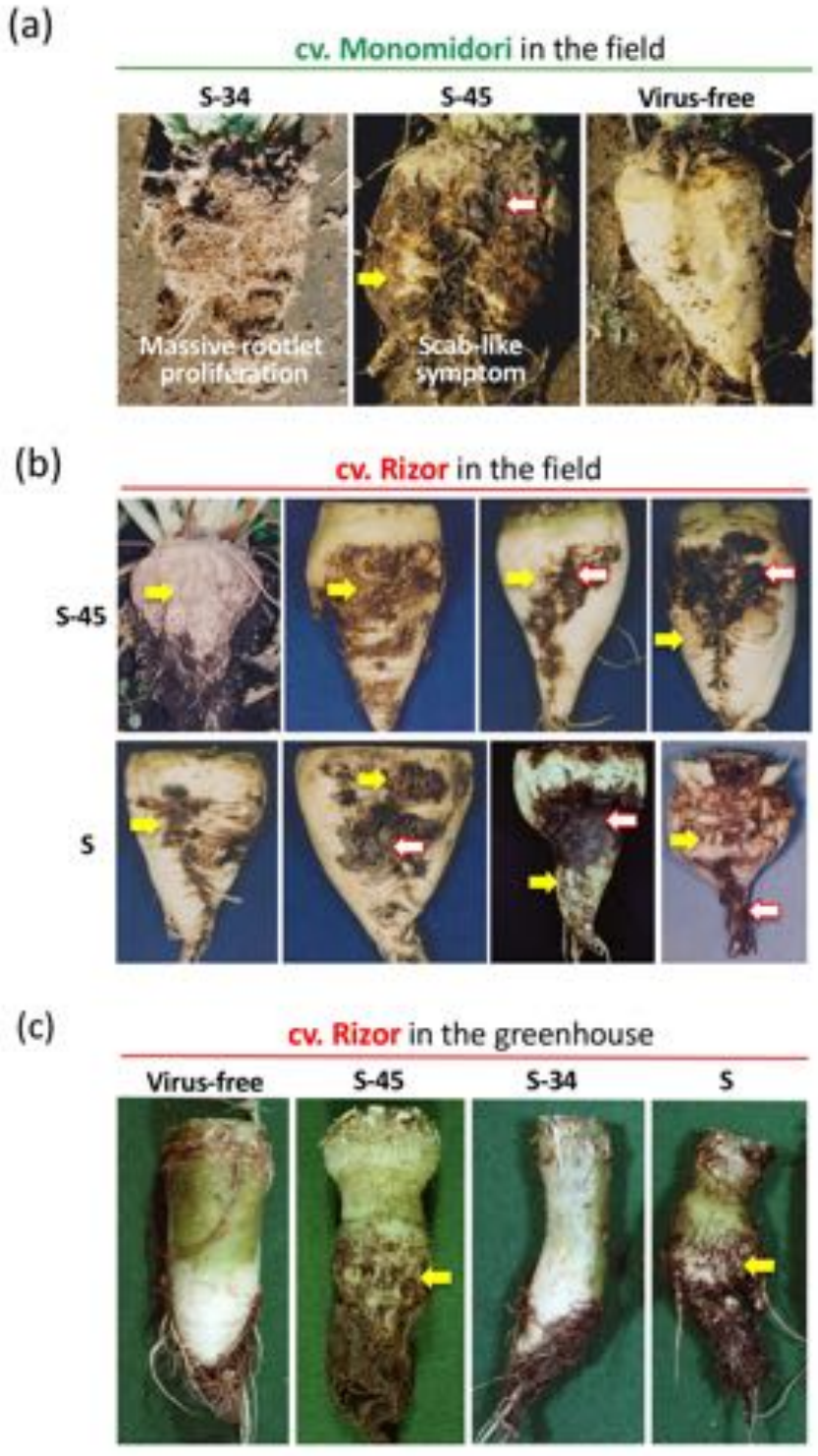

Figure 2. Various types of symptoms produced by infection of BNYVV isolates with RNA3 and/or RNA5 on the roots of sugar beets. (a) Susceptible cultivar Monomidori in the field. (b) and (c) Resistant cultivar Rizor in the field and greenhouse, respectively. Isolates S (RNA-1+2+3+4+5), S-34 (RNA-1+2+3+4) and S-45 (RNA-1+2+4+5) are used. Arrows indicate rough surface (mild scab-like) symptoms (yellow) and progression into scab-like symptoms (red frame).

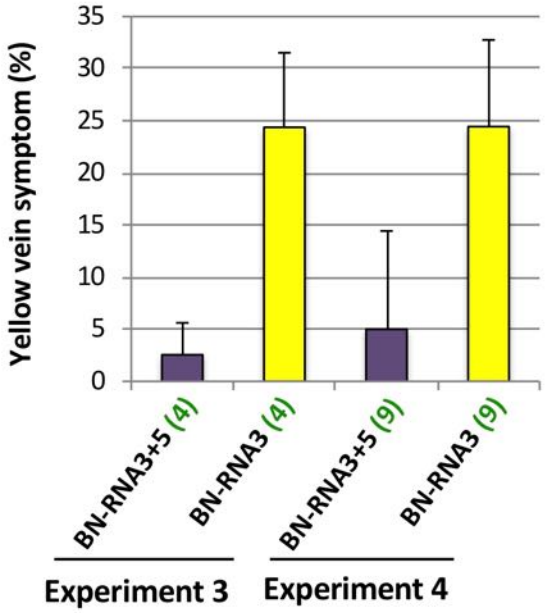

Figure 3. Effect of BNYVV RNA3 and RNA5 on the development of yellow vein symptoms in shoots of susceptible plants grown in the field. Yellow vein symptoms (%) were obtained susceptible cultivars from experiment 3 (Fig. 1c) and experiment 4 (Fig. 1d). In experiment 3, BN-RNA3+5= isolate D104 and BN-RNA3=isolate O11. In experiment 4, BN-RNA3+5= isolates SH1, S44 and T101 and BN-RNA3= isolates H45, K80 and M87. Each bar is the average value for replicates, in which the numbers are indicated in parentheses, and includes standard errors. Note that yellow vein symptom was not observed on sugar beet plants inoculated with isolate S-45 (RNA-1+2+4+5).

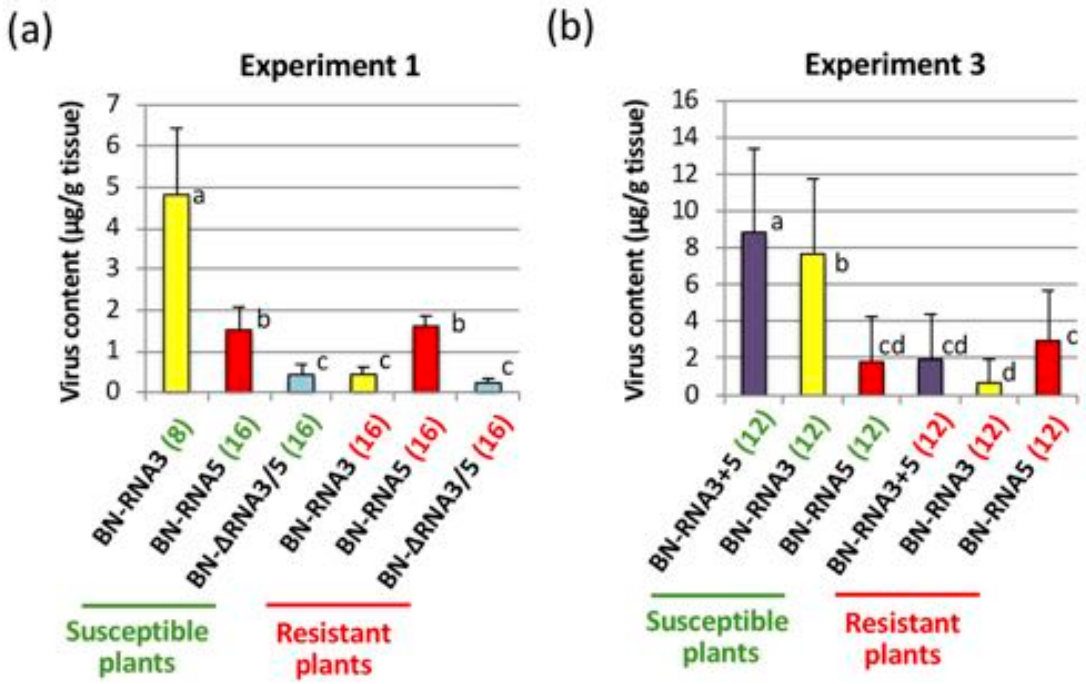

Figure 4. Effect of BNYVV RNA3 and RNA5 on the virus content in taproots of sugar beet plants grown in the field. (a) Data were obtained from experiment 1 (Fig. 1a). Susceptible plants were cultivar Monohikari, and resistant plants were cultivar Rizor. BN-RNA3= isolate S-34, BN-RNA5= isolate S-45 and BN-ΔRNA3/5= isolate S-4. (b) Data were obtained from experiment 3 (Fig. 1c). Susceptible plants were cultivar Mono-ace S, and resistant plants were cultivar Rizor. BN-RNA3+5= isolate D104, BN-RNA3= isolate O11 and BN-RNA5= isolate S-45. Each bar is the average value for samples, in which the numbers are indicated in parentheses, and includes standard errors. The different letters indicate significant differences ($P \leq 0.05$).

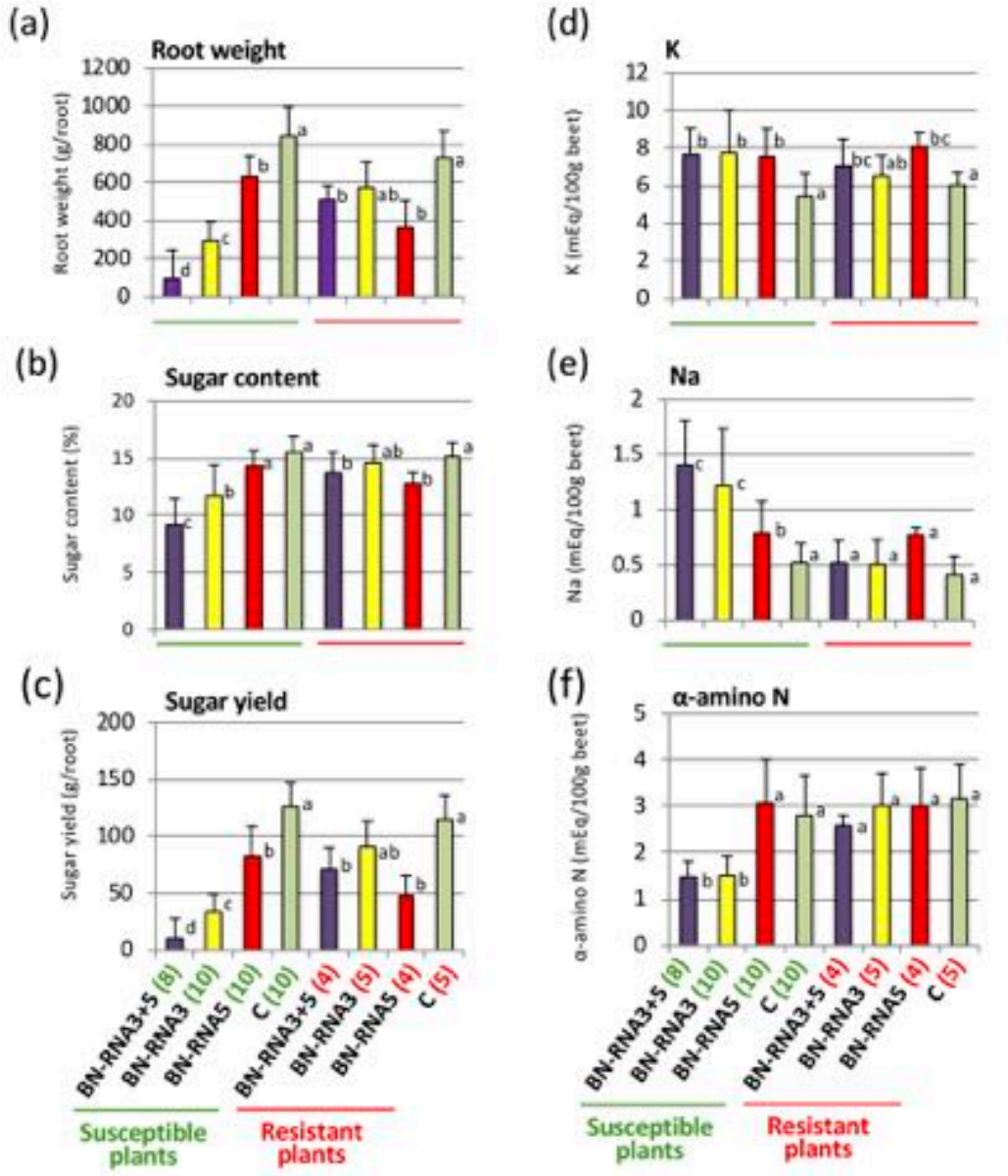

Figure 5. Effect of BNYVV RNA3 and RNA5 on yield parameters (a, b and c) and quality parameters (d, e and f) in roots of sugar beet plants grown in the field. (a) root weight, (b) sugar content, (c) sugar yield, (d) K content, (e) Na content and (f)α-amino N content. Susceptible plants were cultivars Monomidori, Monohikari, Ema (experiments 1 and 5), Mono-ace S, Monohomare (experiments 2, 3 and 5) and Dihill (experiment 3), and resistant plants were cultivars Rizor (experiments 1, 2, 3 and 5) and Schwert (experiment 5). BN-RNA3+5=isolates S and D104, BN-RNA3= isolates S-34 and O11, and BN-RNA5=isolate S-45. Each bar is the average value for replicates, in which the numbers are indicated in parentheses, and includes standard errors. The different letters indicate significant differences ($P \leq 0.05$) between the plots of each of susceptible and resistant plants.

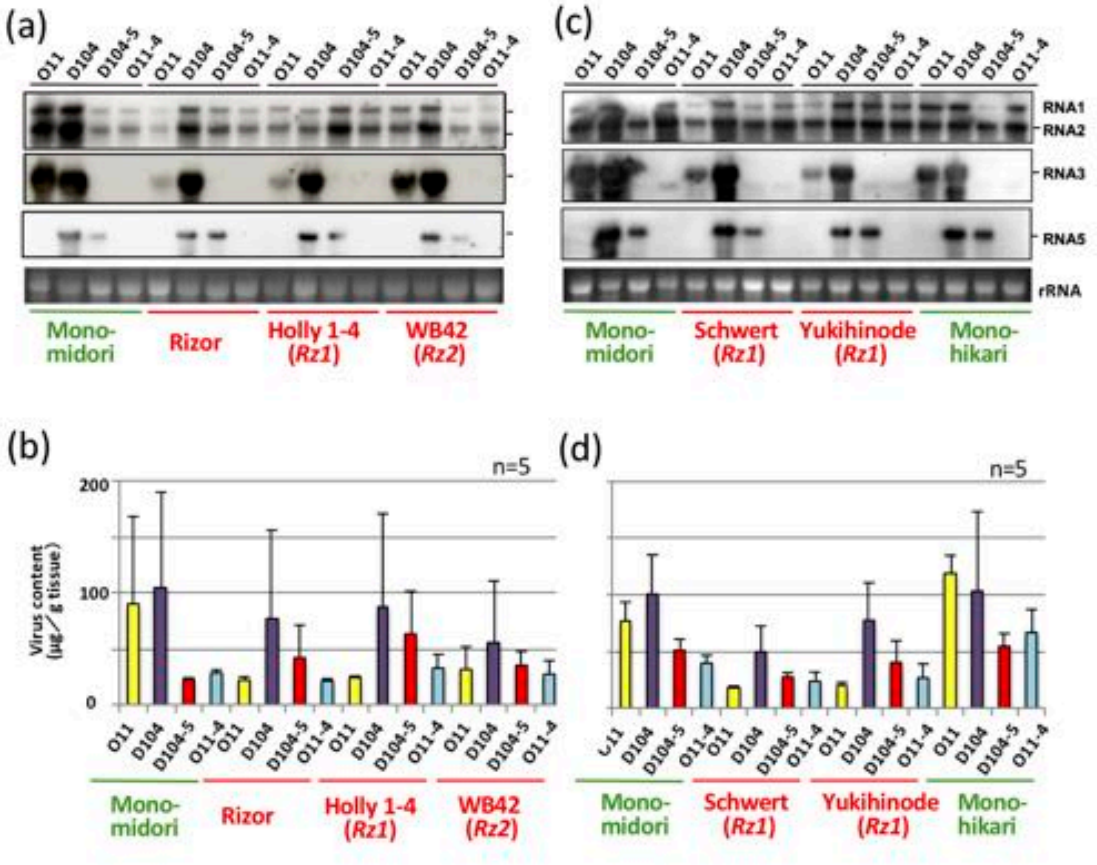

Figure 6. Effect of BNYVV RNA3 and RNA5 on viral RNA accumulation (a and c) and virus accumulation (b and d) in roots of sugar beet and wild sea beet (*Beta vulgaris* subsp. *maritima*) plants grown in sand culture. (a and b) Experiment 1. (c and d) Experiment 2. BNYVV isolates used: D104 (RNA-1+2+3+4+5), O11 (RNA-1+2+3+4), D104-5 (RNA-1+2+4+5) and O-11-4 (RNA-1+2+4). Root samples of seedlings that were inoculated with *Polymyxa betae* cultures carrying each of different BNYVV isolates were collected at 18 or 21 days post-inoculation in experiment 1 or 2, respectively. The rows (a and c) show detection of BNYVV RNAs 1 and 2, RNA3 and RNA5, and a loading control of ethidium bromide-stained 28S rRNA. The virus content (µg/g tissue) by ELISA (b and d) shows the mean values from five plants and includes standard errors.

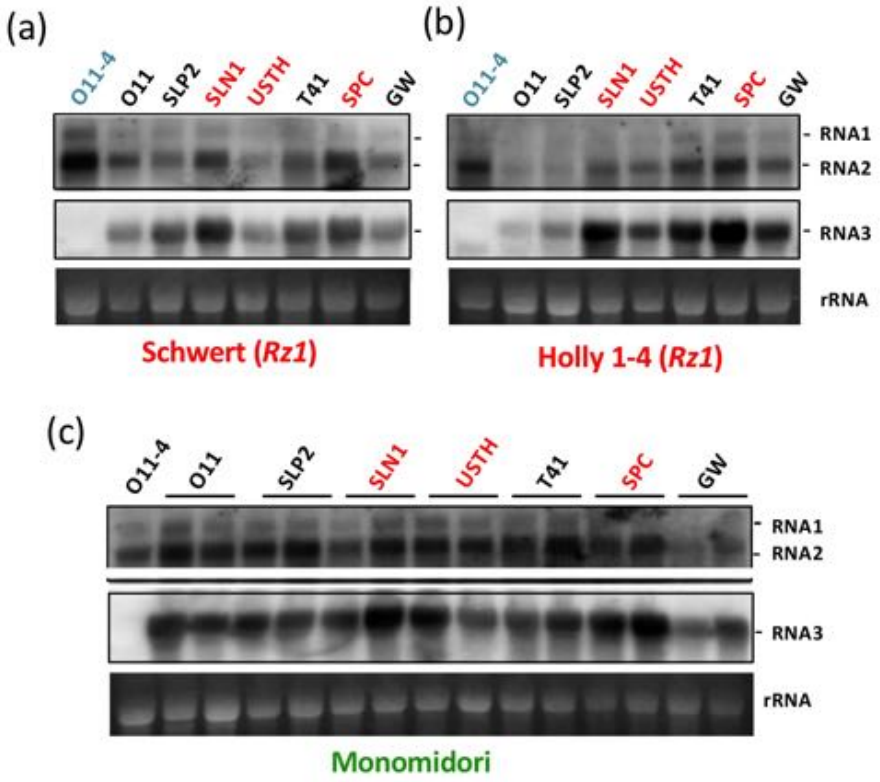

Figure 7. Effect of *Rz1*-resistance breaking BNYVV isolates (without RNA5) on viral RNA accumulation in roots of different sugar beet plants grown in sand culture. (a) Schwert (*Rz1*), (b) Holly-1-4 (*Rz1*) and (c) Monomidori (susceptible cultivar). Non-RB isolates: O11, SLP2, T41 and GW; RB isolates: SLN1, USTH and SPC (see Table 1). Isolate O11-4 (RNA-1+2+4) that lacks RNA3 was used as control. Root samples of seedlings that were inoculated with *Polymyxa betae* cultures carrying each of different BNYVV isolates were collected at 20 days post-inoculation. The rows show detection of BNYVV RNAs 1and 2 and RNA3 and a loading control of ethidium bromide-stained 28S rRNA.

Supporting Information

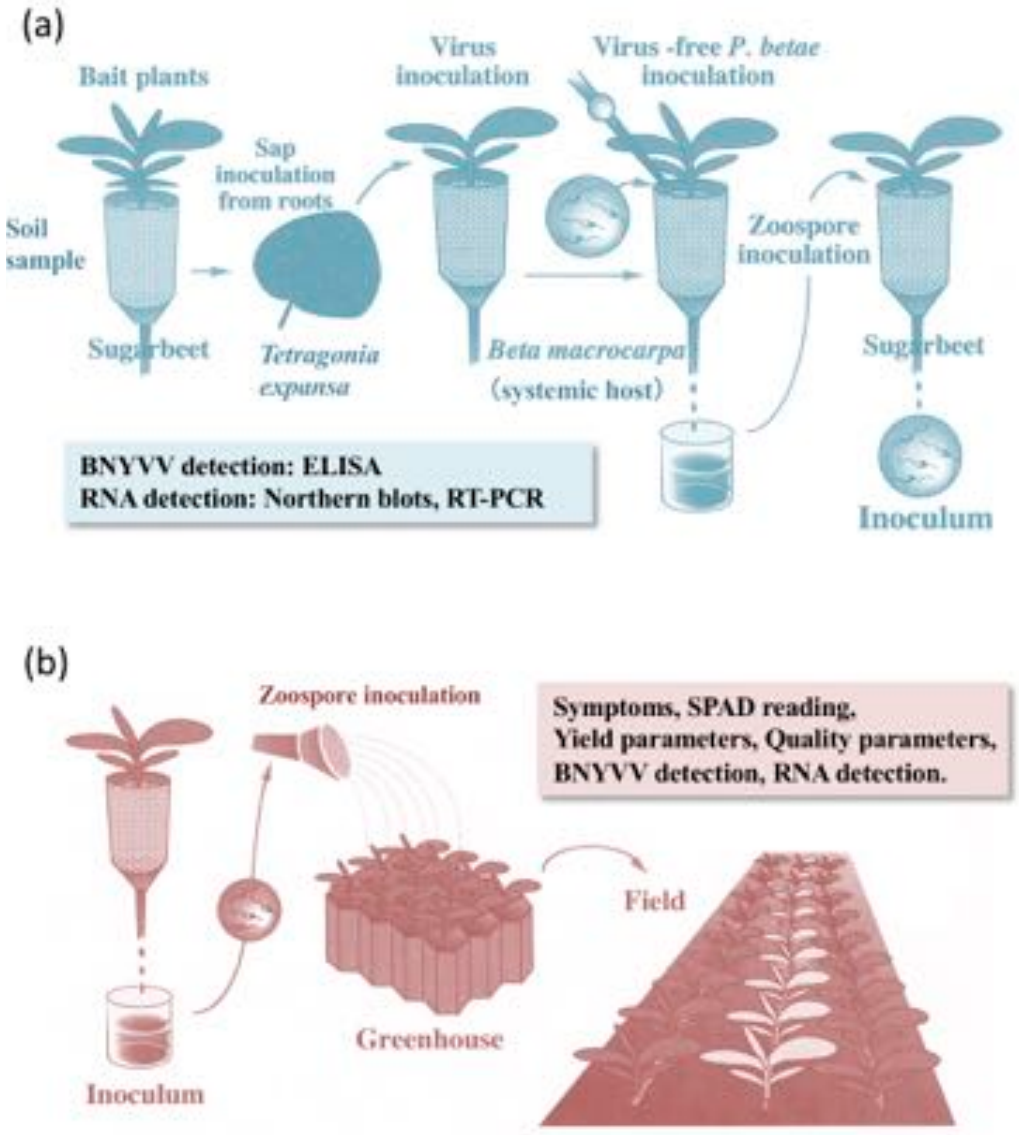

Figure S1. (a) A procedure of virus isolation using bait plants from soil and of preparation of *Polymyxa betae* strains carrying BNYVV isolates. (b) A procedure of *P. betae* zoospore inoculation for field tests. See the text in detail.

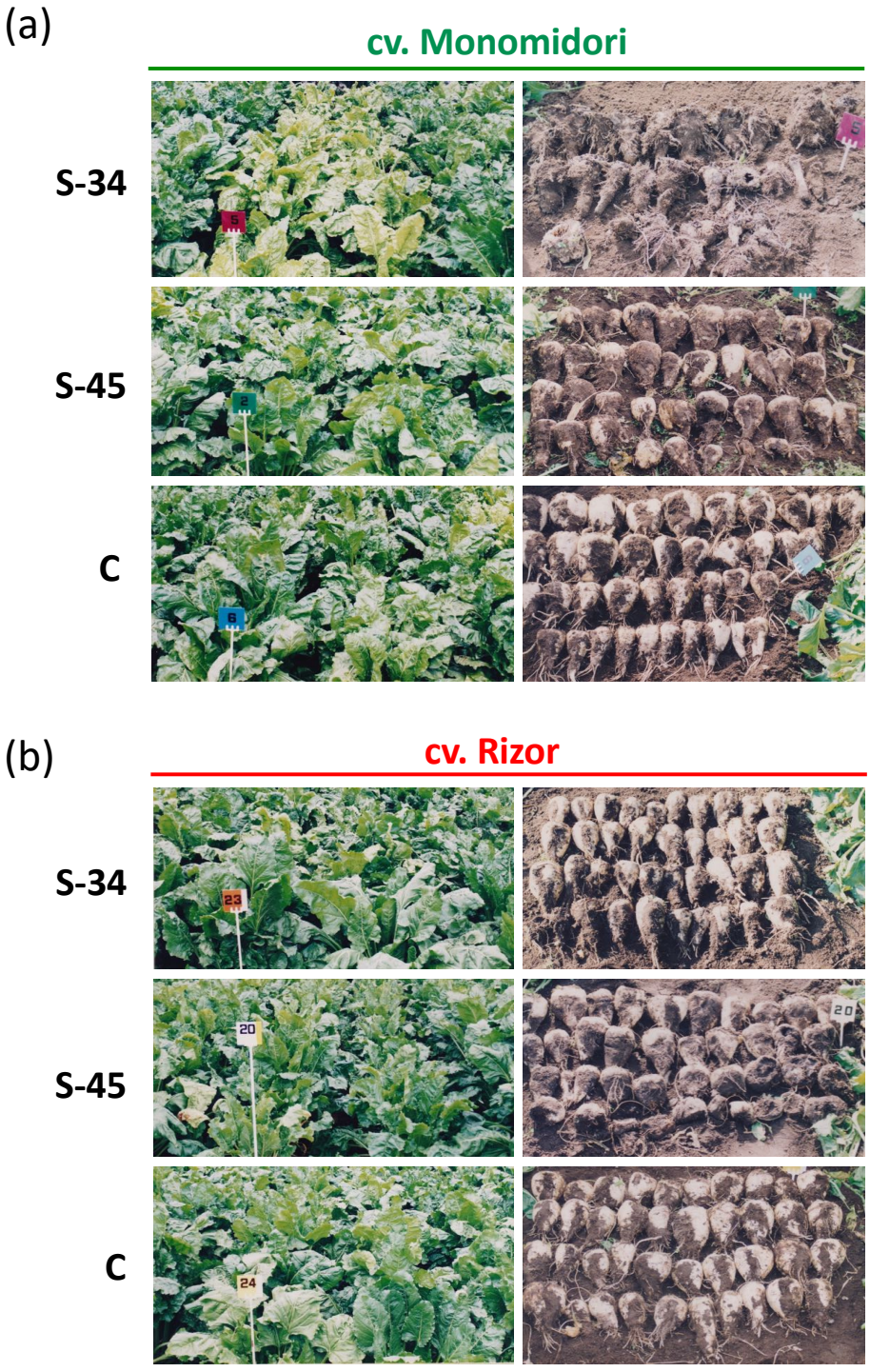

Figure S2. An example of field inoculation plots showing symptoms on shoots and roots of sugar beet plants in susceptible cultivar Monomidori (a) and resistant cultivar Rizor (b) in experiment 1 (Fig. 1a). Plants infected with isolates S-34 (RNA-1+2+3+4) and S-45 (RNA-1+2+4+5) are shown. C shows plots inoculated by BNYVV-free *Polymyxa betae* culture. Photos were taken at harvest time.

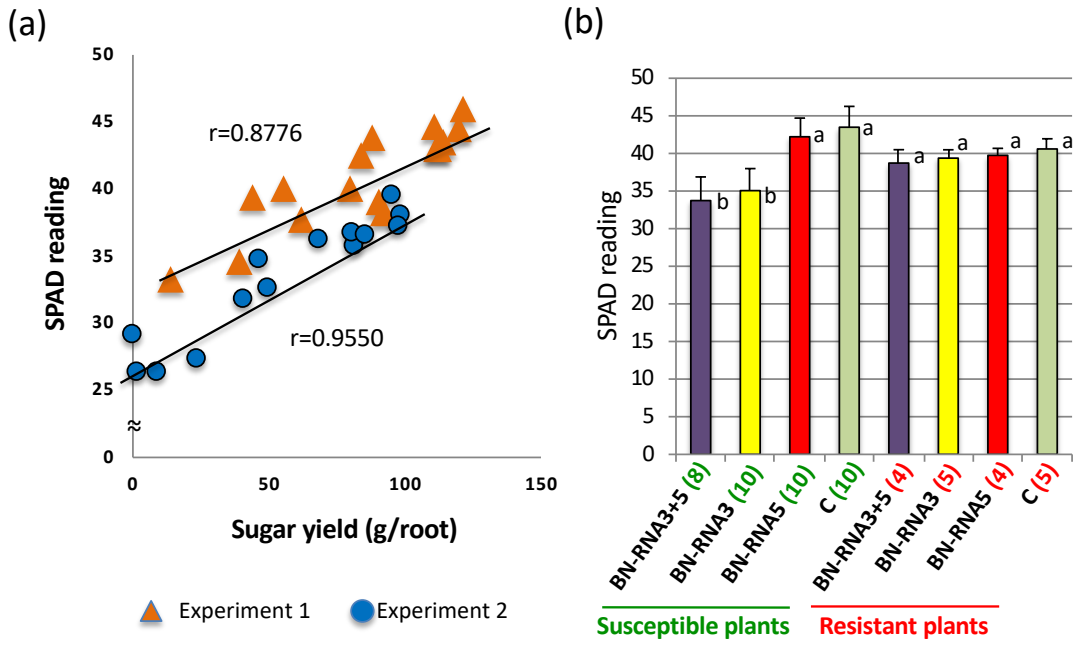

Figure S3. Effect of BNYVV RNA3 and RNA5 on the yellowing intensity (measured using SPAD, see text) in leaves of sugar beet plants grown in the field. (a) Correlation between sugar yields and SPAD values. Data were obtained from experiment 1 (triangle) and experiment 2 (circle). (b) SPAD values in leaves of susceptible and resistant plants. Susceptible plants were cultivars Monomidori, Monohikari, Ema (experiments 1 and 5), Mono-ace S, Monohomare (experiments 2, 3 and 5) and Dihill (experiment 3), and resistant plants were cultivars Rizor (experiments 1, 2, 3 and 5) and Schwert (experiment 5) (see Fig. 1). SPAD values were measured on 18 August (experiment 1), 14 September (experiment 2), 24 August (experiment 3) and 9 September (experiment 5). BN-RNA3+5= isolates S and D104, BN-RNA3= isolates S-34 and O11 and BN-RNA5= isolate S-45. Each bar is the average value for replicates, in which the numbers are indicated in parentheses, and includes standard errors. The different letters indicate significant differences ($P \leq 0.05$) between the plots of each of susceptible and resistant plants.

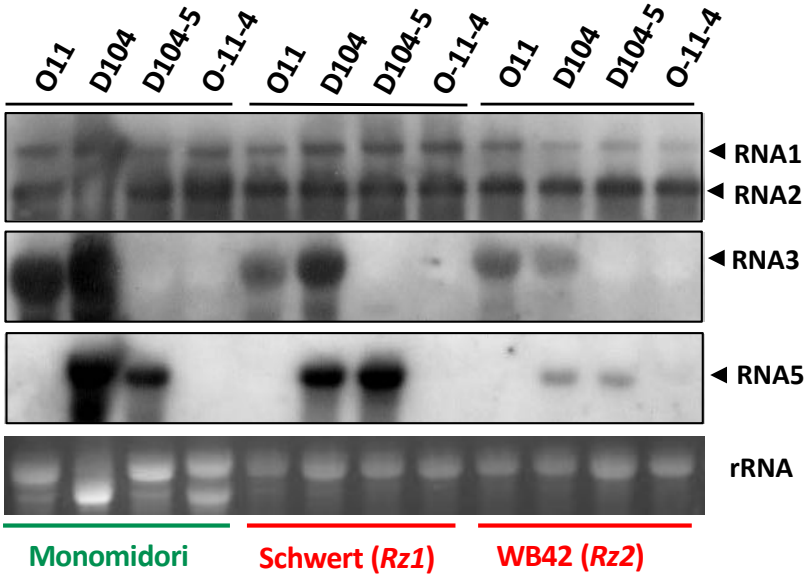

Figure S4. Effect of BNYVV isolates with or without RNA5 on viral RNA accumulation in roots of different sugar beet plants grown in sand culture.
Susceptible cultivar Monomidori and resistant cultivar Schwert (*Rz1*) and accession WB42 (*Rz2*) were used. BNYVV isolates used: D104 (RNA-1+2+3+4+5), O11 (RNA-1+2+3+4), D104-5 (RNA-1+2+4+5) and O-11-4 (RNA-1+2+4). See Table 1 for data about these isolates. Root samples of seedlings that had been inoculated with *Polymyxa betae* cultures carrying each of different BNYVV isolates were collected at 18 days post-inoculation. The rows show detection of BNYVV RNA1/2 and RNA3 and a loading control of ethidium bromide-stained 28S rRNA.

Table S1. List of sugar beet cultivars and wild beet accessions used in this study.

Sugar beet cultivar and accession	Resistance gene	Breeding country and breeding line
Monomidori	no	Japan, T1021
Monohikari	no	Japan, HK55
Mono-ace S	no	Germany, Kawe J338
Monohomare	no	Japan, HK55
Ema	no	Sweeden, HT1
Dihill	no	Sweden, Hill mono 829
Alba P[a]	?	Italy, selection from Alba sources
Rizor	Rizor	Belgium, SES IR2, from Alba sources
Holly 1-4	*Rz1*	USA, selection from Holly sources
WB42	*Rz2*	Denmark, selection from *Beta vulgaris* subsp. *maritima*
Schwert	*Rz1*	Japan, HK70
Yukihinode	*Rz1*	Japan, HK83

[a]Alba P was originally bred for resistance to Cercospora leaf spot, along with rhizomania (Panella & Biancardi, 2016), but this line was thought to be susceptible to rhizomania disease.