Introduction

Breeding background

The major varieties of potatoes cultivated in Japan for table use are ‘Irish Cobbler’ (‘Danshaku Imo’), ‘May Queen’, ‘Nishiyutaka’, and ‘Dejima’. In 2014, these varieties commanded a 67% share of the total planted area in western Japan (Ministry of Agriculture, Forestry, and Fisheries Statistics 2015). ‘Irish Cobbler’ and ‘May Queen’ are typically sold with the name of the variety on display at the point of purchase, and thus, both varieties are familiar to Japanese consumers, but most varieties of potatoes are sold without name displays. As such, it is difficult for consumers to distinguish between varieties solely by their appearance (tuber shape and color). At the same time, consumer awareness of health and food-safety issues, as well as environmental concerns, is increasing, resulting in a growing demand for foods with added value.

‘Inca-no-mezame’ (Mori et al. 2009) and ‘Inca-no-hitomi’ (Kobayashi et al. 2008) are diploid potato varieties released in Japan that contain high levels of carotenoid, which is composed of zeaxanthin and lutein (Kobayashi et al. 2008, Mori et al. 2009). In addition to high carotenoid content, the variety ‘Inca-no-mezame’ has strong antioxidant activity (Ishii et al. 1999) as well as a chestnut-like taste quality of steamed tubers, which is comparable to that of ‘Inca-no-mezame’ tubers, which has high levels of carotenoid, and superior to ‘Nishiyutaka’, another popular double cropping variety. ‘Nagasaki Kogane’ is suitable for French fries, because its tuber has high starch content. The marketable yield of ‘Nagasaki Kogane’ was higher than that of ‘Inca-no-mezame’ in spring cropping, although it was lower than that of ‘Nishiyutaka’ in double cropping regions. ‘Nagasaki Kogane’ tubers are larger on average than ‘Inca-no-mezame’ tubers in spring cropping. Moreover, the ‘Nagasaki Kogane’ variety is resistant to PCN and PVY, and exhibits a high level of resistance to bacterial wilt.

Key Words: potato (Solanum tuberosum L.), Nagasaki Kogane, disease and pest resistance, carotenoid, tetraploid, marker assisted selection.
nutty flavor that enhances the tuber’s eating quality (Mori et al. 2009). Moreover, because the skin color of ‘Inca-no-meaze’ is yellow, consumers can readily distinguish it from other varieties, and as such, it has become a popular variety and is sold at a relatively high price.

Despite these positive traits, ‘Inca-no-meaze’ production in Japan presents several problems. For example, the average weight of an ‘Inca-no-meaze’ tuber is a mere 57 g, considerably smaller than that of tetraploid potato varieties like ‘Nishiyutaka’, a key variety in western Japan. Furthermore, the marketable yield of ‘Inca-no-meaze’ is also markedly lower than that of ‘Nishiyutaka’, and the former is highly susceptible to both the potato cyst nematode (PCN) and potato virus Y (PVY). PCN and PVY are important pests and diseases in potato production. Once PCN enters the field, it can greatly reduce yield due to parasitism of the root, and its control is very difficult. PVY causes diseases of great damage such as mosaic disease and tuber necrosis symptoms. The most effective measure against these pests and diseases is the planting of resistant varieties.

Given the problem of adapting ‘Inca-no-meaze’ to double-cropping systems, we attempted to breed a new variety that incorporates its positive traits (good taste, high carotenoid content, and resistance to bacterial wilt) but which can be grown more efficiently in western Japan. Because ‘Inca-no-meaze’ is a diploid variety (Mori et al. 2009), direct crosses with tetraploid potatoes were not possible (Mukojima et al. 2003). As such, ‘Inca-no-meaze’ was chromosome-doubled using a tuber-disc method (Komura and Ohbayashi 2002) to create TD0101, which possessed improved male and female fertility and could be crossed with tetraploid varieties (Mukojima et al. 2003). ‘Saikai 35’ is a breeding line, which was bred by crossing ‘TD0101’ as female parent and ‘Sakurafubuki’ as male parent (Mori et al. 2012). ‘Sakurafubuki’ is a variety for starch production and possesses H1 and Ry, genes confer resistance to PCN and PVY. Although the carotenoid content of ‘Saikai 35’ is higher than that of ‘Dejima’, which is the predominant double cropping variety in Japan (Mori et al. 2012), ‘Saikai 35’ is not used commercially, because it has a carotenoid content considerably lower than that of ‘Inca-no-meaze’, its yield in spring cropping was 20% or more lower than that of ‘Nishiyutaka’, and there was a case where its taste was harsh. Thus, we tried to breed a new potato variety that contains even higher levels of carotenoid and has added value, as well as a higher yield capability than that of ‘Saikai 35’.

To this end, we have developed a new potato variety called ‘Nagasaki Kogane’, which is characterized by a high carotenoid content (reflected by its yellowish-orange flesh), effective resistance to multiple pests and diseases, and high eating quality. Thus, ‘Nagasaki Kogane’ can be easily differentiated from other varieties, has good taste, and can reduce the need for pesticide application during cultivation.

Materials and Methods

Breeding process

‘Nagasaki Kogane’ was initially designated as ‘T04051-14’, then renamed ‘Chokei 132’, and finally assigned the appellation ‘Saikai 37’. This clone was selected from the progeny of ‘Saikai 35’ and ‘Saikai 33’. The latter was the male parent, and is high yielding, has large tubers, and is resistant to bacterial wilt and PCN. The pedigree of ‘Nagasaki Kogane’ is shown in Fig. 1.

To breed double-cropping varieties, two clonal generations were grown yearly, consisting of spring cropping using clear plastic film-covered rows (mulching) and fall cropping. Selection was conducted in a field in Unzen, a city located in Nagasaki Prefecture. In May of 2004, ‘Saikai 35’ was crossed as a female with pollen of ‘Saikai 33’ and set 752 seeds, 400 of which (‘T04051’ family) were sown in a greenhouse in September 2004, from which 206 tuber-setting genotypes were selected in fall cropping. In February 2005, a single tuber of each genotype was planted in a field, from which 27 genotypes were selected on the basis of general appearance of the tuber (shape and size distribution) in spring cropping. Line selection (eight hills in one row) was conducted during the fall cropping of 2005; agronomic traits were evaluated, and H1 and Ry, genes were identified based on the DNA markers PCN (Tanaka and Komura 2000) and rapid markers 38-580 (Hosaka et al. 2001), respectively (Fig. 2). By fall cropping in 2005, five clones with promising agronomic traits (including clone ‘T04051-14’) were selected.

The selected lines, by marker-assisted selection (MAS), which were expected to have resistance to PCN and PVY, were subjected to a preliminary performance yield trial...
(three rows with 10 hills each) during the spring cropping of 2006. This was followed by several seasons of yield trials (three or four rows with 10 hills each, three replicates) on the basis of their general agricultural traits during double cropping over the period 2007–2015. The selected line was designated as Aikei 151 in 2006, as ‘Chokei 132’ in 2007, and as ‘Saikai 37’ in 2008 (Table 1), and then released as ‘Nagasaki Kogane’ by the Ministry of Agriculture, Forestry, and Fishers of Japan (MAFF) in 2015.

Results

Agronomic characteristics

Agronomic traits of ‘Nagasaki Kogane’, ‘Inca-no-mezame’, and ‘Nishiyutaka’, which is the most prevalent double-cropping variety (Chishiki et al. 1979), were performance-tested from 2013 to 2015. However, ‘Inca-no-mezame’ was performance-tested only in spring cropping, because it is difficult to obtain its seed tuber for fall cropping. Seed tubers were planted in early February for spring cropping and early September for fall cropping. Plot size consisted of three rows, each 2.5 m in length, with a spacing of 0.6 m between rows and 0.25 m between potatoes within rows; three replicates of each plot were included. Fertilizer consisting of 140 kg ha$^{-1}$ N, 112 kg ha$^{-1}$ P$_2$O$_5$, 84 kg ha$^{-1}$ K$_2$O, and 28 kg ha$^{-1}$ MgO was applied to all plots. After planting for the spring cropping, the rows were covered with clear plastic film. Harvesting took place in the middle of May for spring cropping and in late November for fall cropping. Marketable yields (kg a$^{-1}$) were compared; marketable tuber sizes were >30 g in the spring cropping and >40 g in the fall cropping. Starch content was calculated by \((\text{specific gravity} – 1.05) \times 214.5 + 7.5\). Specific gravity was calculated as weight in air \((\text{weight in air} – \text{weight in water}) \times 1\), using 15 tubers weighing approximately 100 g each (Nagao 1994).

Images of ‘Nagasaki Kogane’ are shown in Fig. 3. Emergence of ‘Nagasaki Kogane’ occurred earlier than ‘Nishiyutaka’ but later than ‘Inca-no-mezame’, and its vine shape was semi-erect. The stem length of ‘Nagasaki Kogane’ did not differ significantly from that of ‘Inca-no-mezame’ and ‘Nishiyutaka’. The number of stems of ‘Nagasaki Kogane’ per hill was significantly different from ‘Inca-no-mezame’, but did not differ significantly from that of ‘Nishiyutaka’.

Table 1. Breeding of Nagasaki Kogane

| Year     | Cropping season | Breeding stage                        | No. of Plants and replicates$^a$ | No. of selected lines and tested lines$^b$ | Disease resistance test$^c$ | Name of selection plant |
|----------|-----------------|---------------------------------------|----------------------------------|--------------------------------|--------------------------|------------------------|
| 2004     | Spring          | Crossing                              |                                  | 752                             |                          |                        |
|          | Fall            | Primary individual selection of seedling | 1,1                             | 206/400                         | DNA marker assisted selection (MAS) (PCN, PVY) | T04051-14              |
|          | Fall            | Secondary individual selection of seedling | 1,1                             | 27/206                         | Field test               | Aikei 151              |
|          | Fall            | Line selection                        | 8,1                             | 5/27                           | Field test               | Chokei 132 (2007)      |
| 2006     | Spring          | Preliminary performance yield test     | 30,1                             | 2/5                            | Field test               | Saikai 37 (2008)       |
|          | Fall            | Performance yield test                | 40,3                             | 2/2                            | Field test               |                        |
|          | Fall            | Performance yield test                | 40,3                             | 1/2                            | Field test               |                        |
| 2007–2012| Spring          | Performance yield test                | 40,3                             | 1/1                            | Field test               |                        |
|          | Fall            | Performance yield test                | 40,3                             | 1/1                            | Field test               |                        |
| 2013–2015| Spring          | Performance yield test                | 30,3                             | 1/1                            | Field test               |                        |
|          | Fall            | Performance yield test                | 30,3                             | 1/1                            | Field test               |                        |
| 2016     | Spring          | Registration by MAFF                  |                                  | 40,3                            | Field test               |                        |

$^a$ Number of plants and replicates for each stage; for example, “30,1” represents 30 plants with one replicate.

$^b$ Number of selected lines and tested lines for each stage, for example, “5/27” represents five selected lines and 27 tested lines.

$^c$ MAS and disease-resistance field tests (late blight, bacterial wilt, and common scab) were carried out in Nagasaki Prefecture. Bioassays for potato cyst nematode (PCN) and inoculation tests for potato virus Y (PVY) were carried out in Hokkaido.
Nagasaki Kogane—good taste, high carotenoid content and multiple resistance

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The tuber dormancy period of ‘Nagasaki Kogane’ was shorter than that of ‘Nishiyutaka’ in spring cropping, but did not differ significantly from ‘Dejima’ and ‘Nishiyutaka’ in fall cropping (data not shown), which is the suitable period for double cropping.

Vine maturity of ‘Nagasaki Kogane’ was medium to late, which was slightly earlier than that of ‘Nishiyutaka’. ‘Nagasaki Kogane’ had purple flowers, and its tubers were short ovate with shallow eyes. The flesh and skin were yellowish-orange in color (Table 2, Fig. 3).

‘Nagasaki Kogane’ produced significantly lower marketable yields in spring cropping (3-year mean = 362 kg a⁻¹) than ‘Nishiyutaka’ (3-year mean = 416 kg a⁻¹), but significantly higher than ‘Inca-no-mezame’ (3-year mean = 205 kg a⁻¹), and significantly lower in fall cropping (3-year mean = 274 kg a⁻¹) than ‘Nishiyutaka’ (3-year mean = 330 kg a⁻¹) (Table 3). The number of marketable ‘Nagasaki Kogane’ tubers per hill was not significant in spring cropping, but its mean tuber weight was significantly lower than that of ‘Nishiyutaka’, which resulted in lower yields. However, tubers of ‘Nagasaki Kogane’ were considerably larger and the yield was significantly higher than that of ‘Inca-no-mezame’. The starch content of ‘Nagasaki Kogane’ (15.6% in spring cropping and 12.7% in fall cropping) was significantly lower compared to ‘Inca-no-mezame’ (16.0% in spring cropping), but higher than that of ‘Nishiyutaka’ (12.2% in spring cropping and 9.4% in fall cropping) (Table 3). Although the yield of ‘Nagasaki Kogane’ is lower relative to that of ‘Nishiyutaka’, its agronomic characteristics are satisfactory for commercial cultivation considering the cooking quality of tubers and resistance to disease and pests described in detail below.

**Tuber quality**

Taste quality of steamed potatoes was evaluated 1 month following the spring-cropping harvest and 2 weeks after the fall-cropping harvest via taste tests performed by the same three individuals, who served as sensory panelists. Taste was scored from 1 (bad) to 5 (very good) with ‘Dejima’ as the standard (score 4), and the three-year means were compared. Carotenoid content was measured from 30–100 g tubers by high-performance liquid chromatography, as described by Kobayashi et al. (2008). Data were obtained from tubers harvested from the 2010 spring crop.

The taste of steamed ‘Nagasaki Kogane’ potatoes was deemed to be as good as that of ‘Inca-no-mezame’ (both 4.9 score), and better than that of ‘Dejima’ (a score of 4.0) and ‘Nishiyutaka’ (a score of 2.8) (Table 4). ‘Nagasaki Kogane’ contained carotenoids lutein and zeaxanthin (43.8 ± 5.07 μg and 801.5 ± 59.5 μg, respectively, per 100 g fresh weight), which likely contributed to the yellowish color of the flesh. Carotenoid content of ‘Nagasaki Kogane’, however, was lower than that of ‘Inca-no-mezame’ (79.7 ± 15.54 μg of lutein and 1,131.7 ± 242.60 μg of zeaxanthin). By way of

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**Table 2.** Morphological characteristics of Nagasaki Kogane, Nishiyutaka, and Inca-no-mezame (2013–2015)

| Characteristic               | Cropping season | Nagasaki Kogane | Nishiyutaka | Inca-no-mezame |
|------------------------------|-----------------|-----------------|-------------|---------------|
| Emergence date               | Spring          | March 14ᵗʰ      | March 19ᵗʰ  | March 12ᵗʰ    |
|                              | Fall            | Sep. 26ᵗʰ       | Oct. 3ʳᵈ    | –             |
| Vine shape                   | Spring          | Semi-erect      | Semi-erect  | Medium to semi-erect |
|                              | Fall            | Semi-erect      | Erect       | –             |
| Stem length (cm)             | Spring          | 46 ± 8.2 a      | 37 ± 11.1 a | 33 ± 1.5 a    |
|                              | Fall            | 52 ± 3.0 a      | 48 ± 3.7 a  | –             |
| No. of stems per hill        | Spring          | 1.5 ± 0.11 a    | 1.6 ± 0.57 a| 4.7 ± 0.87 b  |
|                              | Fall            | 2.4 ± 0.18 a    | 2.3 ± 0.41 a| –             |
| Flower color                 | Purple          | White           | Purple      | –             |
| Tuber shape                  | Short ovate     | Short ovate     | Short ovate | Circular  |
| Eye depth                    | Shallow         | Semi-shallow to medium | Semi-shallow to medium |
| Epidermal net                | Few             | Medium          | Few         | –             |
| Skin color                   | Yellow          | Pale beige      | Yellow      | –             |
| Eye color                    | Yellow          | Pale beige      | Yellow      | –             |
| Flesh color                  | Yellow          | Pale yellow     | Yellow      | –             |

ᵃ Values followed by the same letter are not significantly different at p = 0.05 (Tukey’s test).
comparison, ‘Dejima’ contained 48.1 ± 2.46 μg of lutein and no zeaxanthin per 100 g of fresh weight (Table 4). Data for carotenoid content of Nishiyutaka was not available.

Evaluation of the potential of ‘Nagasaki Kogane’ for processing as French fries was carried out by Hokkaido Foods Co., Ltd. (Hokkaido, Japan) (data not shown). ‘Nagasaki Kogane’ French fries were more yellow in color than those produced from ‘Hokkaikogane’ potatoes, which is the major variety for French fry processing in Japan, although generally the appearance of ‘Nagasaki Kogane’ French fries was similar to that of ‘Hokkaikogane’ French fries. Overall, ‘Nagasaki Kogane’ is suitable for French fry processing.

Resistance to diseases and pests

Disease and pest resistance was evaluated using biological assays in the field or by artificial inoculation. Five plants were grown in a row in a field heavily infested with Globodera rostochiensis (pathotype Ro1) in mid-May at shari-cho in 2007–2008, which was carried out by the Hokkaido research Organization Kitami Agricultural Experimental Station. Two replicates were used. Three plants were randomly chosen from each row and evaluated using a rating score of 0 (no cysts) to 4 (many cysts), with the means representing the infection index, $\frac{\sum (\text{the number of infected plants in this index} \times \text{rating score})}{\text{total number of plants investigated} \times \text{the highest rating score}} \times 100$, which ranged from 0 (no cysts) to 100 (many cysts on all tested plants) in 2007–2008. Meanwhile, at the Hokkaido Research Organization’s Central Agricultural Experiment Station, a PVY resistance assay was conducted, in which 10 plants per genotype were grown in pots and inoculated with

| Variety          | Taste evaluationa | Zeaxanthin content (µg 100 g⁻¹)b | Lutein content (µg 100 g⁻¹)b |
|------------------|-------------------|----------------------------------|-----------------------------|
| Nagasaki Kogane  | 4.9a              | 801.5 ± 59.5 b                   | 43.8 ± 5.07 b               |
| Inca-no-mezame   | 4.9 a             | 1131.7 ± 242.6 b                 | 79.7 ± 15.54 a              |
| Dejima           | 4.0 a             | 0 ± 0.00 a                      | 48.1 ± 2.46 b               |
| Nishiyutaka      | 2.8 b             | –                                | –                           |

a Taste was evaluated by sensory panelists using a scoring range of 1 (bad) to 5 (very good). Three-year means were used.

b Carotenoid content was determined by HPLC. Data were obtained from tubers of the 2010 spring crop.

c Same letters indicate no significant differences between the means (Tukey’s test).

–: no data.
Table 5. Disease and pest resistance

|               | Potato cyst nematode | Potato virus Y | Bacterial wilt | Late blight | Common scab |
|---------------|----------------------|----------------|----------------|-------------|-------------|
| Nagasaki      | R (0.0 ± 0.00)       | R (0.0 ± 0.00) | R (7.0 ± 19.80 a) | R (4.6 ± 1.15 a) | M (61.1 ± 13.44 a) |
| Kogane        | S (27.1 ± 2.10)      | S (95.0 ± 5.00) | S (72.2 ± 32.27 b) | S (4.9 ± 0.69 a) | M (72.8 ± 15.22 a) |
| Dejima        | S (no data)          | S (no data)    | S (39.1 ± 32.26 ab) | M (5.5 ± 0.31 a) | S (72.8 ± 15.22 a) |
| Nishiyutaka   | S (no data)          | S (no data)    | R (100.0 ± 0.00 b) | –           | S (100.0 ± 0.00 a) |
| Norin 1       | S (no data)          | S (no data)    | –               | –           | –           |

|               | Spring | Fall |
|---------------|--------|------|
| Naito         | M      | MS   |
| Nishiyutaka   | M      | MS   |
| Dejima        | MS     | S    |
| Norin 1       | S      | S    |

- Transmission percentages from inoculated leaves to upper leaves. Two-year means (2007–2008). Nishiyutaka and Norin 1 were not tested in this study, but are known to be susceptible.
- Transmission indices ranging from 0 (<2% leaflets infected) to 100 (100% leaflets infected). Eight-year means (2007–2014).
- Transmission indices ranging from 0 (no lesions) to 100 (many lesions). Nine-year (two seasons each year) means (2006–2014).
- Transmission indices ranging from 0 (no cyst) to 100 (many cysts on all tested plants). Two-year means (2007–2008). Nishiyutaka and Norin 1 were not tested in this study, but are known to be susceptible.
- Values followed by the same letter are not significantly different among clones at \( p = 0.05 \) (Tukey’s test).

PVY\(^O\) or PVYN on three leaves per plant in a greenhouse. PVY\(^O\) is a common or ordinary strain, which causes systemic infection or hypersensitive response in some potato varieties and tobacco. PVYN is a tobacco vein necrosis strain, which causes systemic infection to some potato varieties and local and systemic vein necrosis to tobacco (Singh et al. 2008). The upper leaves were examined 1 month later with an ELISA test, from which the rates of transmission from inoculated leaves to upper leaves were obtained.

A field test for disease resistance (bacterial wilt, late blight, and common scab) was carried out at the Nagasaki Agricultural and Forestry Development Center. For the bacterial wilt resistance assay, eight plants were grown per row, with two replicates, in a field heavily infested with the pathogen (Streptomyces scabies) in the fall cropping of 2006–2014, from which percentages of diseased plants were obtained. For the late blight resistance assay, five plants were grown per row, with three replicates, in the spring cropping of 2006–2014, under natural conditions and without fungicide application. Plants were individually scored from 0 (<2% of leaflets infected) to 6 (100% of leaflets infected), with the means representing the infection index. Finally, for the common scab resistance assay, five plants were grown in a row, with two replicates, in a field heavily infested with the pathogen (Streptomyces scabies) was dominant) in both the spring and fall cropping seasons of 2007–2014. Harvested tubers were visually inspected and infection indices from 0 (no lesions) to 100 (serious defects) were obtained using the evaluation method described by Kobayashi et al. (2008).

‘Nagasaki Kogane’ was extremely resistant to PCN, G. rostochiensis, and the PVY strains PVY\(^O\) and PVYN, and also exhibited a high level of resistance to bacterial wilt (Table 5). However, ‘Nagasaki Kogane’ was to some extent susceptible to late blight caused by Phytophthora infestans, as well as common scab.

Discussion

‘Nagasaki Kogane’ is the first variety to be selected with linked DNA markers of \( H1 \) and \( Rychc \) in western Japan. Furthermore, ‘Nagasaki Kogane’ is a noteworthy tetraploid variety with high carotenoid content. In spring cropping, the agronomic performance of this new variety, in terms of factors such as yield and mean tuber weight, surpassed that of ‘Inca-no-mezame’. Moreover, the eating quality of steamed ‘Nagasaki Kogane’ was judged to be as high as that of ‘Inca-no-mezame’. Furthermore, this novel variety exhibits good-to-excellent resistance to PCN, PVY, and bacterial wilt, suggesting that farmers can reduce use of pesticides. In addition, consumers will be able to distinguish ‘Nagasaki Kogane’ from other potato varieties by its yellow skin color, providing it with added value. We expect that ‘Nagasaki Kogane’ will contribute to the development of new uses for potatoes and help to meet the expanding demand for new potato products.

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