Overexpression of a Maize SPS Gene Improves Yield Characters of Potato under Field Conditions

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Abstract: We analyzed the yield characters of field-grown transgenic potato plants (Solanum tuberosum) carrying a maize gene for sucrose-phosphate synthase (SPS), the key enzyme in sucrose synthesis. The SPS activity in the leaves of transgenic plants (line Ag1203) was 2 times that of the control (cv. May Queen). There was no difference in the photosynthetic CO2 uptake rates between Ag1203 and May Queen plants, and the leaf starch content of Ag1203 was lower. These observations indicate that the introduction of a foreign SPS gene improved the supply of photosynthate from source (leaves) to sink (tubers). Additionally, leaf senescence of the transgenic potato plants was delayed relative to that of May Queen. The average tuber weight and total yield of Ag1203 plants were at least 20% higher, and the tuber sucrose content, which is related to eating quality, was also higher. Increased translocation of photosynthate and longer period of photosynthetic activity in the leaves may have increased the yield of Ag1203. These results suggest that introduction of the SPS gene improved the yield characters and quality of potato tubers under field conditions.

Key words: Potato, SPS, Transgenic, Yield.

Sucrose-phosphate synthase (SPS, EC 2.4.1.14) is the key enzyme in the synthesis of sucrose, which is the most transportable photoassimilate in plants (reviewed by Ono and Ishimaru, 2006). Particularly in the species that accumulate starch in their leaves (e.g., tomato, potato), higher SPS activity achieved by genetic engineering might improve sucrose synthesis activity and photoassimilation. Transgenic plants with a foreign SPS gene have been produced (e.g., Lycopersicon esculentum and Arabidopsis thaliana; Worrell et al., 1991; Micallef et al., 1995; Signora et al., 1998).

The concentration of leaf sugars may increase during leaf senescence (Crafts-Brandner et al., 1984), although the accumulation of sugars either accelerates or delays senescence (e.g., Crafts-Brandner et al., 1984; Fröhlich and Feller, 1991). Chlorophyll content is used as an indicator of the degree of leaf senescence (Friedrich and Huffaker, 1980). In transgenic rice plants with lower SPS activity than the wild type, the chlorophyll content is maintained at a significantly higher level at the ripening stage (Ono et al., 1999b).

The effect of an introduced SPS gene on yield is dependent on environmental conditions, as shown by studies with transgenic tomato plants. In a growth chamber, there was no difference in yield between transgenic plants and controls (Micallef et al., 1995). Under field conditions, however, the yield characters of transgenic tomato plants were improved (Laporte et al., 2001).

Our group introduced a maize SPS gene into potato (Solanum tuberosum; Tobias et al., 1999). In three lines of transgenic potato plants grown in pots, the dry weight of underground parts (roots and tubers) increased in the line with SPS activity. In addition to the possible effects of SPS on yield, however, the yield of potato plants is strongly influenced by the area each plant covers. In this study, therefore, we analyzed the yield characters of transgenic potato plants grown under field conditions to clarify the effect of an introduced SPS gene on yield.

Materials and Methods

1. Plant materials and cultivation of plants

From the transgenic potato plants overexpressing a maize SPS gene in our previous study (Tobias et al., 1999), we selected line Ag1203, which had the highest SPS activity. In a preliminary study, 15 tubers each of Ag1203 and the control (S. tuberosum ‘May Queen’) were planted in 2002 under the conditions described below, and the yield per plant was measured. Two years later, on 17 March 2004, 30 tubers each of Ag1203 and May Queen were planted at a row spacing of 0.6 m and a plant spacing of 0.5 m in an environmentally isolated field at the National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan (36°N, 140°E). The presence of the maize SPS cDNA in transgenic plants was verified by PCR using the method reported by Ono et al. (1999a).
Virus check of plants and expression of maize SPS
PCR by the method of Sato (2001) and visual examination showed that none of the plants were infected with any of four common potato viruses (potato virus Y, potato leafroll virus, potato virus X, and potato virus S). Total RNA was isolated from the top fully expanded leaves by the use of Qiagen RNeasy columns (Qiagen Co., Valencia, CA, USA), and first strand cDNA was synthesized from 10.0 ng of total RNA with an ExScript RT reagent kit (Takara, Ohtsu, Japan) according to the manufacturer’s instructions. RT-PCR was performed with primers specific to a maize SPS gene.

Physiological analyses
From 21 to 93 days after planting, the relative concentration of chlorophyll in the top leaf of each plant was measured with a chlorophyll meter (model SPAD-502, Konica-Minolta, Tokyo, Japan). The SPAD values (on a scale of 0 to 50) linearly related to the amount of extractable chlorophyll (Markwell et al., 1995).

At 1200 hr on day 40 after planting, the photosynthetic CO₂ uptake rate of the top fully expanded leaves of each plant was measured with a portable gas exchange system (LI-6400; Li-Cor Inc., Lincoln, NE, USA), and leaves were sampled and stored in liquid nitrogen for later measurement of SPS activity and chlorophyll content. SPS activity was measured by the method of Lunn and Hatch (1995), with a slight modification described by Ono et al. (1999a). Chlorophyll content was measured by the method of Arnon (1949). Additional leaves were sampled at the same time and dried at 80°C for 2 d for carbohydrate (starch and sucrose) measurements. Leaf samples of approximately 50 mg (dry weight) were ground to a powder in liquid nitrogen with a mortar and pestle. The powder was then extracted twice with 80% (v/v) ethanol at 80°C. After centrifugation at 12000 × g for 5 min, the pellets were boiled in distilled water for 2 hr and digested with amylloglucosidase for 15 min at 55°C. The starch and sucrose contents were then measured enzymatically according to the method of Ishimaru et al. (2001). All enzymes used in these procedures were obtained from Boehringer Mannheim GmbH (Mannheim, Germany).

Results and Discussion
In all plants of Ag1203, the presence or expression of the maize SPS gene was verified by PCR (data not shown). The SPS activity in the leaves of Ag1203 was 2 times that in the control (Table 1). The starch content of leaves in Ag1203, the main form of carbohydrate accumulated in potato leaves, was only 55% of that in control plants. The sucrose contents of leaves were the same, so the ratio of starch to sucrose contents was significantly lower in Ag1203 leaves than in the control. The photosynthetic rate was almost identical: 19.3 ± 1.1 μmol m⁻² s⁻¹ in May Queen and 19.3 ± 0.9 μmol m⁻² s⁻¹ in Ag1203. These observations indicate that the introduction of a foreign SPS gene increased the supply of carbohydrate from source (leaves) to sink (tubers) in the potato plants.

At 40 days after planting, there was no difference in the chlorophyll content of leaves between control and transgenic potato plants (Fig. 1). The SPAD value (representing chlorophyll content) of leaves of transgenic potato (Ag1203) and control plants (May Queen) during the ripening stage.

Table 1. Maximum SPS activities, starch and sucrose contents, and the ratio of starch to sucrose contents of leaves in control plants (May Queen) and transgenic potato plants with a maize SPS gene (Ag1203).

| Plant type | SPS activity (μmol mg⁻¹ chlorophyll h⁻¹) | Starch content (μmol g⁻¹ FW) | Sucrose content (μmol g⁻¹ FW) | Starch/sucrose |
|------------|-----------------------------------------|-----------------------------|-----------------------------|----------------|
| Control    | 64.5 ± 2.25                             | 345.2 ± 24.7                | 43.5 ± 1.6                  | 8.0 ± 0.8      |
| Ag1203     | 125.5 ± 12.0**                          | 189.6 ± 35.5**              | 59.5 ± 5.0                  | 5.1 ± 1.3*     |

Values are means ± s.e. of 10 plants. * and ** are significant at 5% and 1%, respectively (Student’s test, n=10). Values in parentheses are % of control.

Fig. 1. Changes in SPAD value (representing chlorophyll content) of leaves of transgenic potato (Ag1203) and control plants (May Queen) during the ripening stage. Vertical bars represent s.e. of 30 independent plants in each line.
in chlorophyll content between control and Ag1203 plants (data not shown). At 78 days after planting, the SPAD value (representing chlorophyll content of the leaves) had decreased in control plants, but was maintained in Ag1203 plants (Fig. 1). At harvest (93 days after planting), control leaves were chlorotic, but Ag1203 leaves remained green (data not shown), and the SPAD value of Ag1203 was twice that of the control. Chlorophyll content can be used as an indicator of the degree of leaf senescence (Friedrich and Huffaker, 1980). The transgenic plants appeared to be less senescent than the control plants.

In transgenic rice, a decrease in SPS activity retarded leaf senescence (Ono et al., 1999b). In contrast, by using the same SPS gene, we found that an increase in SPS activity retarded leaf senescence in transgenic potato plants. The content of sucrose in leaves affects the rate of leaf senescence (Ono et al., 2001). In transgenic rice, the lower SPS activity caused the content of the accumulated carbohydrate (sucrose) to decrease, and senescence was delayed (Ono et al., 1999b), whereas in the transgenic potato, lower starch content delayed senescence. These results may reflect the fact that rice leaves and stems accumulate sucrose, whereas potato leaves accumulate starch (Ono and Ishimaru, 2006).

The total yield per plant (Fig. 2B) and the average tuber weight (Fig. 2C) were at least 20% higher in Ag1203 plants than in the controls. The improvement in translocation of photosynthate and the longer period of photosynthetic activity due to the delay of senescence were thus responsible for the higher yield of the transgenic potato plants. The preliminary study in 2002 gave similar yield results (data not shown), suggesting that the effect of the introduced SPS gene on yield is stable.

In most procedures of cooking and processing of potato, the skin is removed. Thus, the larger average tuber size in Ag1203 plants (Fig. 2A) could improve processing efficiency. In addition, the concentration of sucrose is associated with the relative sweetness of a potato tuber. Although there was no difference in tuber starch contents between the lines in this study, the sucrose content of Ag1203 tubers was 2.1 times that of the control tubers (Table 2). This increase in sugar content might improve eating quality.

As mentioned in the introduction, the effect of an introduced SPS gene on tomato yield depended on whether the plants were grown in pots or in the field, the area of each plant (Micallef et al., 1995; Laporte et al., 2001). The previous and present findings indicate a similar improvement in yield characters of transgenic potato under both conditions. This difference between transgenic tomato and potato might depend on species. Further study using various transgenic plant species is needed to clarify this point.

**Conclusion**

The introduction of a foreign maize gene via genetic engineering improved both the quantity and quality of yield characters of field-grown potatoes. An increase in SPS activity increased the photosynthate supply from leaves (source) to tubers (sink) and extended the period of photosynthesis, thus improving yield characters. The SPS activity of leaves may serve as an indicator of translocation competence in the breeding of potato plants to produce higher yields. According to the assessment criteria of the Ministry of

### Table 2. Starch and sucrose contents of tubers in control plants (May Queen) and transgenic potato plants with a maize SPS gene (Ag1203).

| Plant type | Starch (μmol mg⁻¹ FW) | Sucrose (μmol mg⁻¹ FW) |
|------------|------------------------|------------------------|
| Control    | 569.4 ± 39.8           | 12.6 ± 2.7             |
| Ag1203     | 681.0 ± 105.0          | 26.7 ± 4.9**           |

** is significant at 1% according to a t-test.
† Values in parentheses are % of control.

Fig. 2. (A) The five largest tubers collected, (B) yield per plant, and (C) average tuber weight in a transgenic potato line (Ag1203) and control plants (May Queen). Vertical bars represent s.e. of 30 independent plants in each line. ** and *** are significant at 1% and 0.1%, respectively. Values in parentheses are % of control.
Agriculture, Forestry and Fisheries of Japan, there were no differences in characters (e.g., disease resistance) between Ag1203 and May Queen (control) plants. However, Ag1203 produced a higher yield of larger and sweeter tubers than the control, thus improving the economic value of the crop.

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