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Breeding of Tomato with High L-Ascorbic Acid Content by Clonal Selection

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Summary

We developed a variety of tomato with high L-ascorbic acid (AsA) content by clonal selection. Initially, 432 breeding materials of tomato (*Lycopersicon esculentum* Mill.), were evaluated for the following parameters, fruit ascorbic acid (AsA) content, total soluble solids (TSS) content of fruit homogenate, and fruit weight. Their AsA contents ranged from 28 to 639 mg・kg^{-1}; the average was 247 mg・kg^{-1}. Although no clear correlation between fruit AsA content and average fruit weight existed, many varieties with extremely high AsA content bore small fruit weighing less than 10 g. However, several varieties weighing 30-60 g had AsA content higher than 500 mg・kg^{-1}. Moderate positive correlation was observed between AsA content and TSS content, suggesting a probability of developing tomato varieties with high AsA content and TSS. From these breeding materials, 22 and 53, maternal and paternal parents, respectively, were hybridized, resulting in 698 cross combinations. Among their progenies, 10 or 20 individuals from each cross combination were cultured and evaluated for the above characteristics. The average AsA content ranged from 132 to 388 mg・kg^{-1}. Twenty-four outstanding individuals including VT8 that was finally released as a new variety, were selected and multiplied vegetatively and the clones were evaluated under three cropping types: normal, retarding and forcing culture in several farms in Japan using the 'House Momotaro' and 'Momotaro-York' two common varieties, as the controls. Although AsA content of VT8 fluctuated from 220 to 365 mg・kg^{-1}, it consistently exceeded that of the control by 50-100%. TSS content of VT8 varied between 5.2 and 7.2%, in contrast to 4.5 to 6.7% of the control. Average fruit weight of VT8 varied with the cropping type from 57.6 to 72.8 g. Yields of VT8 in retarding culture at three different farms were similar to or slightly higher than those of the control varieties. Thus, VT8 was developed as a variety within three years from evaluation of breeding materials to completion of a year-round trial.

Key Words: ascorbic acid content, breeding, clonal selection, *Lycopersicon esculentum*, tomato.

Introduction

Although the L-ascorbic acid (AsA) content of tomatoes is not exceptionally high compared to other fruits and vegetables, tomatoes are an important source of AsA in human nutrition because of the large consumption (Stevens, 1986; Tigchelaar, 1986). Tomato breeding for high AsA content is, therefore, important for human nutrition, but relatively few such efforts have been made. Varieties, such as 'Double Rich', have been bred with the intention of increasing AsA content. But none have succeeded as a commercial variety because of their poor yields (Stevens, 1986). Today, no commercially developed tomato variety is being cultured with the aim of enhanced AsA content.

We sought to develop fresh market tomato varieties with high AsA content that would be acceptable to both farmers and consumers.

We set a target level of fruit AsA content 50 to 100% higher than those of common regular sized tomatoes. In the fifth revised edition of the Standard Tables of Food Composition in Japan (The Resources Council of the Science and Technology Agency of Japan, 2000) AsA content of regular sized tomatoes is described as 150 mg・kg^{-1}. Therefore, our target AsA content was 225-300 mg・kg^{-1}.

Regarding fruit size, we did not aim at cherry type tomatoes, but those of the regular or medium size. Sweet fruit is desirable for wide acceptance by consumers. We adopted total soluble solids (TSS) content of fruit homogenate as the index of sweetness and set target TSS content to be over 6%. An acceptable yield, that is a crucial parameter for commercial varieties, was set as equivalent to the level of common regular sized tomato varieties for fresh use in Japan.

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Materials and Methods

Evaluation of breeding materials

Initially 432 breeding materials of tomatoes (*Lycopersicon esculentum* Mill.) including genetic resources provided by the Genebank belonging to Ministry of Agriculture, Forestry and Fisheries, Japan or the Asian Vegetable Research and Development Center, purebred and F_1 varieties from domestic and foreign seed companies, and our breeding lines were assembled. Five plants for each breeding material were planted on 10 April, 1995 in plastic greenhouses in the Applied Plant Research Center, Japan Tobacco Inc. The greenhouses were maintained above 13℃ and sufficient fertilizer was applied before planting to raise nutrient levels in the soil to N 1.5 kg-P 2 kg-K 2 kg・a^{-1}. N 0.15 kg・a^{-1} was applied one month after planting and N 0.1 kg・a^{-1} was subsequently applied biweekly. Fruits were harvested in July twice from every plant and their weights, TSS contents and AsA contents were recorded.

Evaluation of progeny from crosses among the breeding materials

Among 22 and 53 maternal and paternal stocks, respectively, 698 cross combinations were made. Their progenies were cultured in plastic greenhouses at a farm in Asahi village in Ibaraki prefecture. Basal fertilizer was applied before planting so that nutrient levels in the soil were N 1 kg-P 2 kg-K 2 kg・a^{-1}. Either 10 or 20 plants of each cross combination were transplanted on 5 July, 1996 and the fruits harvested from September to October thrice from every plant. Their individual weights, TSS contents and AsA contents were recorded.

Selection and Evaluation of VT8

Twenty-four outstanding individuals including VT8, were selected from among the progenies based on its AsA and TSS content as well as other traits important for commercial varieties, such as crop yield, frequency of marketable fruits, and disease occurrence in fields. Virus-free clones of the selected individuals were obtained by apical meristem culture, multiplied by tissue culture and then by stem cutting. Plants of the clones of the selected individuals were grafted on rootstocks 'KCFTN 2' (Musashi Breeding Farm, Tokyo) and cultured for evaluation. 'House-Momotaro' and 'Momotaro-York' (Takii Seed Co., Ltd., Kyoto) that bore regular sized tomatoes were cultivated simultaneously as the controls. Clones of the selected individuals and the controls were evaluated under three cropping types: normal, retarding and forcing culture. In normal culture, the plants were planted in plastic greenhouses in a farm in Yabuki town in Fukushima prefecture on 12 May, 1997. Fertilizer was applied similarly to that described in 'Evaluation of breeding materials'. The fruits were harvested six times from July to September. In the retarding culture, plants were transplanted in plastic greenhouses at three farms on 5-7 July, 1997: one in Asahi village and the others in Choshi city in Chiba prefecture. Fertilizer was applied similarly to that described in 'Evaluation of progeny from crosses among the breeding materials'. The fruits were harvested six times from September to November. In the forcing culture, plants were transplanted to plastic greenhouses at a farm in Itakura town in Gunma prefecture on 25 September, 1997. Nutrient levels in the soil at the planting date were brought to N 2 kg-P 3 k g-K 3 kg・a^{-1} by application of basal fertilizer; N 0.1 kg・a^{-1} was applied biweekly one month after planting. They were harvested seven times from December to April 1998. At each farm, 100 plants each of the clones of the selected individuals and the controls were cultured. In normal and the retarding cultures, plants were trained to a single stem and planted to a density of 200・a^{-1}. In the forcing culture, plants grown at a density of 100・a^{-1} were trained to a double stem. Greenhouse temperatures were maintained above 13℃ in the normal and forcing cultures. TSS and AsA contents of the fruit samples were determined.

Yields of the clones and the controls were recorded in the retarding culture. At each harvest, fruits past the pink stage (30-60% of the fruit surface was pinkish or red) were harvested. Fruits that were malformed, cracked or very small, were discarded. The remaining fruits were weighed.

Determination of TSS and AsA content

Fruits in a table ripeness stage (over 90% red) were harvested and analyzed for TSS and AsA content. Fruits were stored at 4℃ in a refrigerator prior to analysis as follows: 50-100 g sample was homogenized in a blender (Osterizer Blender OB-1; Osaka Chemical Co., Osaka). With small fruit varieties, some fruits were homogenized together. With large fruit varieties, vertically cut fractions of fruits containing all sorts of fruit tissues, locules, pericarps, etc., were homogenized and the TSS content determined by digital refractometer (Atago Co., Ltd., Tokyo).

A 2-g aliquot of the homogenate was mixed with 8 ml 5% metaphosphoric acid in a test tube and re-homogenized (Biomixer ADM; Nippon Seiki Seisakusho, Tokyo). The homogenate was centrifuged at 15000 rpm for 5 min and the supernatant filtered through a membrane filter (SJLH013NS, pore size 0.5 μm; Millipore Corp., Tokyo). A 20-μl aliquot of the filtrate was analyzed by high performance liquid chromatography (HPLC) equipped with a column, Mightysil RP-18 GP Aqua (250 mm × 4.6 mm i.d.; Kanto Chemical Co., Tokyo). The column was maintained at 35℃ and eluted with 1% metaphosphoric acid at a flow rate of 1.0 ml・min^{-1}. A UV/VIS detector with wavelength set at 242 nm detected L(+)- ascorbic acid.

Results

Evaluation of breeding materials

AsA contents of fruits from the 432 breeding materials ranged 28-639 mg·kg^{-1} of which 76% ranged between 140-300 mg·kg^{-1} (Fig. 1); the mean was 247 ± 94 mg·kg^{-1}. No clear correlation was found between the AsA content and the average fruit weight of each material, but many of those with very high AsA content were the cherry type (Fig. 2). Moderate positive correlation (r = 0.52) was observed between the fruits' AsA content and the TSS content (Fig. 3).

The majority of the breeding materials, especially the foreign ones had some defects, such as poor fruit set, high frequency of malformed or cracked fruits (data not shown).

Considering TSS content, fruit weight and other important traits such as crop yield and frequency of marketable fruits besides AsA contents, 69 materials were selected as the parents for crossing; 16 of them were used as maternal parents, 47 as paternal parents and 6 were as both maternal and paternal parents. Table 1 and 2 shows the parents and their AsA and TSS contents and fruit weights.

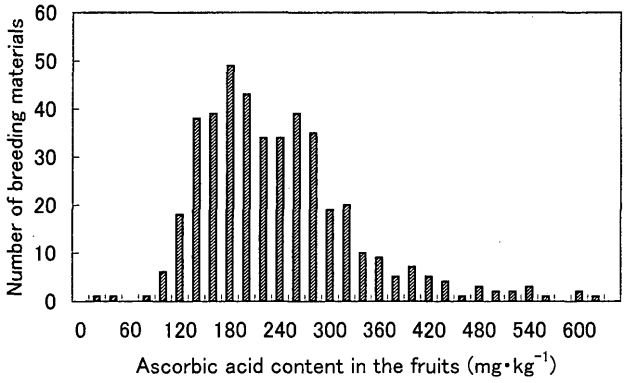

Fig. 1. Distribution of ascorbic acid content of the fruits from 432 breeding materials of tomato.

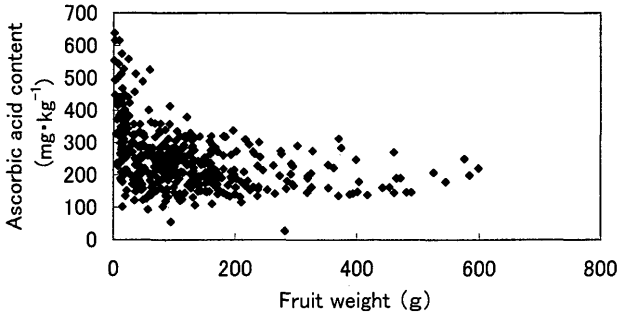

Fig. 2. Plot distribution between ascorbic acid content and average weight of fruits from 432 breeding materials of tomato.

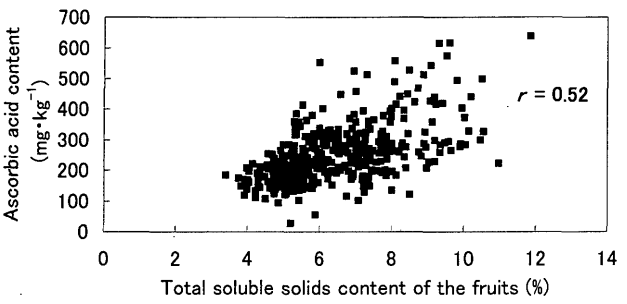

Fig. 3. Plot distribution between ascorbic acid and total soluble solids contents of fruits from 432 breeding materials of tomato.

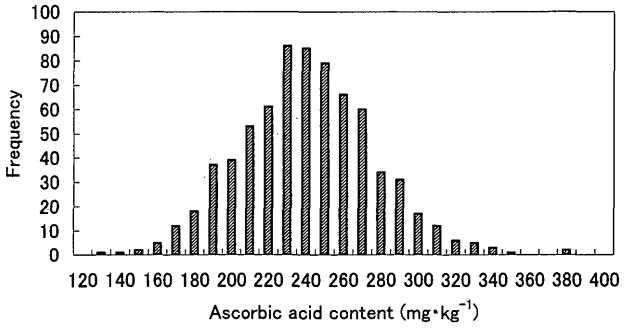

Fig. 4. Distribution of mean ascorbic acid contents of progenies from 698 cross combinations of breeding materials of tomato.

Table 1. Ascorbic acid and total soluble solids contents and the fruit weight of the strains selected as the maternal lines.

Strain	Ascorbic acid (mg.·kg^{-1})	Total soluble solids (%)	Fruit weight (g)
A00059*	321	6.8	117
A00063	269	7.8	127
A30118	279	8.3	64
A30119*	370	6.8	19
A30120*	489	8.1	48
A30123	278	9.9	13
A30125	287	7.0	101
A30126	297	6.5	84
A30127*	359	9.1	92
B00010	287	9.9	41
B00013	285	10.1	55
B00014	300	10.5	30
B00016	295	9.6	49
B00017	265	9.7	66
B20057	291	9.5	44
B30006	293	5.7	48
B30007	268	7.1	75
B60001	276	9.0	112
B60002*	327	10.6	49
B60007*	319	7.7	142
B60008*	284	8.0	115

* Strains selected also as the paternal parents.

Table 2. Ascorbic acid and total soluble solids contents and the fruit weight of the strains selected as the paternal lines.

Strain	Ascorbic acid ($mg \cdot kg^{-1}$)	Total soluble solids (%)	Fruit weight (g)
A00020	336	5.9	95
A00057	313	6.5	126
A00062	364	6.9	80
A20093	513	8.9	13
A20210	306	7.8	103
A30124	332	7.1	61
A30125	347	7.0	101
A30133	349	7.4	65
A30134	525	6.9	60
A30163	402	6.1	18
B00011	329	10.1	75
B30015	303	7.2	138
B50003	318	7.7	106
B50004	294	7.6	163
C30049	616	9.3	9
C30050	575	9.5	14
C30057	528	8.5	16
C30063	558	8.1	24
ABUNDANCE	359	5.3	77
BRANDYWINE	338	6.0	196
BREHM'S SOLID RED	380	5.8	121
BURPEE'S MATCHLESS	330	6.0	126
CHEROKEE PURPLE	321	6.4	181
CHERRY GOLD	426	9.1	12
CORONA	320	5.8	161
COSTOLUTO GENOVESE	341	5.3	85
DOUBLE RICH	413	5.5	93
EVERGREEN	337	6.9	75
GARDEN PEACH	440	8.2	24
GOLIATH	302	7.0	239
JT104	438	9.2	16
LILLIAN'S YELLOW	305	7.3	159
PERON	311	5.6	132
RED CURRANT	639	11.8	2
REVERMUM	320	5.4	110
SCHELLENBERG'S FAVORITE	322	7.1	130
STRIPED GERMAN	313	6.0	370
SUGAR CHERRY	616	9.6	2
SUN GOLD CHERRY	499	10.5	6
SUPER SWEET 100	416	9.2	6
SWEET 100 HYBRID	415	8.1	7
SWEET MILLION	544	9.1	8
SWEETIE	427	9.1	6
TIFFEN MENNONITE	318	6.8	185
VERNA ORANGE	311	5.8	128
WINS ALL	310	5.8	217
YELLOW PIGMY	440	10.2	9

Evaluation of progenies from crosses between breeding materials and the selection of a clone VT8

Average AsA contents of the progenies from the 698 cross combinations ranged 132-388 $mg \cdot kg^{-1}$ with an average of 245 $\pm$ 36 $mg \cdot kg^{-1}$; 68% of them ranged from 210 to 280 $mg \cdot kg^{-1}$ (Fig. 4).

VT8 was selected from the progeny of the cross between B00010 (maternal parent) and B60007 (paternal parent). B00010 and B60007 are both our breeding lines. B00010 is a line originated from selfing of a commercial F_1 variety 'Kansuke' and B60007 is that

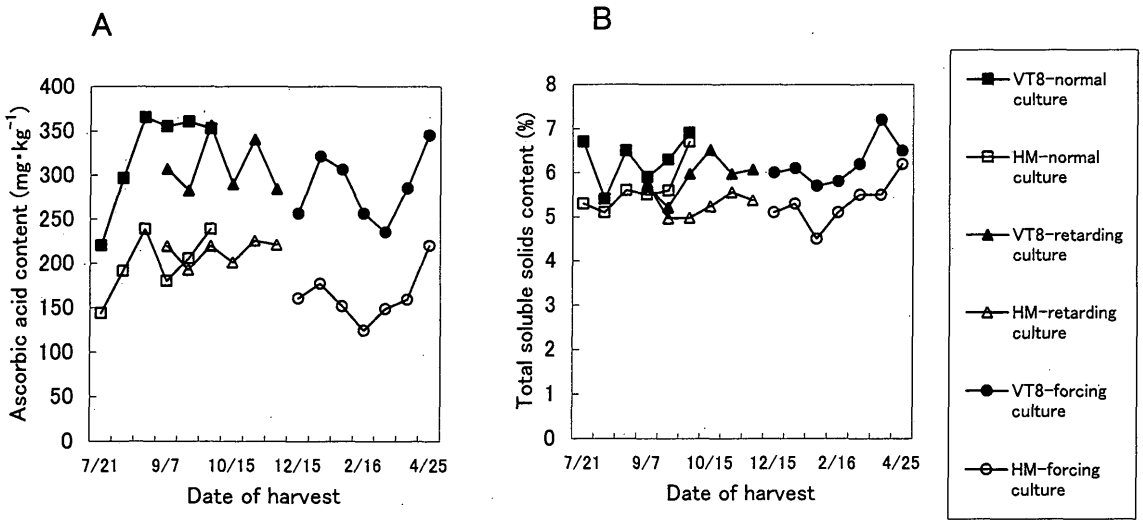

Fig. 5. Time course of ascorbic acid (A) and total soluble solids contents (B) of the fruits of VT8 and 'House-Momotaro' (HM) during year-round culture.

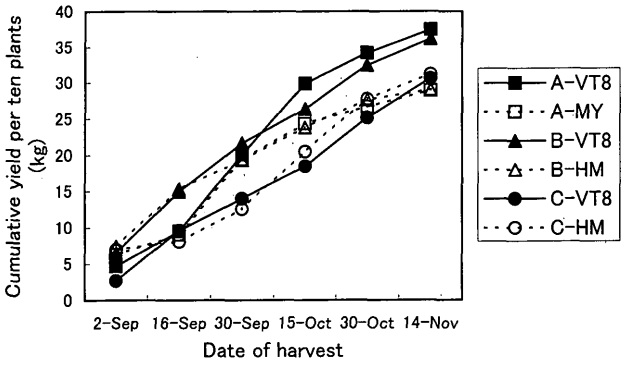

Fig. 6. Cumulative yield of marketable VT8 and control cultivars 'House-Momotaro' (HM) and 'Momotaro-York' (MY) fruits in retarding cultures at sites, A, B and C.

originated from the cross between 'Ark 60' and 'Momotaro'.

Mean AsA and TSS contents and fruit weight of the progeny of B00010 × B60007 were 268 ± 43 mg·kg^{-1}, 6.4 ± 0.53% and 67 ± 21 g, respectively. Mean AsA and TSS contents and fruit weight of VT8 itself at the selection were 324 ± 25 mg·kg^{-1}, 6.8 ± 0.4 % and 83 ± 18 g, respectively, in contrast to those of the control, 'House-Momotaro', 155 ± 33 mg·kg^{-1}, 5.4 ± 0.45 % and 153 ± 18 g, respectively.

Evaluation of VT8

AsA content of VT8 varied from 220 to 365 mg·kg^{-1} during normal-, retarding- and forcing cultures (Fig. 5A). The mean is ca. 1.5-2 times higher than that of 'House-Momotaro' throughout the whole culture period. TSS content of VT8 varied between 5.2 and 7.2% being higher than that of the control during most of the culture period (Fig. 5B). Average fruit weights of VT8 under normal, retarding, and forcing cultures were 65.6 ± 21.0 g, 72.8 ± 21.8 g, and 57.6 ± 17.5 g, respectively. Yields of marketable fruits of VT8 were almost equal to or somewhat higher than those of control varieties in retarding cultures at three farms (Fig. 6).

Discussion

Breeding materials with high AsA contents were inferred to be necessary to breed tomato varieties with high AsA content. The range of 28-639 mg·kg^{-1} among 432 breeding materials in this study was almost the same as those reported previously for *L. esculentum* by Maclinn et al. (1937), whereas wild species, such as *L. pimpinellifolium* and *L. peruvianum* have been reported with very high AsA content up to ca. 1200 mg·kg^{-1} (Lincoln et al. 1943). We used only *L. esculentum* because the wild species engender many undesirable traits.

Fruit size is an important trait for marketing tomatoes. Our breeding target was not a cherry tomato, but a medium to regular-sized tomato weighing no less than 50 g. Stevens (1986) and Tigchelaar (1986) reported an inverse relationship and a linkage or pleiotropy, respectively, between AsA content and fruit size. Most breeding materials in our experiment with extremely high AsA contents were of the small fruit varieties, although quite a few bore fruit weighing 100 g or more and having AsA content over 300 mg·kg^{-1}, suggesting that breeding a regular sized tomato with AsA content over 300 mg·kg^{-1} is possible.

Sweetness of fruit is an important characteristic for fresh use tomatoes in Japan. Tasteless tomatoes would not sell at a good price even if their AsA contents were double those of common tomatoes. For these reasons, our aim was to attain tomatoes with a minimum TSS content over 6%. Fritz et al. (1976) reported the existence of a highly significant positive correlation between AsA contents and monosaccharides in tomato. A mod-

erate but positive correlation between TSS content and AsA content was observed also among our breeding materials, which suggests that obtaining tomatoes having high AsA content and sweetness is possible.

AsA content is influenced considerably by environmental conditions such as light, growing stage and harvest season (Matthews, 1973, Shinohara et al., 1982; Stevens, 1986). Hence, breeding of high AsA varieties of tomato would be meaningless if the range of AsA content fluctuation by environmental conditions were greater than the genetic variability in AsA content between the specially bred high AsA variety and common varieties. However, even though fruit AsA content of the clone VT8 fluctuated during the year-round culture, it consistently exceeded that of the control by 50-100%, confirming that breeding a tomato with a high AsA content is both possible and feasible.

Stevens (1986) reported that there appears to be a relationship between high AsA levels and relatively poor yields. Several AsA-rich varieties of tomatoes, including 'Double Rich', were developed in the 1950s but none of them thrived because of their poor yields (Stevens, 1986). However, yields of VT8 were similar or slightly higher than those of control varieties that are widely cultured in Japan, suggesting that high AsA content does not always accompany poor yield.

Using clonal selection, VT8 was developed very shortly within three years from evaluation of breeding materials to completion of a year-round trial. First among the advantages of clonal selection over F_1 breeding is its outstanding rapidity. The second advantage is that much more breeding materials could be examined and used in clonal selection than in F_1 breeding. In addition, new and improved VT8 types may be obtained through selection within the F_2 generation. The disadvantages of the clonal selection are: 1) it requires a much larger-scale plant culture for selection than the usual F_1 breeding method; 2) in varieties bred by clonal selection, seeds, as source of seedlings, are unavailable. Thus, a system of seedling multiplication and mass-production is necessary.

Such a propagation system for tomato and sweet pepper was established by Shirai and Hagimori (2004a, b, c) so that seedlings of VT8 are now in commercial production. VT8 is currently registered as a new tomato variety under the name 'Tomapple' in Japan.

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アスコルビン酸高含有性トマト品種の栄養系選抜による育成

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摘　要

アスコルビン酸含量の高いトマト品種を栄養系選抜により育成した．最初に *Lycopersicon esculentum* Mill. に属する432種類の育種素材について果実のアスコルビン酸(L-アスコルビン酸)含量，可溶性固形分含量，1果実重量(果重)を測定した．アスコルビン酸含量は28 mg・kg^{-1}から639 mg・kg^{-1}の範囲に分布し全素材の平均値は247 mg・kg^{-1}であった．アスコルビン酸含量と果重の間には明確な相関関係は認められなかったが含量が極めて高いものには果重が10 g程度以下のものが多かった．しかし果重が30 g程度から60 g程度のものでアスコルビン酸含量が500 mg・kg^{-1}以上のものも幾つか見いだされた．アスコルビン酸含量と可溶性固形分含量には正の相関関係が認められ，高ビタミンかつ高糖度の品種が育成可能であることが示唆された．これらの素材から22系統を母親，53系統を父親として選びこれらの間で694通りの組合せで交配を行った．次代を1組合せにつき10または20本栽培し，全個体のアスコルビン酸含量，可溶性固形分含量を測定した．それぞれの交配組合せ後代毎の平均アスコルビン酸含量は132 mg・kg^{-1}から388 mg・kg^{-1}の範囲に分布していた．これらの後代の中から24個体を選抜した．これを生長点培養によるウィルスフリー化後栄養増殖してクローンとし，国内の複数の農場において夏秋栽培，抑制栽培，促成栽培の3つの作型で栽培し，年間を通してのアスコルビン酸含量，可溶性固形分含量，果重と収量を調査した．対照品種として‘ハウス桃太郎’または‘桃太郎ヨーク’を同じ場所で栽培し比較した．その結果最終的にVT8を新品種として選定した．VT8のアスコルビン酸含量は220 mg・kg^{-1}から365 mg・kg^{-1}の間を推移したが，常に対照品種の含量の1.5倍から2倍を維持した．VT8の可溶性固形分含量は5.2%から7.2%の間を推移したが概ね6%以上であった．対照品種の可溶性固形分含量は4.5%から6.7%の間を推移した．VT8の平均果重は作型により変動したが57.6 gから72.8 gであった．VT8の3つの農場での抑制栽培での収量は対照品種とほぼ同等かそれ以上であった．VT8は栄養系選抜法によって素材の評価から3つの作型での試作の完了まで3年間という非常な短期間で育成された．

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