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Improvement of Satsuma Mandarin (Wase Group) Using the Appearance of Clones with Desirable Characteristics

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Abstract

An evaluative comparison of the Wase Satsuma group with four subgroups of what are possibly mutant clones of the Wase group was carried out. All the clones were grafted onto *Citrus aurantium* L. rootstock. For the comparative study of the clones, characteristics such as vigour, yield, fruit quality and other morphological characteristics were evaluated.

The results showed that the four mutant clones have significant morphological differences from the Wase group. Major characteristics such as yield level, fruit morphology and juice quality were found to be better over 3 consecutive years in the mutant clones. Clone A gave a greater yield and larger fruit size compared to the Wase group. Clones B and C showed improved juice quality characteristics such as low acidity, higher soluble solids and vitamin C content compared to the Wase group; these clones also have a thinner peel with the result that the ratio of flesh: peel is greater than that of the Wase group and the other clones. Clone D is characterized by late ripening, 15 days after the Wase group and the other 3 clones studied, while the other characteristics are typical of the Wase group.

Introduction.

The mandarin is one of the major cultivated Citrus species. Among the cultivated Satsuma (*C. unshiu* Marc.) varieties, the Wase (early) group is distinguished by high yield, early ripening and cold tolerance (4, 5, 6). During the past twenty years, the cultivation of this group has spread in the prefectures of Ag. Saranta and Delvino where low temperatures are common.

Improvement in yield, quality and ripening period of this group as well as in other desirable characteristics is of increasing importance with the continuous increase in the needs and demands of consumers. For many years, in orchards where mandarins of the Wase group are grown, it has been observed that under the influence of various internal and external factors, canopy and leaf shape of individual trees differ greatly from those typical for the Wase group and between individual trees. Under the same climactic, edaphic and cultivation conditions, it has been observed that some individual trees had greater yields than is characteristic of the Wase group. Fruit weight and morphology, flesh and peel weight, flesh: peel and other quality characteristics were found to be different in these trees from usual fruit characteristics of the Wase group. Likewise, the ripening period of fruit on some of these trees is later than that usually associated with the Wase group.

These observations of differing characteristics led to the systematic study of these trees in order to determine how stable the appearance of these characteristics is and whether new, improved clones can be developed.

Various researchers such as Tanaka (1) report that the Wase group is characterized by genetic instability and multiple mutations thus creating groups within the same variety in which the differences in characteristics are so distinct as to warrant each group being designated as a variety itself. Other researchers describe clones which have arisen from bud mutation (2, 3, 5).

The aim of this investigation was to identify new clones within the Wase

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group having characteristics which can contribute to improvements in yield, fruit quality, cold tolerance, storage quality and in socio-economic factors such as farm income.

Materials and Methods

Research was carried out for three consecutive years (1989-1991) as part of an ongoing program, in an orchard of the former agricultural company Stiari of a total area of 3.5 ha with 2000 trees. Observations were made during the ripening period (15-30 October) on the trees which were of the Wase group 'Satsuma' mandarins (20 years old) on *Citrus aurantium* L. rootstock planted at 4 x 4 m distance. The soil is of medium texture (sandy-clay) pH 6.8-7.4. Trees were irrigated during the summer months and pruned each year. Annual fertilizer applications of 0.48 units P, 0.96 units N and 0.70 units K per tree were made and sprays against common pests and diseases were also carried out.

In order to determine which trees exhibit morphological differences from the Wase group, observations have been made twice a year on 800 trees for the past three years. The first observations have been made during spring and include: anthesis (light, medium, heavy); shoots (vigorous, medium, small); colour and morphology of leaves and canopy; pest and disease status. The second observations have been made during fruit ripening and include external morphological characteristics of tree and fruit as well as ripening period and pest and disease status. From these observations it was found that trees differing from those of the Wase group largely fell into four groups. Individual trees in each group show similar characteristics and this has led to the belief that these groups may in fact be different clones; for simplicity in this text they are hereafter referred to as clones though this has yet to be genetically confirmed.

For the comparative study of the adaptation and behaviour of the clones under investigation with the Wase group the following observations were made using four trees per clone which were in accordance with the observations made above, most representative:

1. *Vigour, general growth and tree canopy morphology.*

Canopy dimensions were measured (height, diameter) and the canopy shape was described.

2. *Leaf size, shape, colour and petiole size.*

Random samples of 40 leaves per variety and clone were taken from one year old shoots.

3. *Yield level.*

Total fruit weight was measured for each tree, variety and clone. The weight of fallen fruit in good condition was added to the above.

4. *Fruit weight (g)*

Forty fruit from each variety and clone were randomly selected and the following measurements made: average fruit, peel and flesh weight, number of segments and seeds, fruit morphology, juice volume.

5. *Juice quality characteristics.*

Twenty fruit from each variety and clone were randomly selected and the following analyses carried out:

(a) Determination of acidity (%). The total volumetric acidity of the juice was determined by titration with 0.1N NaOH and expressed as % citric acid.

(b) Determination of vitamin C (mg 100^{-1}). The chemical method for this determination is based on the reduction of coloured 2,6 dichlorophenol-indophenol to the colourless form.

(c) Total soluble solids. Total soluble solids, mostly in the form of sugars, were determined using a hand refractometer and expressed in ° Brix.

(d) Total dry weight of fruit (%). Fruit weight was measured (raw) and fruit was then placed in an oven at 70 °C until there was no further weight reduction. The oven-dry weight was then expressed as % of fresh weight.

6. *Ripening period.*

Observations were made for each tree variety and clone. Fruits were considered to be ripe when the colour change, from green to orange, had taken place over

Table 1. Tree dimensions and shape of canopy of the Wase group and its different clones.

Group of Wase and clones	1989 Canopy diameter (cm)	1989 Canopy height (m)	1989 Canopy shape	1990 Canopy diameter (cm)	1990 Canopy height (m)	1990 Canopy shape	1991 Canopy diameter (cm)	1991 Canopy height (m)	1991 Canopy shape	Branches orientation
Wase	3.53 a	2.14 a	cup-sph*	4.44 a	2.27 a	cup-sph	3.85 a	2.38 a	cup-sph	sideways-downwards
Clone Á	3.64 a	2.20 a	cup	3.83 a	2.35 a	cup	3.99 b	2.45 a	cup	downwards
Clone Â	4.34 c	3.13 c	cup	4.51 a	3.25 c	cup	4.65 d	3.43 c	spherical	upwards
Clone C	3.97 b	2.82 b	cup	4.13 a	2.83 b	cup	4.27 c	3.20 b	spherical	upwards
Clone D	3.60 a	2.13 a	cup-sph	3.80 a	2.27 a	cup-sph	3.98 b	2.41 a	cup-sph	sideways-downwards

* cup-sph. = cup-spherical shape.
Duncan test was used for the comparison of means. Level of significant difference p = 0.05.

25% of the fruit surface in 105 of the tree's fruit.

Results

1. Tree dimensions and morphological characteristics.

Macroscopically clones A, B and C were distinguished from the trees of Wase and clone D as regards vigour. In Table 1 it can be seen that the height of the canopy of clones B and C is statistically greater than that of trees from the Wase group for all three years of measurement; these trees are vigorous and uniform in their canopy growth. In contrast, clones A and D were less vigorous. Similar results were found for the canopy diameter. Differences are noticeable in the shape and orientation of branches. In the Wase group and clone D the canopy is spherical cup-shaped and the branches are oriented sideways and downward. In contrast in clones B and C the canopy is spherical with multiple branches oriented upwards with heavy shoots and anthesis. Clone A has a cup-shaped canopy with branches oriented downwards and with medium flowering.

With regard to leaf size, significant differences from the Wase group and between clones is observed (Table 2). Clone A, which was not significantly different for leaf size from the Wase group, has large deep green leaves while the other clones, with small leaves of a light green colour, were significantly different in size from those of the Wase group and each other. Petiole size differed similarly.

Table 2. Leaf length and width of the Wase Satsuma group and its different clones.

Group of Wase and clones	1989 Leaf length cm	1989 Leaf width cm	1989 Petiole length cm	1989 Length: width ratio	1990 Leaf length cm	1990 Leaf width cm	1990 Petiole length cm	1990 Length: width ratio	1991 Leaf length cm	1991 Leaf width cm	1991 Petiole length cm	1991 Length: width ratio
Wase	11.40 d	4.46 c	1.73 c	2.55:1	11.51 d	4.53 c	1.84 c	2.54:1	11.36 d	4.49 c	1.71 c	2.53:1
Clone Á	11.52 d	4.54 c	1.74 c	2.53:1	11.60 d	4.67 c	1.83 c	2.48:1	11.47 d	4.46 c	1.77 c	2.57:1
Clone Â	10.36 c	3.66 b	1.53 b	2.83:1	11.49 c	3.80 b	1.62 b	2.73:1	11.16 c	3.53 b	1.47 b	2.87:1
Clone C	9.35 a	3.42 a	1.33 a	2.75:1	9.53 a	3.53 d	1.40 a	2.69:1	9.44 a	3.36 a	1.40 a	2.80:1
Clone D	9.90 b	3.62 b	1.50 b	2.75:1	9.92 b	3.74 b	1.57 b	2.63:1	9.82 b	3.57 b	1.53 b	2.75:1

Duncan test was used for the comparison of means. Level of significant difference p = 0.05.

Table 3. The annual yield of the Wase Satsuma group and its different clones.

Group or clones	1989 kg/tree	1990 kg/tree	1991 kg/tree
Wase	40.87 a	45.17 a	39.32 a
Clone Á	57.70 c	61.27 b	54.90 c
Clone Â	49.27 b	56.97 b	45.30 b
Clone C	45.55 a	56.67 b	41.95 ab
Clone D	39.20 a	49.32 a	40.37 a

Duncan test was used for the comparison of means. Level of significant difference p = 0.05.

2. Production.

(a) Yield level per tree.

Table 3 shows that clone A had greater yields per tree for the three years in comparison with the initial group and the other clones. Clone B was next in yield with small differences from clone C which were not significant. Clone D had the lowest yield, comparable with that of the Wase group.

(b) Average fruit weight.

The high average fruit weight of clone A (Table 4) is one of the factors contributing to the superior yield of this clone and is greater then the Wase group by 23-25 g.

Table 4. Fruit weight and other characteristics of the Wase Satsuma group and its different clones.

Group or clones	Fruit weight (g)	Endocarp weight (g)	Peel weight (g)	Endocarp: Peel ratio	Segment number	Ripening date	Fruit shape
			1989				
Wase	60.07 b	42.17 a	17.90 c	2.35:1	10-11	20-25/10	hemi-oblate
Clone	85.20 c	61.47 c	23.72 d	2.59:1	10-11	20-25/10	oblate
Clone Â	63.85 b	49.67 b	14.20 b	3.49:1	10-11	20-25/10	sph.med-oblate
Clone C	54.20 a	39.67 a	11.62 a	3.41:1	10-11	20-25/10	sph.sl-oblate
Clone D	52.87 a	39.25 b	13.67 b	2.87:1	10-11	10-15/11	sph.med-oblate
			1990				
Wase	62.77 c	44.87 b	17.95 c	2.49:1	10-11	15-50/10	hemi-oblate
Clone A	88.75 e	65.15 d	23.62 d	2.75:1	10-11	15-20/10	oblate
Clone B	68.52 d	52.92 c	15.65 b	3.38:1	10-11	15-20/10	sph.med-oblate
Clone C	52.42 a	39.47 a	11.45 a	3.44:1	10-11	15-20/10	sph.sl-oblate
Clone D	56.45 b	41.77 ab	14.57 b	2.86:1	10-11	5-10/11	sph.med-oblate
			1991				
Wase	60.00 a	42.17 a	17.67 c	2.38:1	10-11	20-25/10	hemi-oblate
Clone A	83.54 c	59.57 b	24.45 d	2.43:1	10-11	20-25/10	oblate
Clone B	60.00 a	38.57 a	14.00 b	2.75:1	10-11	20-25/10	sph.med-oblate
Clone C	49.87 b	38.05 a	11.85 a	3.21:1	10-11	20-25/10	sph.sl-oblate
Clone D	48.92 b	36.05 a	13.17 a	2.73:1	10-11	10-15/11	sph.med-oblate

hemi = hemispherical; sph = spherical; med = medium; sl = slightly.
Duncan test was used for the comparison of means. Level of significant difference p = 0.05.

Table 5. Qualitative characteristics of fruit of the Wase Satsuma group and its different clones.

Group or clones	Juice content per fruit (ml)	Acidity %	Total soluble solid content ° Brix %	Vitamin C mg/100ml	Dry weight %
			1989		
Wase	19.77 a	1.68 c	4.14 a	24.84 a	9.97 a
Clone A	23.32 b	1.85 d	4.24 b	26.20 b	9.87 a
Clone B	20.27 a	1.57 b	4.47 c	29.05 c	10.07 a
Clone C	19.90 a	1.47 a	4.82 d	31.87 d	10.07 a
Clone D	19.92 a	1.66 a	4.26 b	27.32 b	10.02 a
			1990		
Wase	21.12 c	1.63 c	4.21 a	20.00 a	10.05 a
Clone A	24.05 c	1.83 d	4.32 a	21.55 a	9.87 a
Clone B	20.50 bc	1.54 b	4.45 b	24.72 b	9.86 a
Clone C	19.75 ab	1.42 a	4.48 b	25.22 b	10.02 a
Clone D	19.00 a	1.64 c	4.34 a	21.90 a	10.15 a
			1991		
Wase	19.77 a	1.67 a	3.19 ab	27.60 a	9.82 a
Clone A	23.32 b	1.96 c	3.03 a	27.30 a	9.77 a
Clone B	20.27 a	1.58 d	3.42 ab	29.72 b	10.15 a
Clone C	19.90 a	1.42 b	3.90 c	28.79 c	10.17 a
Clone D	19.92 a	1.63 a	3.54 bc	27.76 a	10.12 a

Duncan test was used for the comparison of means. Level of significant difference p = 0.05.

With the exception of 1990, where there were differences between the clones and the Wase group, the other two years showed that the average fruit weight for clone B was similar to that of the Wase group. Average fruit weight of clones C and D was similar but 10-12 g less than that of the Wase group.

(c) Other fruit characteristics.

Peel weight in fruit of the clones was again significantly different from that of the Wase group, while differences existed between the clones (Table 4). The clones can be divided into three groups (i) clone A: large fruit size, average fruit weight exceeding 80 g, average peel weight 23-25 g, thick wrinkled peel, oblate shape; (ii) clones C and D: small sized fruit with average weight of approx. 50 g, smooth thin peel 11-14 g in weight, spherical often slightly necked; (iii) clone B: fruit similar to the Wase group, average fruit weight of approx. 60 g, medium peel 14-17 g, medium oblate.

Positive characteristics observed primarily in clones B and C of consumer value include the ratio between flesh and peel which was 2.75:1 to 3.49:1, compared to 2.35:1 to 2.49:1 observed in the Wase group. In the clones and Wase group the number of segments was 10-11 per fruit. The fruit were seedless and the presence of seeds was rare, one seed only

being found in the Wase group and clones A and D.

3. Quality characteristics of juice.

In comparison with the Wase group and other clones, clone A can be seen to have the highest juice content (Table 5). Clones B, C and D had similar juice content to the Wase group in 1991 and 1992, although different in 1990.

Clone A was found to have a higher acidity compared to the Wase group. The other clones B, C and D have significantly lower acidity than the Wase group and there are significant differences between these clones.

Clone C is characterized by having the lowest acidity (1.42-1.47% compared to 1.63-1.68% in the Wase group).

A basic indicator of internal fruit quality is the total soluble solids content. Clones B and C have the greatest t.s.s. content compared to the Wase group and the other clones. Clone D does not differ significantly from the Wase group.

As regards vitamin C content, clones B and C have the highest content (24-30 mg/100ml and 25-32 mg/100ml compared to 22-28 mg/100ml in the Wase group). Clones A and D, except in 1989 where content was significantly different from the Wase group, but not between clones, were similar in vitamin C content to the Wase group.

While total dry weight of fruit for clones was not significantly different from that of the Wase group, differences between the clones existed.

4. Ripening period.

Clone D was found to be later ripening (after 10 November) (Table 4). For clones A, B and C ripening was the same as for the Wase group and took place in the middle of October.

5. Resistance to pests, diseases and low temperatures.

No real differences between the clones was found as regards pest/disease resistance and cold tolerance. In spite of this the Wase group and the clones showed tolerance to the low temperatures often experienced in the study, which can reach as low as –8 °C.

6. Compatibility with the rootstock.

In no case was there any incompatibility between the Wase group or the clones and the rootstock.

Conclusions

Based on the morphological features studied and the analyses carried out on the Wase group and the four clones it is concluded that there are important statistical differences between them. Many of the morphological characteristics mentioned above are more positive in the clones than in the Wase group.

Clone A presents greater yield, larger fruit and greater juice content. Clones B and C have better indicators of fruit quality, while clone D is a later ripener.

The results, which were consistent over the three years, indicate interesting genetic material which further study could lead to stabilization of these positive characteristics and to the continuation of these clones.

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