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Assessment of beet leaf yellowing virus tolerance based on leaf yellowing in sugar beet

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Abstract

Sugar beet (*Beta vulgaris*) production in Japan faces major challenges from virus yellows (VY), caused by beet leaf yellowing virus (BLYV) and transmitted by aphids. Outbreaks have reduced sugar yields, and breeding for tolerant varieties has not been conducted in Japan. This study marks the first step toward developing VY-tolerant varieties by testing three hypotheses: (1) leaf yellowing can be induced by artificial inoculation, (2) tolerance varies among breeding materials, and (3) differences in tolerance to yellowing disease can be evaluated. From 2017 to 2020, four sugar beet materials were grown in inoculated and uninoculated plots and assessed using two methods: the yellowing index (YI), scored as 0–3, and the yellowing area (YA), calculated from digital images. Triple antibody sandwich–enzyme-linked immunosorbent assay confirmed that all BLYV-inoculated plants were infected and exhibited marked yellowing compared with uninoculated plants. Analysis of variance (ANOVA) applied to YI and YA data revealed that inoculation significantly influenced yellowing, symptoms progressed over time, and yellowing progression varied by material. Additionally, YI and YA were significantly correlated, with a Spearman correlation coefficient (rs) of 0.718. The significant correlations between YI or YA values and sugar yield loss (rs = 0.86–0.87) and root weight loss (rs = 0.80–0.83), but no significant correlation with Brix loss (rs = 0.32–0.46). These results validate the tested methods for evaluating BLYV tolerance and highlight the potential for breeding sugar beet varieties with enhanced tolerance. Moreover, the findings offer valuable insights for future VY tolerance breeding programs.

Plain Language Summary

Sugar beet (*Beta vulgaris*) farming in Japan faces major challenges due to virus yellows (VY), caused by the beet leaf yellowing virus and spread by aphids. This disease

Abbreviations: ANOVA, analysis of variance; BChV, beet chlorosis virus; BLYV, beet leaf yellowing virus; BMYV, beet mild yellowing virus; BWYV, beet western yellows virus; BYV, beet yellows virus; HARC, Hokkaido Agricultural Research Center; OD, optical density; TAS-ELISA, triple antibody sandwich–enzyme-linked immunosorbent assay; VY, virus yellows; YA, yellowing area; YI, yellowing index.

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reduces sugar yields, and there has been no breeding of tolerant varieties in Japan. This study is the first step toward developing VY-tolerant sugar beets. The researchers tested three ideas: (1) artificial infection can cause leaf yellowing, (2) different materials have different levels of tolerance, and (3) tolerance can be measured. From 2017 to 2020, four types of sugar beets were grown in both infected and noninfected areas. They measured yellowing using two methods: a yellowing index and yellowing area. The results showed that infected plants had more yellowing, and suffered greater yield losses. The study confirmed that these methods can be used to evaluate VY tolerance and provided useful information for breeding more tolerant varieties in the future.

1 | INTRODUCTION

Sugar beet (*Beta vulgaris*), one of the major sugar crops cultivated worldwide alongside sugarcane, is grown exclusively in Hokkaido, Japan's northernmost island. In recent years, Japan's sugar self-sufficiency rate has reached ~35%, with sugar beet contributing 82% of domestic sugar production (MAFF, 2024). Four major diseases threaten sugar production in Japan: *Cercospora* leaf spot, *Aphanomyces* root rot, *Rhizoctonia* root rot, and rhizomania. Additionally, virus yellows (VY) has emerged as a new disease requiring attention. Indeed, a major VY outbreak in Japan has already caused substantial damage to sugar beet production. From 2009 to 2014, when VY was prevalent, annual sugar production averaged 583,000 tonnes, well below the 659,000 tonnes average from 1999 to 2023 when VY had less impact (FAOSTAT, 2024). Although factors such as high summer temperatures and the co-occurrence of other diseases, including *Cercospora* leaf spot and *Aphanomyces* root rot, contribute to yield declines, the VY epidemic is considered a primary factor in declining sugar production.

Major VY outbreaks in Japan have occurred every 10–20 years since the 1950s (Kuroda et al., 2016). Although the cause of these outbreaks is unknown, aphids, as the virus vectors, are a key factor. Controlling VY in Japan currently depends on spraying neonicotinoid insecticides, which are highly effective against aphids (Hauer et al., 2017; Qi et al., 2004). However, the European Union banned most neonicotinoids in 2018 due to their environmental impact (Goulson, 2018; Jones et al., 2014), leading to substantial economic losses during subsequent VY outbreaks (Dewar & Qi, 2021). Aphids, being polyphagous pests infesting over 50 plant families and transmitting more than 100 viruses (Blackman & Eastop, 2000; Kennedy et al., 1962), complicate outbreak control.

VY causes yellowing symptoms in sugar beets and involves several pathogenic viruses worldwide (Stevens et al., 2005), including beet yellows virus (BYV), beet western yellows virus (BWYV), beet mild yellowing virus (BMYV), beet chlorosis virus (BChV), and beet leaf yellowing virus (BLYV). Although all five viruses are taxonomically distinct species transmitted primarily by the peach aphid (*Myzus persicae*) (Kozłowska-Makulska et al., 2009; Watson, 1940), they differ in genetic characteristics and host plants (Stevens et al., 2005; Yoshida & Tamada, 2019). Furthermore, virus distribution varies regionally: BYV, BWYV, BMYV, and BChV are reported in Europe and the United States (Duffus, 1960, 1961; Duffus & Liu, 1991; Stevens et al., 2006). In Japan, BYV and BWYV were previously identified (Sugimoto & Murayama, 1972; Sugimoto et al., 1970), but surveys in recent years indicate that only BLYV is present. BLYV, belonging to the *Polerovirus* genus of the Luteoviridae family, was previously classified as BWYV, native to the United States, but was renamed owing to host-specificity differences and an 86% amino acid sequence identity with BWYV (Yoshida & Tamada, 2019).

BLYV infection causes interveinal yellowing in the middle to lower sugar beet leaves (Figure 1a,b). Affected leaves thicken and become brittle, whereas immature central leaves remain largely unaffected. Infected fields exhibit circular patches several meters in diameter from around July (Figure 1c), eventually turning entirely yellow. The importance of breeding tolerant (or resistant) varieties arises not only from pesticide regulations but also from aphid resistance, as well as the costs and environmental impact of chemical controls (Bass et al., 2014; Zhang et al., 2008). Resistance breeding began in Europe in the 1940s and the United States in the 1950s (Biancardi et al., 2012), but no such efforts have been made in Japan.

To advance VY tolerance breeding, three conditions must be met (Kuroda et al., 2016). First, methods must be established to infect plants, induce symptoms, and evaluate yellowing. Second, tolerant genetic resources must be identified. Third, tolerance heritability must be elucidated.

Although the third condition is crucial for breeding efficiency, the first and second conditions are fundamental for initiating variety development.

Previous studies suggest that the first and second conditions are achievable. Yellowing symptoms can be induced via sap inoculation using infected aphids (Takeda et al., 1970). Our preliminary tests confirmed the onset of yellowing and suggested variation in material tolerance (Y. Kuroda, unpublished data). Yellowing symptoms are easily identified, and prior evaluations have used graded scores based on symptom severity, ranging from no yellowing to all mature leaves affected (Lewellen & Skoyen, 1984; Takeda et al., 1970). In recent years, digital imaging has been applied for disease diagnosis (Abbas et al., 2023; Jafar et al., 2024; Kouadio et al., 2023).

The present study aims to take the first step in breeding VY-tolerant sugar beet varieties in Japan. Over four years, outdoor cultivation tests were conducted to test three hypotheses: (1) leaf yellowing can be induced by artificial inoculation, (2) tolerance varies among breeding materials, and (3) tolerance differences can be evaluated.

Core Ideas

- Sugar beet production in Japan is impacted by virus yellows (VY), highlighting the need for tolerant varieties.
- The study confirmed that inoculation can induce yellowing and that tolerance varies among breeding materials.
- The methods used for evaluating yellowing are effective and can aid in breeding VY-tolerant sugar beet varieties.

2 | MATERIALS AND METHODS

2.1 | Plant materials

Field trials to evaluate VY tolerance were conducted from 2017 to 2020. Four materials with varying VY tolerance were selected based on a 2016 preliminary experiment (Y. Kuroda, unpublished data). The materials, listed by tolerance levels, were as follows: CR951-210 (strong tolerance), NK-210BR (medium tolerance), Lycka (medium–weak tolerance), and NK-323 mm-O (weak tolerance).

The origin and characteristics of each material are as follows. CR951-210 is a multigerm, self-compatible sugar beet strain tolerant to VY caused by combinations of BYV and BChV. It exhibits high sugar concentrations and yields under diseased conditions (https://npgsweb.ars-grin.gov/gringlobal/accessiondetail?id=1750192; Panella et al., 2015). This strain was registered and released by the United States Department of Agriculture in 2008. NK-210BR is a multigerm, self-incompatible strain with high sugar content and combining ability. Bred from TA-30 by the Hokkaido Agricultural Research Center (HARC) in 1987, it served as the pollen parent of the high-yielding variety Kabutomaru, registered in 1999. Lycka is a high-yielding F1 variety bred by Syngenta and reed in 2008 in Japan. During severe VY outbreaks, it displayed prominent yellowing symptoms. NK-323 mm-O is a monogerm, self-compatible strain with high sugar content. Bred from TA-30 by HARC in 2001, it was selected as a weak-tolerance material owing to its pronounced leaf yellowing in preliminary experiments.

2.2 | BLYV inoculation and field trials

Field trials were conducted during 2017–2020 in two plots: inoculated with BLYV and uninoculated. In 2017, the plots were located ~1 km apart, whereas in 2018–2020, they were adjacent within the same field. In both plots, four materials were sown in paper pots (Nippon Beet Sugar Manufacturing Co. Ltd.) in early April, grown in a greenhouse for approximately 40 days, and then planted in the field. The seedlings used in the inoculated plots were inoculated with BLYV following the method of Takashino et al. (2017). Green peach aphids propagated on BLYV-infected plants were introduced at a ratio of three to five aphids per seedling approximately 30 days after sowing (BBCH stage 12–14) in a ventilated box for around 5 days. Seedlings were then sprayed with neonicotinoid insecticide and grown in a greenhouse for an additional 5 days; thus, planting occurred approximately 40 days after sowing. Specifically, inoculated and uninoculated seedlings were planted in isolated fields in the Sapporo station of HARC in late May.

Infection status was confirmed using triple antibody sandwich (TAS)–enzyme-linked immunosorbent assay (ELISA) tests for all plants in both plots each year, except uninoculated plots in 2017. ELISA Test Kits for BWYV (Agdia) were used, as they also reliably detect BLYV (e.g., Yoshida & Tamada, 2019). Optical density (OD) values were compared to three times those of uninfected negative controls after 1 h. Samples exceeding this threshold were judged positive; others were deemed negative. Tests were conducted twice annually, in June and August, with the second test limited to plants testing negative in the first test.

Field trials had a random blocking design with four replications per material. Five plants per material were grown at a density of 120.0 cm between rows × 22.5 cm between plants in adjacent inoculated and uninoculated plots with one row of uninoculated plants placed as a buffer between the two plots. No physical barrier such as a windbreak was installed between the plots; however, in 2017, as an exception, the

inoculated and uninoculated plots were located approximately 1 km apart.

Fertilizer and cultivation management followed conventional methods. However, weeding (herbicide application and hand weeding) was frequently conducted to distinguish sugar beet leaves from weeds in aerial photographs, facilitating leaf size estimation. Insecticides were applied 10 times, including three to four applications of neonicotinoids, to prevent natural infection from the inoculated to the uninoculated plot.

Roots were harvested in late October and yield-related traits were investigated. The yield-related traits assessed included root weight (g), Brix (%), and sugar yield (g). Sugar yield was calculated by multiplying root weight by Brix. The values for each of these traits were measured in both inoculated and non-inoculated plots, and the percentage of yield loss due to inoculation was calculated using the following formula:

$$\text{Yield loss}\,(\%) = (1 - \text{value in inoculated plot} / \text{value in uninoculated plot}) \times 100$$

2.3 | Evaluation of leaf yellowing

Yellow symptoms are typically characterized by yellowing between the veins of mature leaves (Figure 1a). Two methods, the yellowing index (YI) and yellowing area (YA), were used to evaluate symptoms.

YI is evaluated using four main scores, ranging from 0 to 3: 0 denotes no disease symptom, 1 indicates partial yellowing of mature leaves, 2 represents extensive yellowing of mature leaves, and 3 shows full yellowing of mature leaves (Figure 2). Each score, from 0 to 3, corresponds to approximately 0%, 30%, 60%, and 90% of yellowing in mature leaves, respectively, excluding unexpanded young leaves at the center of the plant. Additionally, two to three minor scores were included between the main scores to capture intermediate degrees of yellowing. In early infection stages, yellowed leaves often appear at the plant periphery, under overlapping leaves. Therefore, evaluation involved manually separating yellowed leaves from the total leaves of each plant.

YA was determined by processing aerial digital images of the field in late September, when yellowing symptoms were most pronounced. Images were HSV-converted, and the number of pixels representing total leaf area was counted within a hue range of 30–125. Pixels representing the yellowed area were identified within a hue range of 30–70. The ratio of yellowed pixels to total pixels was calculated as a percentage (Figure 3). Image processing was performed using ImageJ software.

Based on our preliminary test indicating that yellowing symptoms become visible by late July (Y. Kuroda, unpublished data), this evaluation was conducted monthly from late June through late September. However, for YA, an additional survey was conducted in early October, just before harvest, when the values were more pronounced.

2.4 | Statistical analysis

Analysis of variance (ANOVA) was conducted on the OD values from TAS-ELISA tests, with inoculation status (two types), survey year (3 years: 2018–2020), and material (four types) as factors. ANOVA was also performed on YI and YA values, using survey date, inoculation status (two types), material (four types), and survey year (4 years) as factors. Tukey–Kramer multiple comparison tests were employed to identify differences among materials. YI values were Box–Cox transformed, and YA percentages were arcsine transformed for statistical analyses. Spearman's rank correlation coefficient was calculated to assess the relationship between YI and YA, and the relationship between YI or YA and each yield-related trait (sugar yield, root weight, and Brix). For this calculation, the values of YI and YA from the final survey were used. However, when calculating correlations between each yield-related trait, the year 2017, in which the two plots were far apart, was excluded from the analysis. Statistical analyses were performed using R version 4.3.1.

3 | RESULTS

3.1 | BLYV inoculation

TAS-ELISA analysis confirmed that all plants in the inoculated plots were infected across all years (2017–2020), with a significant increase in the OD values observed for all materials during the early growth stage (June). In contrast, no infection was detected in uninoculated plots, even during the late growth stage (August). The OD values differed significantly between inoculated and uninoculated plants. Although year-to-year variation was significant, no significant differences were observed among the materials (Table 1).

3.2 | Factors affecting leaf yellowing

ANOVA for the YI revealed that "inoculation" (Factor A), "evaluation date" (Factor B), "material" (Factor C), and "evaluation year" (Factor D) were all significant (Table 2). The effects of these factors were ranked by F value as follows: Factor A, Factor B, Factor C, and Factor D. Interactions involving multiple factors were not significant when combining Factors C and D. Regarding YA, results were similar to those for YI, although significant interactions were observed when



FIGURE 1 Photographs of mature leaves showing (a) the typical symptoms of leaf yellowing caused by beet leaf yellowing virus (BLYV) infection and (b) an unaffected leaf with no symptoms. (c) Photograph of a commercial sugar beet field taken in September, showing yellowing plants growing in a circular pattern.

TABLE 1 Effects of inoculation status, year, and material on the optical density (OD) values based on the triple antibody sandwich–enzyme-linked immunosorbent assay (TAS-ELISA) method.

Effect	Sum square	*df*	*F* value	Significance
Inoculation (A)	544.9	1	7562.9	**
Year (B)	14.2	2	98.2	**
Material (C)	0.5	3	2.1	
A × B	14.5	2	100.5	*
B × C	1.2	6	2.9	**
A × C	0.5	3	2.2	
A × B × C	1.2	6	2.8	
Residuals	50.1	695		

* and ** denote significant difference at the 5% and 1% levels.

multiple factors were combined, except when all four factors were included (Table 3). These findings indicate that (1) inoculation is primary determinant of yellowing, (2) yellowing intensifies over time, (3) materials exhibit differences in yellowing progression, and (4) year-to-year variation has the weakest effect on yellowing progression.

3.3 | Variation in leaf yellowing and yield loss

In the uninoculated plots, no visible disease symptoms were recorded during any survey period. Conversely, inoculated plots showed clear yellowing symptoms on mature leaves starting in July, with YI increasing over time. Materials with higher tolerance exhibited slower YI increases throughout the 4 years (Figure 4). By late September, CR951-210 (strong tolerance) consistently had the lowest YI, generally <1.0, whereas NK-210BR (medium tolerance) was around 1.5, and NK-323 mm-O (weak tolerance) reached the highest index of ~ 2.5.

YA displayed similar trends to YI. In uninoculated plots, YA remained under a few percent across all survey periods



FIGURE 2 Representative images of plants given yellowing index scores from 0 to 3. Ringed areas highlight the yellowing symptoms.

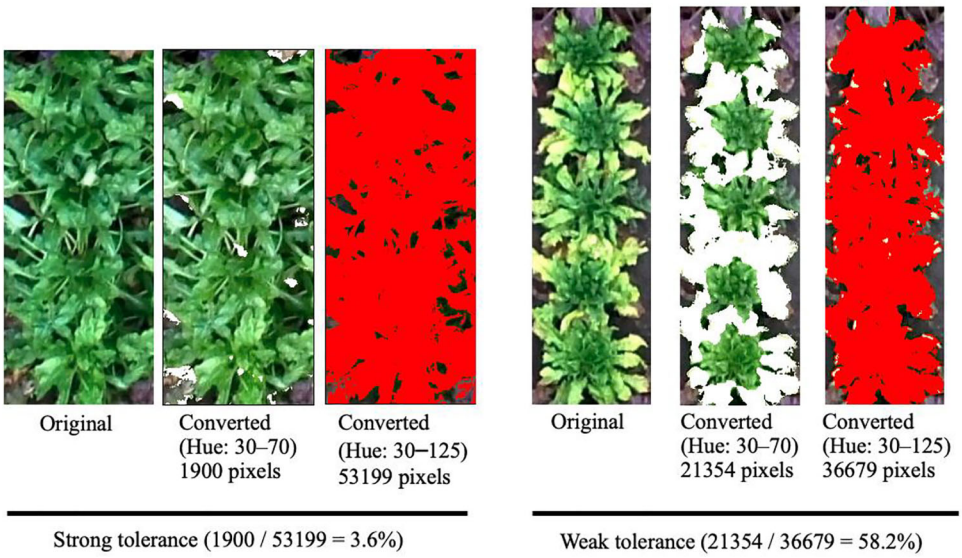


FIGURE 3 Representative examples of yellowing area percentage calculations using digital images of strains with strong and weak beet leaf yellowing virus (BLYV) tolerance.

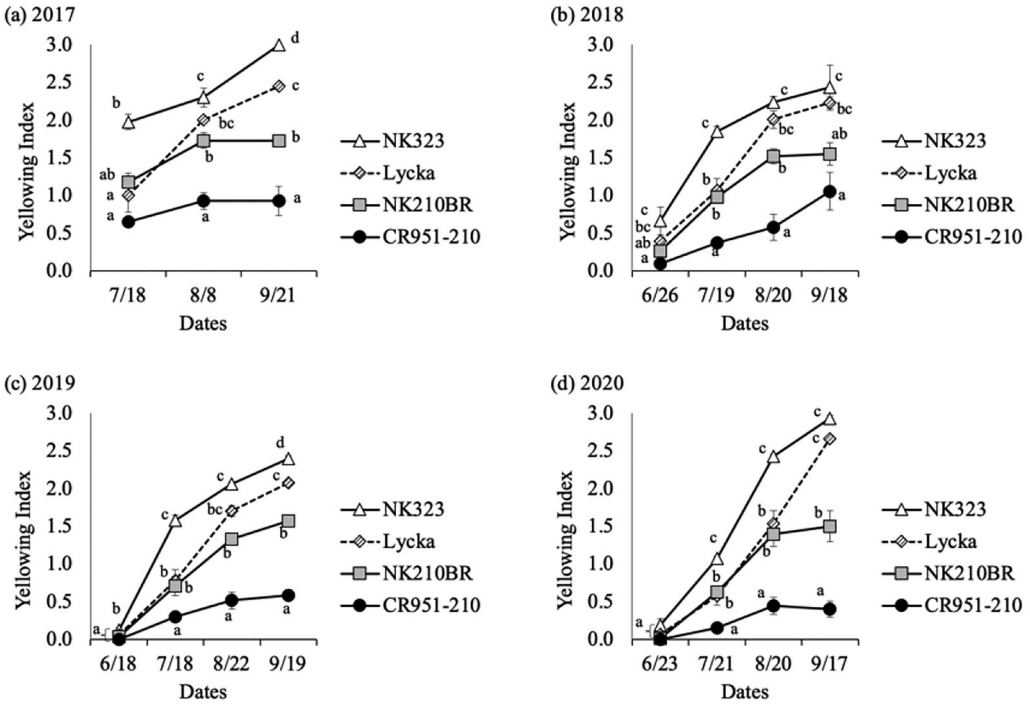


FIGURE 4 Changes in yellowing index values based on material over 4 years (inoculated plots, 2017–2020). Different letters indicate a significant difference at the 5% level based on a Tukey–Kramer multiple comparison test.

TABLE 2 Effects of inoculation status, evaluation date, material, and evaluation year on the yellowing index based on analysis of variance (ANOVA).

Effect	Sum square	*df*	*F* value	Significance
Inoculation (A)	8892.4	1	10384.0	**
Date (B)	404.5	3	157.5	**
Material (C)	98.9	3	38.5	**
Year (D)	44.6	3	17.4	**
A × B	363.4	3	141.5	**
A × C	79.8	3	31.1	**
B × C	32.5	9	4.2	**
A × D	24.3	3	9.5	**
B × D	51.9	8	7.6	**
C × D	5.5	9	0.7	
A × B × C	39.9	9	5.2	**
A × B × D	70.5	8	10.3	**
A × C × D	9.2	9	1.2	
B × C × D	11.4	24	0.6	
A × B × C × D	11.7	24	0.6	
Residuals	308.3	360		

** denotes significant difference at the 1% level.

TABLE 3 Effects of inoculation status, evaluation date, material, and evaluation year on yellowing area based on analysis of variance (ANOVA).

Effect	Sum square	*df*	*F* value	Significance
Innoculation (A)	4561.2	1	421.3	**
Date (B)	5119.0	4	118.2	**
Material (C)	3035.7	3	93.5	**
Year (D)	102.9	3	3.2	*
A × B	2759.4	4	63.7	**
A × C	2424.9	3	74.7	**
B × C	2227.8	12	17.1	**
A × D	443.8	3	13.7	**
B × D	218.5	11	1.8	**
C × D	367.2	9	3.8	**
A × B × C	1681.6	12	12.9	**
A × B × D	251.3	11	2.1	*
A × C × D	342.7	9	3.5	**
B × C × D	775.4	33	2.2	**
A × B × C × D	350.7	33	1.0	
Residuals	4936.6	456		

* and ** denote significant difference at the 5% and 1% levels.

(data not shown). In inoculated plots, YA increased progressively from late July onward (Figure 5). By late September to early October, NK-323 mm-O exhibited the highest YA at approximately 30%, whereas CR951-210 showed the lowest at <10%, demonstrating a marked difference. However, no significant differences were observed between CR951-210 and NK-210BR in any year or between CR951-210 and Lycka in 2 out of 4 years.

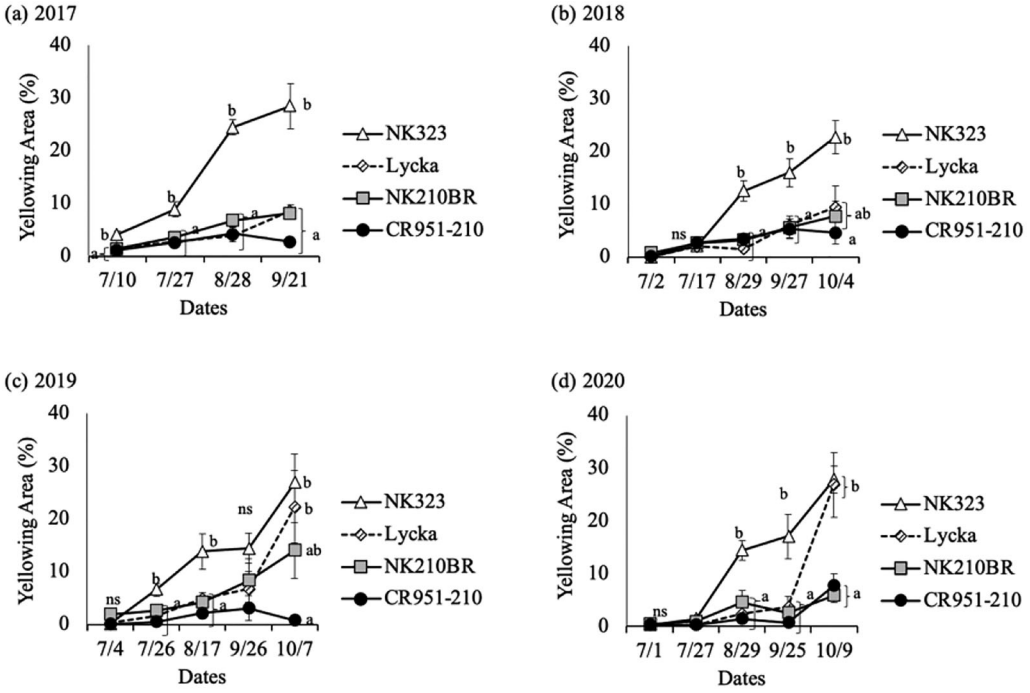


FIGURE 5 Changes in yellowing area values based on material over 4 years (inoculated plots, 2017–2020). Different letters indicate a significant difference at the 5% level based on a Tukey–Kramer multiple comparison test.

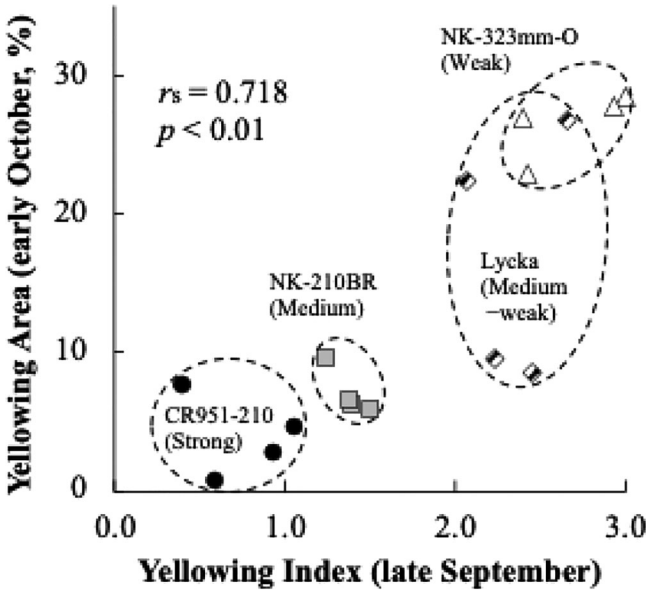


FIGURE 6 Spearman rank correlation coefficients for yellowing index (late September) versus yellowing area (early October) by material over 4 years (inoculation plots, 2017–2020).

The Spearman's rank correlation coefficient between YI (late September) and YA (late September to early October) for the final survey period across 4 years was 0.718. Thus, a significant relationship existed between the two traits ($p < 0.01$; Figure 6).

The Spearman's rank correlation coefficients calculated between each yield-related trait and the yellowing symptom index revealed that the relationship between Brix value and the yellowing symptom index (YI or YA) was not significant, but the higher the YI and YA values, the greater the loss in sugar yield and root weight ($p < 0.01$; Table 4).

4 | DISCUSSION

Yellowing of sugar beet leaves has been reported in Japan since the 1950s. In the 1960s, it was confirmed that the yellowing was caused by a virus (Sugimoto et al., 1970), but no breeding trials for VY tolerance have been conducted. The present study aimed to obtain essential information for breeding programs by testing three hypotheses: (1) leaf yellowing can be induced through artificial inoculation, (2) variation in tolerance exists among breeding materials, and (3) differences in tolerance to yellowing disease can be evaluated. The results, discussed below, provide support for the validity of these hypotheses. We also discuss the implications for future tolerance breeding.

4.1 | Leaf yellowing is induced through artificial inoculation

The comparison of inoculated and uninoculated plots revealed and yellowing of mature leaves was rarely observed in uninoculated plants, suggesting that the yellowing observed in this study was primarily due to BLYV infection. The accurate

TABLE 4 Yield loss (%) calculated from both inoculated and uninoculated plots, and Spearman's rank correlation coefficient between loss and yellowing index (YI) or yellowing area (YA).

Trait	Materials	Tolerance	Reduction ratio (%)				*rs*	*rs*
			2017	2018	2019	2020	YI	YA
Sugar yield (g)	CR951-210	strong	55.6	35.7	11.9	17.3	0.86**	0.87**
	NK-210BR	medium	56.2	28.4	40.4	33.3		
	Lycka	medium–weak	64.5	34.7	46.4	45.8		
	NK-323 mm-O	weak	72.1	50.9	41.4	51.0		
Root weight (g)	CR951-210	strong	46.9	28.1	7.4	15.0	0.80**	0.83**
	NK-210BR	medium	46.8	18.5	33.9	29.0		
	Lycka	medium–weak	59.5	26.5	42.8	39.9		
	NK-323 mm-O	weak	65.6	38.6	32.7	46.4		
Brix (%)	CR951-210	strong	16.0	13.1	3.7	1.6	0.46 ns	0.32 ns
	NK-210BR	medium	17.6	11.3	7.7	5.4		
	Lycka	medium–weak	11.8	11.0	7.4	8.9		
	NK-323 mm-O	weak	18.8	16.8	7.5	9.1		

Note: The year 2017, in which the two plots were far apart, was excluded from the analysis of Spearman's rank correlation.
** denote significant difference at the 1% level.

establishment of inoculated and uninoculated plots was critical for assessing the differences in yellowing caused by the virus. Previous studies indicate that early infection typically exacerbates VY symptoms (e.g., Borgolte et al., 2024). In the current study, inoculated plots were designed to facilitate rapid infection, whereas uninoculated plots were managed to minimize natural infection. These measures resulted in a clear distinction between the two treatments.

The success of this study is attributed largely to the application of an artificial inoculation method (Takashino et al., 2017). Physical inoculation methods, such as the carborundum method, are ineffective for Japanese BWYV. However, the virus' persistent retention in aphid vectors makes aphid-mediated inoculation highly effective (Sugimoto et al., 1970). Although aphid-based inoculation is not novel, this method has two distinct advantages over conventional methods. First, the method is labor-efficient. Conventional field-based inoculation requires releasing aphids over large areas, leading researchers to study techniques that involve inoculating a smaller proportion of plants (e.g., 10%; Borgolte et al., 2024; Hossain et al., 2020). In contrast, Takashino's method targets seedlings grown in paper pots, streamlining the process. A single seedling fits into a paper pot measuring 2 cm in diameter and 13 cm in length, and approximately 300 seedlings can be accommodated in a polystyrene foam box with around 100 cm per side. By releasing three to five aphids per seedling, large numbers of seedlings can be reliably and efficiently inoculated simultaneously. Second, this method reduces the risk of unintended virus spread. Aphids can be easily eradicated with insecticides after inoculation, ensuring that the uninoculated plots remain virus-free. Additional precautions, such as frequent insecticide applications and careful plot management, further reduced the likelihood of natural infection. This approach resulted in uninoculated plots that remained infection-free, even when adjacent to inoculated plots in the same field (except in 2017). Importantly, no infection was detected in uninoculated plots until the second sampling period in August.

4.2 | Tolerance varies among breeding materials

In this study, VY symptoms were evaluated using YI and YA criteria, revealing that inoculation, survey date, material, and year were the main factors influencing symptoms. Symptoms, primarily caused by viral infection, intensified over time and varied across materials and years, underscoring the need to prevent infection. However, tolerant materials remain valuable where prevention is impractical.

This study evaluated four materials presumed to exhibit varying levels of BLYV tolerance based on our preliminary tests. Consistent differences in disease severity, across materials in YI and YA evaluations, along with uniform disease progression over 4 years, indicate their potential as provisional standards for future BLYV tolerance assessments.

From a breeding perspective in Japan, the strongly tolerant material CR951-210 warrants particular attention. Developed in the United States as a VY-tolerant material (Panella et al., 2015), this strain reflects decades of breeding efforts for VY tolerance in regions such as California, where VY has long been prevalent (McFarlane & Skoyen, 1968; McFarlane et al., 1969). Although the Japanese- and US-based viruses belong to the same genus, *Polerovirus*, they are distinct

species. Therefore, given that CR951-210 showed tolerance to the Japanese viruses, its tolerance mechanism may not be species-specific within the *Polerovirus* genus.

In Europe, *Polerovirus* species, such as BMYV, are widespread, and materials resistant to BMYV with low virus accumulation have been reported (Asher et al., 2001; Grimmer, Bean, Luterbacher, et al., 2008; Grimmer, Bean, Stevens, et al., 2008). However, in the present study, TAS-ELISA testing revealed that virus accumulation in CR951-210 was comparable to that in materials with weaker tolerance. This indicates that CR951-210 possesses a tolerance mechanism, as it does not suppress virus accumulation but rather prevents disease symptoms even when virus levels are high. Tolerance is a complex genetic trait involving multiple molecular mechanisms operating simultaneously, many of which are associated with signal transduction pathways integral to basal innate immune defenses (Paudel & Sanfacon, 2018). The ability of CR951-210 to mitigate symptoms despite high viral loads likely reflects the coordinated activity of such pathways.

4.3 | Tolerance differences to yellowing disease can be evaluated

This study demonstrated that the two criteria used to evaluate yellowing symptoms, YI and YA, yielded similar overall results. However, each criterion exhibited unique advantages and disadvantages. One critical distinction is evaluation accuracy. The YI method provided superior accuracy compared with the YA method. For instance, the YI method distinguished between strong and weak tolerance as early as July, whereas the YA method failed to differentiate between strong and medium tolerance throughout the growing season. This suggests that the YI method holds potential for evaluating differences in tolerance during the early stages of infection, particularly in July. This discrepancy arises because, in the early stages of infection, yellowing leaves may be obscured by healthy leaves. The YI method allowed for manual separation of overlapping leaves to identify symptomatic ones, whereas the aerial photography–based YA method struggled to detect concealed symptoms.

Leaf color variability and diverse causes of yellowing, such as drought, aging, pest damage, and nutrient deficiency (Draycott, 2006), further complicated the evaluations. In September 2018, which experienced high precipitation, partial yellowing of the aboveground parts was observed in CR951-210, a material sensitive to root rot, highlighting that the effects of nonviral factors, such as wet damage, could not be completely eliminated. Despite this limitation, the YI method focused on typical disease symptoms, specifically the retention of green veins when leaves turn yellow, minimizing the influence of inherent leaf color differences. Conversely, the YA method, which relied on mechanical hue-based analysis, was more susceptible to inaccuracies. Although physiological yellowing was largely absent in the early to middle growth stages, some aging-related yellowing appeared during later stages. Although this physiological yellowing did not significantly affect overall results, it contributed to minor increases in YA percentages in uninoculated plots during certain years.

In contrast to YI, the YA method was less labor-intensive. Image analysis from ground-level photographs required less effort compared with manually inspecting and separating overlapping leaves. However, to maximize accuracy when using the YA method, specific considerations are necessary. For accuracy, images should be taken during the later growth stages (September–October), when disease symptoms are more visible above ground. Early-stage images have lower accuracy due to leaf overlap and less pronounced symptoms. One of the cultivation conditions used was a row width of 120 cm, double the standard width. This width extension reduced leaf overlap between rows and enhanced leaf color visibility. Additionally, photography was primarily conducted on cloudy days to minimize issues with sunlight reflection and leaf shadows, which can increase the difficulty of hue detection.

Furthermore, the study found a significant positive correlation between the YI and YA values of the final survey and sugar yield loss (rs = 0.86–0.87, $p < 0.01$). A similar correlation was observed for root weight loss (rs = 0.80–0.83, $p < 0.01$), but no significant correlation was found for Brix loss (rs = 0.32–0.46, $p > 0.05$). These results suggest that the impact of virus infection on yield loss may be more pronounced on root weight than on Brix.

4.4 | Conclusions and future perspectives

This study represents the first step toward breeding for VY tolerance in Japan. By verifying three hypotheses, the research successfully fulfilled two critical conditions necessary for advancing tolerance breeding: confirming that yellowing of mature leaves is caused by BLYV infection and demonstrating variation in tolerance among breeding materials. These findings represent progress in the pursuit of VY tolerance breeding. However, several key challenges remain to be addressed to progress further.

One critical issue is determining the economic impact of yellowing. Although only four materials used in this study, a clear relationship was shown between YI and YA and a loss in sugar. It is reasonable to assume that yellowing of the main leaves due to BLYV infection reduces photosynthetic capacity, which could, in turn, lower sugar beet yields. Previous studies have shown that infections with related viruses, such as BYV, BWYV, BMYV, and BChV, negatively impact productivity (Bennett, 1960; James et al., 2012; Lewellen & Skoyen, 1984; McFarlane & Bennett, 1963; McFarlane et al.,

1969). The number of materials used in this study was relatively small, so it remains to be determined whether similar trends would be observed if a greater variety of materials were used. Establishing a clear relationship between the evaluation methods used in this study, YI and YA, and yield reductions in sugar beet is critical for advancing tolerance breeding efforts.

Another important question is whether tolerance to BLYV is genetically controlled (McFarlane et al., 1969). Previous research has demonstrated that tolerance to some VY-related viruses, such as BWYV and BYV, is polygenic and selective (McFarlane et al., 1969). Furthermore, quantitative trait loci (QTLs) associated with virus-induced vein clearing and mottling symptoms have also been identified in BYV (Grimmer, Bean, Stevens, et al., 2008), as well as QTLs for yellowing symptoms in BMYV (James et al., 2012). These findings suggest that tolerance to BLYV may also have a genetic basis. Understanding the heritability and identifying genetic markers associated with tolerance could provide a foundation for more efficient breeding strategies in the future.

Finally, it is essential to determine the scope of tolerance across different virus species. This study revealed that CR951-210, a material tolerant to US-based viruses, also exhibits tolerance to Japanese virus strains. Investigating whether this tolerance extends to other problematic viruses, such as BMYM, BChV, and BYV, prevalent in Europe and the United States, would markedly enhance the breeding value of tolerant materials.

AUTHOR CONTRIBUTIONS

Yosuke Kuroda: Conceptualization; data curation; funding acquisition; investigation; project administration; writing—original draft; writing—review and editing. **Kazuyuki Okazaki**: Data curation; investigation. **Kenji Takashino**: Data curation; investigation. **Shigenori Ueda**: Conceptualization; investigation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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