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ORIGINAL RESEARCH PAPER

Evaluation of the potential yield and primary symptoms of *Xanthomonas campestris* pv. *campestris* infection in Asian vegetables grown in the Czech Republic

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Abstract

Selected cultivars of Asian brassicacean vegetables were evaluated for their yield potential and susceptibility to bacterial infection. Chinese broccoli, two cultivars of Chinese cabbage ('Dwarf milk cabbage' and improved 'Tahtsai'), and mizuna were grown in the conditions of the Czech Republic. Morphological and yield parameters for the field conditions (plant height, plant diameter, and marketable weight) were verified. In addition, genotypes were tested for resistance to black rot [*Xanthomonas campestris* pv. *campestris* (Xcc)] at the planting stage. The results show that a very promising genotype of mizuna, which yielded best (1,478 g per plant). Chinese cabbage (cultivar 'Dwarf milk cabbage') also showed high yield values (2,839 g per plant), especially when compared to the reference value for Napa cabbage. Chinese Cabbage 1 ('Dwarf milk cabbage') and mizuna also showed a low susceptibility to infection by bacterial black rot at the planting stage. Both cultivars reached Level 2 (median) of infection, which corresponds to the extent of the symptoms on 25% of the leaf surface.

Keywords

Brassicaceae; Chinese cabbage; mizuna; marketable weight; plant diameter; Xcc

Introduction

For some time, there has been a growing interest in the enrichment of the vegetable species composition offered for human consumption, which is also facilitated by an increased awareness of foreign assortments. The biological value of commonly grown vegetables is predominantly known, but we know less about other kinds of vegetables [1]. The results of a project carried out at the University of Wageningen in 2007–2010 show that the vegetable market is changing. Consumers require more new vegetables, new exotic products and forgotten types of vegetables [2]. The region of East Asia is referred to as one of the largest genetic centers in terms of species diversity. Approximately 130 species of cultivated crops, especially vegetables and fruit trees, come from there [3]. For example, vegetable production is a very important agricultural sector in China. It has been reported that the consumption of vegetables per person in China is higher compared to Western countries [4].

Cabbage is still one of the most grown and consumed vegetables in the Czech Republic. However, it is noticeable that consumers prefer to spend less time on cooking basic raw materials, which is why the use of time-consuming vegetables in cooking is decreasing (such as cabbage, celeriac, kale, leek, and red beet). There is an increase in the consumption of species that can be prepared in a shorter time or can be consumed raw and immediately without thermal processing (e.g., tomatoes, peppers, radishes, and lettuce) [5].

Potentially interesting vegetables for Europe could include species from the family Brassicaceae, e.g., Chinese cabbage, Chinese broccoli, or mizuna. When selecting new vegetable species, it is necessary to consider potential diseases and pests, as well as appropriate growing conditions. One of the major diseases of brassicacean plants is bacterial black rot caused by bacterium *Xanthomonas campestris* pv. *campestris* (Xcc). Typical symptoms of this pathogen are necrotizing V-shaped lesions starting from the leaf edges and blackening vascular bundles of plants [6,7]. Testing for resistance to Xcc should form part of an assessment of how suitable it is to grow these species under given climatic conditions. New genotypes may also be a source of resistance to this pathogen.

The aim of this study was to evaluate the potential market yield of selected Asian vegetables grown under the Czech Republic conditions and to verify their symptomatic response to the major pathogen of this group of vegetables, Xcc.

Material and methods

Plant material

The trial was carried out during 2017 on an experimental field at the Faculty of Horticulture in Lednice, Mendel University in Brno, the Czech Republic. The following potential genotypes obtained during visits to China in previous years were selected for testing:

- Chinese broccoli (*Brassica alboglabra*, *B. oleracea* var. *alboglabra*), which is a leaf vegetable grown for its young flowering stems. It is a perennial herb, but is often commercially grown as a 1-year crop. It is suitable for growing in a mild climate and has a certain tolerance to freezing conditions.
- Chinese cabbage (*Brassica rapa* subsp. *chinensis*) – there are many types and forms, which differ in appearance and size. The genotypes marked “Chinese Cabbage 1” (characterized as ‘Dwarf milk cabbage’ has larger leaves) and “Chinese Cabbage 2” (described as improved ‘Tahtsai’, which forms a tiny rosette with dark leaves) were selected for testing.
- Mizuna (*Brassica rapa* var. *nipposinica*, *B. rapa* var. *japonica*) – labeled “Jing Shui Cai” – creates a thick tuft of finely pinnate, jagged leaves that are dark green and smooth.

All the species were grown in seedling trays with a cell volume of 49 cm^3. They were sown on July 4. Profimix (Klasman, Germany) was used as the substrate. The seedlings were fertilized twice with NPK in liquid form (Kristalon). They were transplanted onto the trial plot on August 8. The plant spacing, 0.3×0.3 m, was selected and the plants were covered with polypropylene nonwoven fabric (19 g m^{-2}). The experimental set up in a fully randomized design with four replications. During the whole period of the trial, the plants were irrigated as needed with a sprinkler Meganet (Netafim, Israel). No fertilization or chemical protection was performed. The harvest took place on October 18. The selected plants were adapted to suit consumer needs (removing the root and old and damaged leaves) and further evaluated.

Testing the susceptibility of the genotypes to Xcc infection

Three genotypes were evaluated in the trial for resistance to bacterial black rot: Chinese Cabbage 1, Chinese Cabbage 2, and mizuna. The Chinese broccoli plants had not developed sufficiently and were excluded from the evaluation. The genotypes tested were sown on October 26, 24 plants per variety, and grown in a glasshouse under standard conditions. Inoculation of 18 plants per variety took place when the seedlings were 1 month old. The control was six plants of each. The inoculation was performed using the ISTA methodology [8] by drilling two fully developed leaves using toothpicks soaked in the bacterial suspension. A mixed suspension of four Xcc bacteria isolates was used, specifically 3811, 3971A (both Race 11), and 1279A (Race 4) from the Horticultural

Research International Warwick (HRIW) collection and the local isolate SU1 from the region of Svijanský Újezd, the Czech Republic. The bacterial inoculum concentration was standardized to 108 CFU mL^{-1}. A visual evaluation of the symptoms took place 14 days after inoculation, according to the recommendations by Peňázová et al. [9], using a 5-point infection scale (Tab. 1).

Tab. 1 Point scale for assessing the extent of Xcc symptoms.

Degree of infection	1	2	3	4	5
Affected leaf surface	0%	<25%	25–50%	50–75%	>75%

Statistical analysis of the data

The data obtained were subjected to ANOVA. Post hoc analysis was performed using the Kruskal–Wallis test (Statistica CZ software, StatSoft, USA). Statistical differences ($\alpha = 0.05$) between means were determined using the multiple comparisons test. To assess the resistance of the genotypes to Xcc infestation, based on the Wilcoxon pair test results, the mean values of the plants infected were used. The data were further evaluated using the Kruskal–Wallis ANOVA test.

Results

Evaluation of the potential yield

The evaluation of the edible parts of the selected species is shown in Fig. 1. The greatest weight was achieved by Chinese Cabbage 1, the value of which was significantly higher than Chinese Cabbage 2 and Chinese broccoli. On the other hand, the lowest values were for Chinese Cabbage 2 and Chinese broccoli, and there was no statistically significant weight difference between them.

The results of the morphological measurements are shown in Tab. 2. It can be stated that mizuna achieved the highest plant height, followed by Chinese broccoli and Chinese Cabbage 1. Chinese Cabbage 2 was the shortest, corresponding to a lower morphological type. This cabbage produces small-leaved rosettes of low growth.

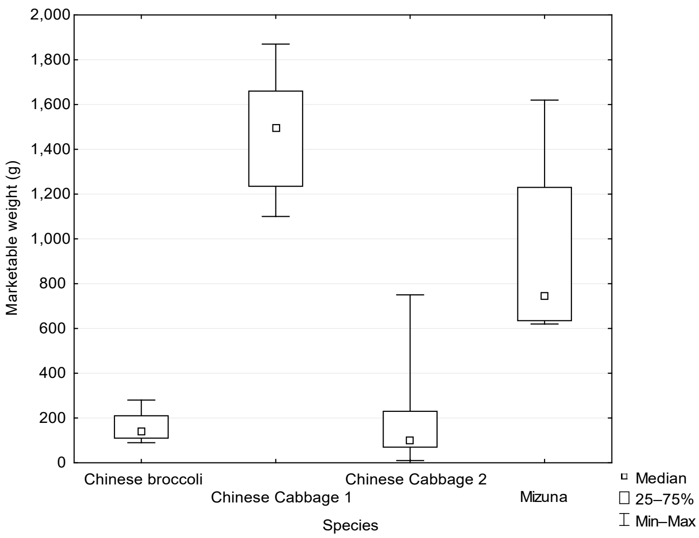

Fig. 1 Marketable weight of one plant for the Asian vegetable species (according to Kruskal–Wallis test; $p < 0.001$).

Tab. 2 Evaluation of the morphological parameters in the Asian vegetable species.

Species	Plant height (mm)	Diameter of the edible part (mm)
Chinese broccoli	590 ±23 [b]	22 ±2 [a]
Chinese Cabbage 1	377 ±5 [b]	116 ±3 [b]
Chinese Cabbage 2	154 ±8 [a]	70 ±8 [a]
Mizuna	635 ±12 [b]	155 ±23 [b]

Mean values with ± standard error are tabulated. Statistical differences between means (α = 0.05) according to the multiple comparisons test.

Tab. 3 Comparison of the plant consumption weights in the Asian vegetable species with the reference species.

Species	Consumption weight (g)	Reference species*	Consumption weight of reference species (g)	Percentage difference
Chinese broccoli	240.0	Leaf lettuce	390–482	49–61
Chinese Cabbage 1	2,839.0	Napa cabbage	1,240–1710	166–228
Chinese Cabbage 2	215.3	Napa cabbage	1,240–1710	12–17
Mizuna	1,477.5	Leaf lettuce	390–482	306–378

* [13].

Tab. 3 shows the values of the consumption weight achieved and comparisons with the selected reference species.

Symptom evaluation after Xcc infection

All the genotypes tested displayed a degree of Xcc infection in the grades of 1 to 4. Chinese Cabbage 1 and mizuna showed the lowest level of infection. The highest recorded degree of infection was Grade 3, indicating the presence of visual symptoms with an infection on 25–50% of the leaf surface. Some Chinese Cabbage 2 plants also had leaves with fourth degree infection (50–75% of the surface). The median value for all genotypes ranged from 2 to 2.5 (Fig. 2). There were no statistically significant differences in symptom expression between the genotypes.

Discussion

Evaluation of the potential yield

Lower yields can be expected with Chinese broccoli according to the morphological measurements. The values obtained are higher than those found in the study of Kopta and Pokluda [10]. They evaluated five cultivars in 2 years, and a weight of 13–40 g was achieved but, in this case, it was only the weight of the stems without leaves. Tindall [11] reported that the yield of Chinese broccoli can vary between 0.8–3.5 kg m^{-2}, which is consistent with the recalculated yield in this experiment, which was 2.7 kg m^{-2}. There was an interesting yield for mizuna, which is due to the fact that it is a fast-growing vegetable, with the potential for continuous harvesting. The values correspond to the results of Enikő [12], who, during standard production and at the same harvest term, achieved an average weight of 1,105 g per plant.

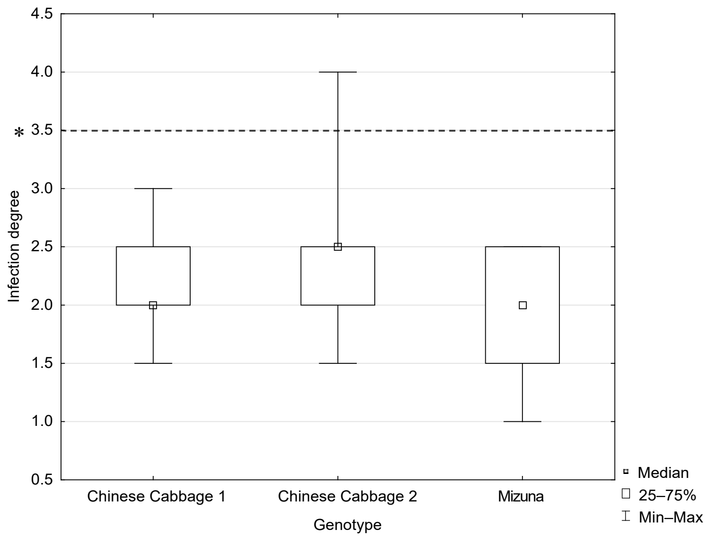

Fig. 2 Average level of infection of individual genotypes inoculated with Xcc bacteria (according to the Kruskal–Wallis test; $p = 0.187$). * Median for cv. 'Sonja' cabbage, data from Peňázová et al. [9].

Tab. 2 also shows that the broadest plants were found in mizuna, which is obviously due to its growth form. Therefore, further enlargement of the planting space could be considered due to the large rosettes formed by the mizuna leaves. Chinese broccoli achieved lower values, where the stem was evaluated as a consumable part. As a suitable qualitative measure, a minimum stem diameter of 10 mm [13] can be used, which suits this cultivar. Chinese Cabbage 1 achieved a greater width of the edible part compared to Chinese Cabbage 2. This fact once again shows the different morphological types of the two Chinese cabbages tested.

Due to the fact that the species tested are not part of a commonly grown selection of leafy crops for the Czech Republic conditions, species used in similar ways were selected for comparison in terms of their potential yield. For mizuna and Chinese broccoli, the leaf and stem vegetables, leaf lettuce was selected as an equivalent. In spite of certain differences, the value of the biomass is comparable to leaf lettuce in the brassicas tested. For both Chinese cabbages, the consumption weight was compared with a related type of Napa cabbage. The values of the two reference species are the averages of the multiyear evaluation of several cultivars, which were found from previous trials under comparable conditions and in comparable growing spaces [14].

Where biomass production is considered, mizuna significantly exceeded the production of leaf lettuce. It reached values exceeding 300% of the weight of the reference leaf lettuce. On the other hand, Chinese broccoli, compared to the leaf lettuce, attained roughly half the biomass production. Both Chinese cabbages showed totally different values. Whilst Chinese Cabbage 1 exceeded the reference consumption weight of the Napa cabbage by a factor of 2, Chinese Cabbage 2 achieved only 12–17% of the Napa cabbage biomass. This fact could lead to the exclusion of Chinese Cabbage 2 from cultivation. However, it is necessary to take into account the different morphological measures that determine the formation of the total biomass.

Symptoms evaluation after Xcc infection

Our results were compared with the cultivar evaluation of the cabbage (*B. oleracea* var. *capitata*) 'Cerox' (declared resistant to Xcc by Bejo, NL) and 'Sonja' (susceptible cultivar) by Peňázová et al. [9], which were evaluated by the same method and inoculated with the same mixture of isolates. The infection of cv. 'Cerox' plants reached the third level, with a median of 1.5. On the other hand, the plants of cv. 'Sonja' exhibited symptoms of third to fifth levels of infection with a median of 4.5. Reaction of the genotypes tested to Xcc infection was similar to that of the resistant cultivar, indicating a lower susceptibility to Xcc infection at the juvenile stage of development.

Because of the low degree of infection in individual genotypes examined in this work, the occurrence of resistant genes was considered. The bacterial isolates of Races 1 and 4 used are responsive to the *R1* and *R4* genes for resistance based on the gene-for-gene model [15]. Resistance to Race 1 is, however, very rare and has so far been found only in brassicas containing the B-genome (*B. carinata*, *B. juncea*, and *B. nigra*) [16,17]. On the other hand, sources of resistance to Race 4 have already been described in species containing genomes A, B, and even C [18,19]. When the *R4* gene was present, the symptoms of the infection could be caused by other isolates of Race 1 or an isolate of an unspecified race. It was also possible to have a certain level of horizontal (race nonspecific) resistance, which has led to less symptoms involving all isolates being expressed. The role of polygenes as part of Xcc resistance testing in *B. rapa* has already been demonstrated in studies by Soengas et al. [20] or Artemyeva et al. [21]. To elucidate the response of the genotypes tested, it would be necessary to verify the level of resistance at the adult stage of development in future experiments. To maintain a low level of symptoms, it is possible to perform separate inoculations with individual races or directly test the presence of resistance genes using molecular markers.

Conclusions

The difference between the evaluated measurements for both Chinese cabbages genotypes shows that general recommendations concerning the species may not be reliable in case of specific genotypes, and therefore the available cultivars within one species should be tested. If the production of biomass corresponding to that of Napa cabbage is expected, only the genotype Chinese Cabbage 1 ('Dwarf milk cabbage') can be recommended. However, if the production of small leaves suitable for the baby leaf sector or for "designer foods" is preferred, Chinese Cabbage 2 (improved 'Tahtsai') can be a possibility. Mizuna can be included in the list of very promising species as it achieved the highest yield values in our trials. This species was able to produce the greatest biomass from all the vegetables assessed during 2 months of growth. Due to the possibility that it can be harvested continuously and that it withstands low temperatures, this species can clearly be recommended for production under field conditions in the Czech Republic. For Chinese broccoli, the levels of the observed values were only average. The production of biomass was not very large, but it is also possible to carry out a gradual harvesting of this broccoli. Genotypes of Chinese Cabbage 1 and mizuna showed a low susceptibility to infection by Xcc causing bacterial black rot in the seedling stage, and thus proved their suitability for use in the baby leaf sector. At the same time, they can be potentially useful for breeding for resistance to Xcc. However, whether these genotypes can be recommended for the breeding process depends on confirmation of resistance by further studies.

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Ocena plonowania oraz symptomów infekcji bakteryjnej *Xanthomonas campestris* pv. *campestris* wybranych odmian azjatyckich warzyw z rodziny kapustowatych uprawianych w Republice Czeskiej

Streszczenie

W doświadczeniu oceniano pod względem plonowania oraz podatności na infekcję bakteryjną wybrane odmiany azjatyckich warzyw z rodziny kapustowatych. Materiał badawczy stanowiły brokuł chiński, kapusta chińska w typie Tahtsai (odmiany 'Dwarf milk cabbage' i ulepszona 'Tahtsai') oraz kapusta chińska *mizuna*. W stadium rozsady analizowano również podatność wymienionych warzyw na *Xanthomonas campestris* pv. *campestris* (Xcc). Najwyższy plon uzyskano w przypadku kapusty chińskiej *mizuna* (1478 g z rośliny). Kapusta chińska 'Dwarf milk cabbage' również wydała wysoki plon (2839 g z rośliny), szczególnie w porównaniu do

powszechnie uprawianej kapusty pekińskiej. Odmiany kapusty chińskiej 'Dwarf milk cabbage' i *mizuna* wykazały w stadium rozsady niską podatność na infekcję bakteryjną wywołaną przez *Xanthomonas campestris* pv. *campestris* (Xcc). Dla wymienionych odmian odnotowano 2-gi (mediana) poziom infekcji, który odpowiada wystąpieniu symptomów chorobowych na 25% powierzchni liści.

