JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Technique for Rapid, Small-Scale Analysis of Vitamin C Levels in Fruit and Application to a Tomato Mutant Collection

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We present a technique for easy, rapid analysis of both total and reduced forms of vitamin C in fruits using microplates and a plate reader. This technique has been compared with a spectrofluorometric technique classically used for assaying vitamin C in fresh tomato. We have applied these methods to a population of 118 tomato mutant lines and controls in search of variability for this trait. Six lines, identified as having high vitamin C levels, and four lines having low vitamin C levels have been chosen for further study. The vitamin C levels have been compared with sugar concentration, dry matter content, fruit weight, titratable acidity, and firmness. The correlations that often exist in tomato varieties between sugar and vitamin C content (positive correlation) or fruit weight and vitamin C content (negative correlation) can be uncoupled in the lines selected for further analysis.

KEYWORDS: Vitamin C; tomato; genetic variation

INTRODUCTION

Vitamin C (ascorbic acid) is an essential vitamin found mainly in fresh fruit and vegetables. It is of interest first from a nutritional point of view as an important antioxidant and second because the molecule ascorbate is involved in numerous fundamental processes within a plant such as cell division and expansion, the control of stomatal opening, and protection against oxidative stress (*1*−*3*). Furthermore, vitamin C mutants have severely altered growth and development as shown by the *Arabidopsis* mutant *vtc1*, which is altered in defense transcripts, growth, and stress tolerance (*2*, *4*, *5*).

Tomatoes contain moderate amounts of vitamin C, but especially in the summer, the large quantities consumed mean that this fruit provides us with significant quantities of the vitamin. Research on tomato has developed rapidly due to the identification of this plant as a model for fruit development and is set to continue with the current genome sequencing project (*6*). The plant lends itself to studies of fruit architecture, ripening, and all aspects of fruit quality; for this reason, different populations have been created and evaluated for organoleptic or nutritional quality (*7*, *8*). Improvement of vitamin content in species of agronomic interest is also cited as an important criterion (*9*−*11*). Indeed, wild tomato accessions are rich in vitamin C, a quality that has been lost in many commercial varieties, although cherry and cocktail varieties are richer in vitamin C than standard varieties. Growth and harvesting conditions are also factors affecting tomato nutritional quality (*12*). Mutant collections exist (*13*), which are not only a resource for exploring gene function but can be used as sources of genetic diversity to search for plants with useful quality characteristics, via Tilling, for example, as has been shown in wheat (*14*).

A variety of methods exist for measuring the reduced or oxidized (dehydroascorbate) forms of vitamin C. Many of these methods rely on the reduction or oxidation of a compound by ascorbate leading to a detectable color change. Methods to detect one or both forms include the reduction of indophenol by reduced vitamin C (*15*), the complexing of oxidized vitamin C with phenylenediamine to produce a fluorescent quinoxaline (*16*), or the formation of a formazan precipitate following reduction of nitrobluetetrazolium (*5*). The two forms of ascorbate can also be assayed using high-performance liquid chromatography (HPLC) (*17*). The disadvantage of many techniques, including HPLC, is the time taken to process each sample: Vitamin C is an unstable molecule that degrades easily. When working with vitamin C, the rapidity and ease of the assay method are of utmost importance as the molecule is unstable, particularly in the absence of an acid buffer or following freezing and thawing. Measurements of vitamin C are also simpler in leaves where concentrations of ascorbate reach 5 mM. In fruit, such as tomato, levels are often lower. In modern tomato varieties, concentrations are 5−10 times lower, reaching about 0.5−1 mM (about 10−20 mg/100 g fresh weight). In contrast, wild *Solanum lycopersicum* accessions, for example *Solanum peruvianum*, contain up to 120 mg vitamin C/100 g fruit.

We present a technique for easy, rapid analysis of both oxidized and reduced forms of vitamin C using microplates and a plate reader. The technique involves the reduction of iron 3+

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10.1021/jf061241e CCC: $33.50
Published on Web 07/27/2006

to iron 2+ by ascorbate. Concentrations of vitamin C can be measured in 0.5−1 g of frozen ground powder from tomato fruit. This technique has been compared with a spectrofluorometric technique classically used for assaying vitamin C in 5 g of fresh tomato. In addition, a subset of plants with an identifiable fruit phenotype from a saturated mutation library of tomato, which has been previously screened (*13*), has been screened in the M3 generation for fruits exhibiting high or low vitamin C levels. Both techniques have been applied to the analysis of this tomato mutant population (118 lines including controls) to select plants with high or low vitamin C levels. Six lines, identified as having high vitamin C levels, and four lines having low vitamin C levels have been chosen for further study. The vitamin C levels have been correlated with sugar levels, dry matter content, fruit weight, titratable acidity, and firmness for fruits of these plants.

MATERIALS AND METHODS

Microplate Assay. *Extraction.* Tomato fruits were ground in liquid nitrogen to a fine powder. Approximately 1 g of frozen powder or up to 100 mg of dried powder was added to 600 μL of ice cold 6% trichloroacetic acid (TCA) in a 2 mL eppendorf. In both cases, the quantity of powder was sufficient to give absorption values toward the middle of the standard curve. The volume of TCA was not limiting (data not shown), and increasing the volume did not increase the yield of vitamin C but had the disadvantage of no longer allowing extractions to be carried out in eppendorfs, thus reducing the practicality of the method. Once extracted, samples were stable for a few hours on ice but extractions could not be frozen. It was found that each freeze−thawing cycle led to up to 20% losses of vitamin C activity (data not shown); therefore, extractions had to be analyzed the same day.

For the calculation of ascorbate concentration, an adjustment had to be made for the volume increase due to the sample (see calculation section below). Samples were vortexed for 10 s or until the sample thawed. Samples were then left on ice for 15 min to quench the metabolism followed by centrifugation for 15 min at 25000*g* and 4 °C. The supernatant, which was clear of plant material, was transferred to a clean tube. Twenty microliters of this supernatant was used in each assay.

The ascorbate standard was prepared fresh on the day of analysis. A solution of 1 mg/mL (Na) ascorbate in 6% TCA was diluted to give a concentration in 20 μL of 0, 5, 10, 15, 20, and 30 nmol.

These quantities allowed a standard curve of absorption values between 0 and 1 to be generated. Two assays were carried out on each sample to measure the total and reduced forms. Each time it was reduced ascorbate that was assayed. The total ascorbate was measured by including an incubation with dithiothreitol (DTT), which reduced the oxidized form present in the sample. A second assay, without DTT, allowed the measurement of the reduced ascorbate only and gave the amount of oxidized form by comparison with the DTT measurement.

Total Ascorbate Assay. Twenty microliters of sample or standard was distributed into two wells for the two repetitions. Twenty microliters of 5 mM DTT (in 0.4 M phosphate buffer, pH 7.4) was added to reduce the oxidized ascorbate. The covered plate was incubated for 20 min at 37 °C. Ten microliters of N-ethyl maleimide (NEM; 0.5% w/v in water) was added, mixed, and left for 1 min at room temperature. Eighty microliters of color reagent was added (see below). The covered plate was incubated for 40 min at 37 °C, and the absorbance was read at 550 nm using a microplate reader.

The color reagent was as follows: solution A: 31% orthophosphoric acid, 4.6% w/v TCA, and 0.6% w/v iron chloride; solution B: 4% 2,2-dipyridyl (w/v made up in 70% ethanol); and solutions A and B were mixed 2.75 parts (A) to 1 part (B).

For the reduced ascorbate assay, the DTT and NEM were omitted and the procedure was carried out as above. The DTT and the NEM (which eliminates the excess DTT) were replaced by the same volumes of 0.4 M phosphate buffer, pH 7.4.

Calculations. A standard curve can be obtained from the standard solution values (x = nmol in well, and y = absorbance; **Figure 1**), and

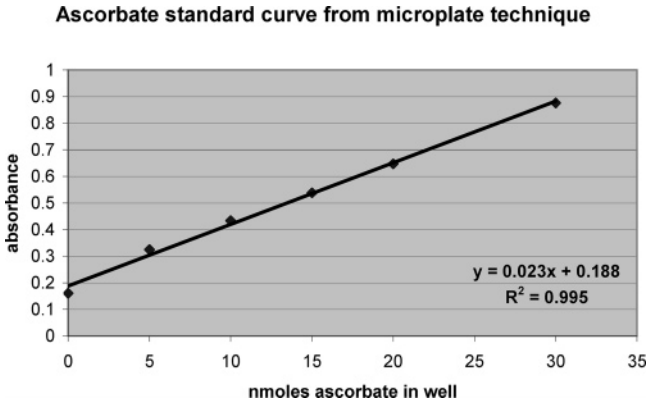


Figure 1. Example of standard curve obtained. Ascorbate standard curve generated from microplate technique standards. The linear regression produced allows the calculation of the nmol of ascorbate in 20 μL of each sample.

from these data, two calculations were carried out: nmol of ascorbate in 20 μL of each sample using the equation of the line, and μmol in the sample.

For fresh (nondried) samples of tomato fruit, the volume was corrected for the quantity of water introduced by the sample. The corrected volume was equal to: volume of buffer + [weight of powder × (1 − percentage dry weight)]. Therefore, the dry weight of any sample was required. Calculations were as follows: μmol/g fresh weight, mg/100 g fruit, redox state = ascorbate (without DTT)/total ascorbate (DTT), and oxidized ascorbate (dehydroascorbate) = total ascorbate − ascorbate.

A similar calculation was carried out for dried (lyophilized) samples. In this case, only 100 mg maximum of material was required and there was no volume correction. The original fresh weight can be calculated as long as the dry weight is known.

Sample Calculation. For a total ascorbate absorbance, A_t, of 0.5, a standard curve equation of $A = 0.023x + 0.188$ and $R^2 = 0.995$, an initial volume of TCA, T, of 0.6 mL, and a sample of weight, P, of 1 g and a sample dry weight, D, of 5%:

$$\text{nmol in 20 } \mu\text{L of sample} = \text{nmol in well} = x = (A_t - C)/M = (0.5 - 0.188)/0.023 = 13.6 \text{ nmol}$$

$$\text{total volume} = T + \text{volume of water in sample} = T + P(1 - D/100) = 0.6 + 1(1 - 5/100) = 1.55 \text{ mL}$$

$$\mu\text{mol in sample} = \mu\text{mol in well} \times \text{total volume} = 13.6/(0.02 \times 1000) \times 1.55 = 1.05$$

$$\mu\text{mol/g fresh weight} = \mu\text{mol in sample}/P = 1.05$$

$$\text{mg/100 g} = \text{mmol/100 g fresh weight} \times \text{RMM ascorbate} = 1.05/1000 \times 100 \times 198 = 20.8 \text{ mg/100 g}$$

Similarly, the reduced ascorbate concentration for an absorbance value of 0.45 was 17.4 mg/100 g. The redox state can therefore be defined as 17.4/20.8 = 0.8, and the quantity of the oxidized form can be defined as 3.4 mg/100 g.

Spectrofluorometric Method. The method was based on the reaction of oxidized ascorbate (dehydroascorbate) with orthophenylenediamine producing a fluorescent quinoxaline and has been previously described (*16*). A set of standards containing between 5 and 50 μg ascorbic acid/mL were prepared in extraction buffer (3% metaphosphoric acid and 25% methanol) and processed in the same way as the test samples. Five grams of frozen tomato powder was then mixed with 50 mL of extraction buffer and incubated with 1 g of activated charcoal (Norit) for 10 min to oxidize the reduced ascorbate present in the sample. If the endogenous oxidized form in the sample was required, then a small quantity was removed before addition of the activated charcoal. The mix was filtered, and the samples were dialyzed and then mixed with 3.7 M sodium acetate and 0.05% orthophenylenediamine dihydrochloride before incubation in a water bath at 37 °C. The

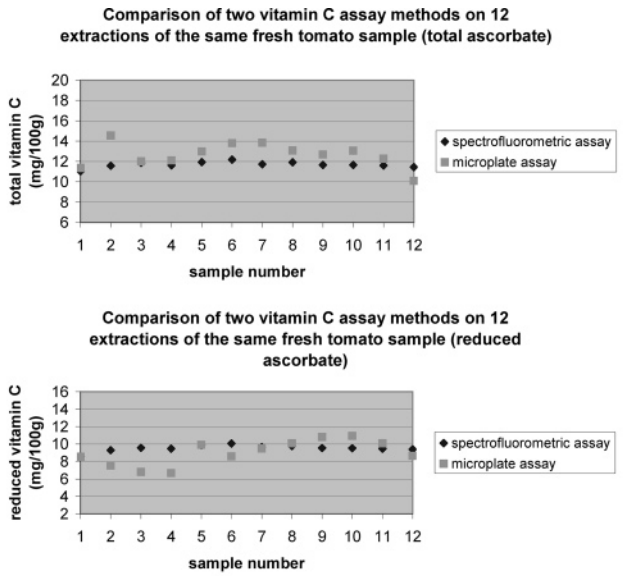
orthophenylenediamine reacted with the oxidized form of ascorbate producing a fluorescent quinoxaline whose fluorescence at 435 nm was detected by a spectrofluorometer after excitation at 350 nm, at the rate of 30 samples per hour. Results appeared in the form of peaks, and the concentration of vitamin C was calculated by reference to the standards.

Tomato Mutant Series Harvesting and Phenotypic Analyses. A total of 111 M3 families and seven controls were planted in the field (SE France), and fruits were harvested in July 2004. A plot of six plants represented each mutant line, and the seven control lines (M82 parent; six plants per plot) were interspersed randomly among the plots. At harvest, a total of 30 ripe tomatoes were harvested per line (five red ripe fruits of uniform size per plant). Half the sample was used for nondestructive tests on a fruit-by-fruit basis (fruit weight and color) and finally for texture assays using a durofel apparatus (Setop Giraud Technologies, France). A 1 cm central slice of the remaining 15 tomatoes was immediately frozen at −30 °C before grinding in liquid nitrogen. The ground powder was used to measure chemical traits including vitamin C content using the two techniques described in this article and sugar concentration, titrable acidity, pH, and dry matter content as described in ref *21*. For vitamin C content, the powders were immediately assayed using the spectrofluorometric technique and aliquots of the powder were also immediately lyophilized and conserved at −80 °C while the microplate technique was being developed. The conserved lyophilized samples were then tested using the microplate technique 1 year later.

RESULTS

Development of a Microplate Method for Assaying Vitamin C in Tomato Fruits. A protocol for assaying vitamin C in 1 g of freshly ground powder has been developed for tomato and other fruits. The protocol uses the spectrophotometric detection of Fe^{2+} complexed with dipyridyl following the reduction of Fe^{3+} to Fe^{2+} by ascorbate used in leaves or blood (*18*−*20*). The sensitivity of the method has been defined as 0.044 mol/g fresh weight (*18*) or 0.87 mg/100 g fresh weight. It has been adapted for tomato fruit on a small scale, using eppendorfs and a microplate reader. On a 96 well plate, 20 samples can be assayed for both the reduced and the oxidized form with two repetitions per sample (40 wells per form plus 12 wells for the standards). Several plates can be processed within a day, the rate-limiting step being the acid extraction. The detailed methods for the extraction, assay, and calculation of vitamin C concentrations in tomato are presented in the Materials and Methods.

Comparison with a Spectrofluorometric Method. A comparison between the microplate assay method and the spectrofluorometric method widely used for assaying vitamin C by continuous flow (see Materials and Methods) was carried out on a finely ground frozen tomato powder from a pool of red ripe M82 processing tomatoes. The assay was repeated 12 times on the same powder, the same day, using each technique (extractions were carried out separately). The results are shown in **Figure 2**. The tomato powder contains between 11 and 13 mg vitamin C per 100 g fresh weight with 11.7 mg/100 g for the spectrofluorometric technique and 12.6 mg/100 g for the microplate technique. For the reduced ascorbate assay, the ripe tomatoes contain between 9 and 10 mg reduced vitamin C per 100 g fresh weight, 9.5 mg/100 g for the spectrofluorometric technique, and 9.0 mg/100 g for the microplate technique. By deduction, there is between 2.2 and 3.6 mg/100 g of oxidized ascorbate (dehydroascorbate) and the redox state is between 0.7 and 0.8 (reduced divided by total ascorbate) for red ripe tomatoes. The microplate technique has therefore been validated, by comparison with a standard technique, as a way of assaying vitamin C content in tomato. The standard deviation of the microplate technique is higher (1.2 mg/100 g as compared to 0.3 mg/100 g for total ascorbate and 1.5 mg/100 g as compared to 0.4 mg/100 g for reduced ascorbate) and shows that this technique is less precise as compared to the spectrofluorometric technique.



	spectrofluorometric assay		microplate assay	
	total ascorbate	reduced ascorbate	total ascorbate	reduced ascorbate
mean (mg/100g)	11.7	9.5	12.6	9.0
standard deviation	0.3	0.4	1.2	1.5
% standard deviation	2.5	3.9	9.5	16.2

Figure 2. Comparison of two vitamin C assay methods on a sample of fresh tomato. Total ascorbate levels (top) and reduced ascorbate levels (bottom) are shown for 12 individual extractions of the same frozen ground tomato powder using each assay method (spectrofluorometric, diamonds; microplate, squares). The table shows the mean, standard deviation, and percentage standard deviation of the 12 assays for each technique on total or reduced ascorbate.

Table 1. Estimated Sources of Error Due to Different Steps of the Microplate Method

source of error	% error	% error squared
absorbance	0	0
fresh weight of powder	1	1
dry weight	1	1
600 μL of TCA	1	1
weight of ascorbate standard	1	1
pipet 20 μL of extract	5	25
total % error excluding powder error	5.4	29.0
measured experimental error	9.5	89.7
hypothetical calculated error due to powder	7.8	60.7

The estimated sources of error are shown in **Table 1**, and while the quality of the frozen ground powder is an important issue for both techniques (a heterogeneously ground powder leads to heterogeneous results), a source of error that can be introduced into the microplate technique is imprecise pipetting of the small volumes that are used. An error of 1 μL on 20 μL introduces a 5% error into the final result. This error exists for the sample and the standard, and these are the two major sources of error that result in a final standard deviation of 1.2 mg/100 g. Although volumes can be increased slightly (and the other components of the reaction have not been found to be limiting for the ascorbate concentrations used; data not shown), it is important not to overfill the wells. This error could also be reduced by using automated pipetting techniques. The remaining error, present for both techniques, is supposedly due to the sample extraction and the homogeneity of the powder. This error

Table 2. Advantages and Disadvantages of the Microplate Method

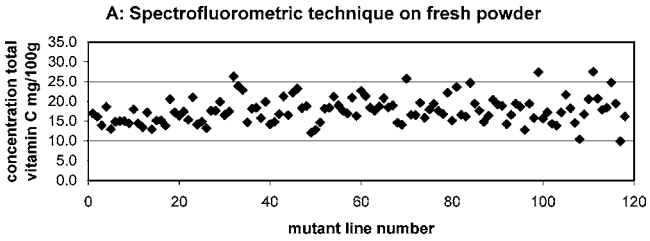
	fluorometric assay	microplate assay
cost	1 euro for 1 assay of both forms	2.50 euros per plate (20 assays, both forms)
time required for 40 analyses of both forms[a]	1 person day	1 person day
quantity of material	5 g of fresh material	0.5–1 g of fresh material
equipment	specialized set up, not routine laboratory equipment	plate reader 550 nm, eppendorfs and pipets
major sources of error	interference between samples	errors on small volumes mean less precision

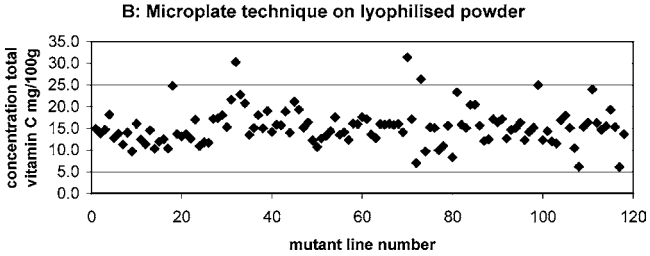
[a] For both methods, the extraction time is the limiting factor.

is calculated by taking the square root of the differences of the square of two errors: The first error is the calculated estimated error excluding the powder error (**Table 1**), and the second error is the measured experimental error from **Figure 2**. Thus, the error due to the powder is estimated at 7.8% (**Table 1**) for the microplate method, which only uses 1 g of powder and so represents $7.8/(\sqrt{5}) = 3.5\%$ error for the alternative technique, which uses five times the quantity of powder. This percentage correlates well with the standard deviations shown calculated for the spectrofluorometric technique, if we consider the major source of error for this technique to be the powder (in the absence of any interference; data shown in the summary table of **Figure 2**).

Although the microplate technique is less precise than the spectrofluorometric technique, it has numerous advantages as shown in **Table 2**: The cost per sample is five times lower; the only specialized equipment required is a plate reader with a filter at 550 nm; the rest of the material is found in a standard laboratory. Also, smaller quantities of powder and materials are required as compared to the alternative method. The major error of the spectrofluorometric technique, which comes into play when using a very heterogeneous sample population, is interference due to the continuous flow so, for example, a highly concentrated sample will often mask a subsequent low vitamin C sample.

Application to a Population of M3 Mutagenized Lines Selected for Fruit Phenotype. Both techniques have been applied to a collection of tomato mutants sown in the field in July 2004. The production of these mutants by ethyl methane sulfonate or fast neutron mutagenesis is described in ref *13*. A subset of the M3 families (111 families), identified as having a fruit phenotype, and seven M82 parent controls were analyzed for vitamin C levels in ripe fruit of the M3 generation to establish the variation obtained in this trait following random mutagenesis of the genome (see Materials and Methods for harvesting conditions). The spectrofluorometric technique was used immediately on each mutant line (for each line the central slices of 15 tomatoes were ground in liquid nitrogen), and the results are shown in **Figure 3a** (values from 9.9 to 27.5 mg/100 g). The second series of assays were carried out 1 year later, while the microplate method was developed, on the same tomato powder that had been immediately lyophilized and conserved at −80 °C. The results are shown in **Figure 3b** (values from 6.1 to 31.4 mg/100 g). Seven internal controls of wild-type M82 processing tomatoes were present among the 111 mutant lines, and the variation found was 18.8 ± 1.7 mg/100 g for the spectrofluorometric technique and 16.2 ± 1.7 mg/100 g for the microplate technique. The number of lines falling within different standard deviation ranges is shown and is consistent between techniques. The values from the spectrofluorometric technique are more clustered possibly due to slight interference between samples during the assay.





Internal control variation (M82 processing tomatoes) 18.8+/-1.7mg/100g using the spectrofluorometric technique and 16.2 +/- 1.7mg/100g using the microplate technique

	number of samples in standard deviation range	
	spectrofluorometric assay	microplate assay
<2SD	74	74
2-3SD	29	22
3-4SD	9	11
>4SD	6	11

Figure 3. Total vitamin C content of the 118 tomato lines harvested in the field in 2004. The total vitamin C content of 111 mutant lines and seven control lines is shown using the spectrofluorometric technique on fresh powder (top) and the microplate technique on lyophilized powder (bottom). The number of fruit samples appearing in different standard deviation ranges is shown in the table.

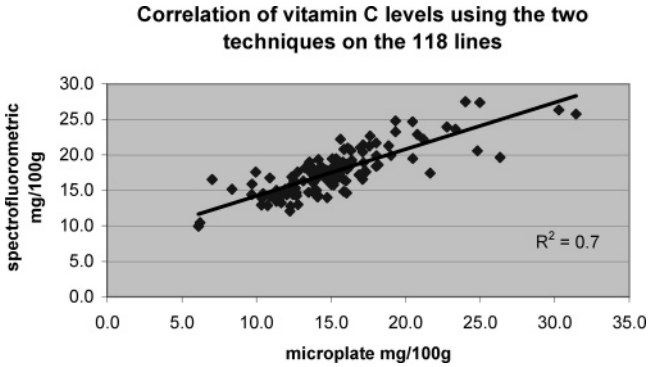


Figure 4. Correlation of the two vitamin C assay techniques over the 118 lines. The ascorbate concentration of each line using both techniques is compared, and the R^2 is given.

The standard deviations are identical for the seven control samples because probably the interference between samples that can occur for the spectrofluorometric technique, which uses continuous flow, is more apparent in this kind of experiment, where high and low vitamin C tomatoes are mixed, than for the validation experiment in **Figure 2**. When results from the two techniques are correlated, as shown in **Figure 4**, the trend is very apparent ($R^2 = 0.7$) and no high vitamin C tomato is confused with a low vitamin C tomato and vice versa showing a good correspondence between the two methods. If the 10 tomatoes with the highest vitamin C content are selected by each technique, six of the 10 tomatoes are common to both sets and four of the 10 lowest vitamin C content tomatoes (raw data not shown). It can be noted that the microplate technique gives consistently lower values than the spectrofluorometric method.

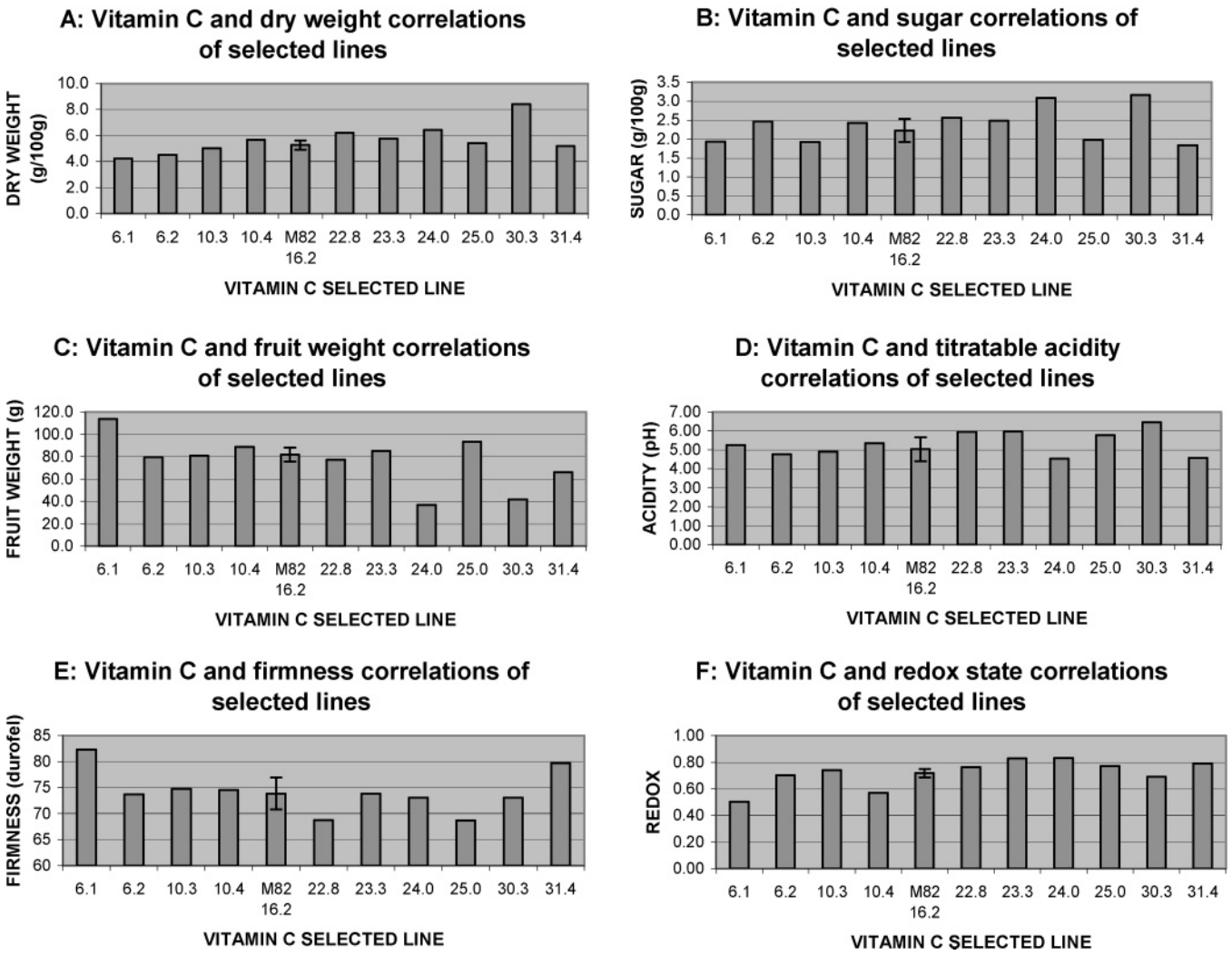


Figure 5. Characterization of mutant lines selected for low or high vitamin C content and controls. Further phenotypic characterization of selected mutant lines is carried out for four low vitamin C lines and six high vitamin C lines. The data from the seven control (M82) plants are also shown with error bars. The values on the *x*-axis correspond to the vitamin C contents of the selected lines. For each analysis, the average of 15 fruit is shown (fruit weight and firmness). Otherwise, the analyses were carried out on 15 fruits ground in liquid nitrogen. (**A**) Dry weight of selected lines. (**B**) Sugar concentration of selected lines. (**C**) Fruit weight of selected lines. (**D**) Titratable acidity of selected lines. (**E**) Firmness of selected lines. (**F**) Redox state of selected lines.

This difference was not seen in the initial experiment comparing the two techniques (**Figure 2**), so we reasoned that some ascorbate may be lost or degraded during the lyophilization process, which was carried out immediately following grinding in liquid nitrogen. To check this hypothesis, we carried out an independent experiment on lyophilized and nonlyophilized samples of 10 different frozen tomato powders. For each sample, when the lyophilized powder was compared with the nonlyophilized powder, an average of 20% less vitamin C (total and reduced forms) was measured in the lyophilized samples as compared to the corresponding nonlyophilized powder (raw data not shown). Therefore, during lyophilization, vitamin C is lost or degraded and thus vitamin C assays on lyophilized powders are suitable for comparative purposes but unsuitable for the calculation of exact concentrations.

Phenotypic Characterization of Selected Lines. Four low vitamin C lines and six high vitamin C mutant lines were selected for further study. These 10 lines were identified by both methods as having particularly low or high vitamin C contents. Control lines were also included for study, and error bars are shown for these samples. As vitamin C levels are often highly correlated to sugar levels and dry weight and negatively correlated to fruit weight, these phenotypes were also studied, along with total acidity, firmness, and redox state. The results are shown in **Figure 5**, and the lines are identified by their total vitamin C content. In the case of dry weight, there may be a slight positive correlation between vitamin C content and this character (**Figure 5A**). The same is not true of sugar and vitamin C content, often highly correlated, as two lines (25 and 31.4) have relatively low sugar contents and yet high vitamin C (**Figure 5B**; results significant as compared to the control lines). **Figure 5C** shows the average fruit weights of the selected lines, two lines with high vitamin C contents have very low fruit weights (24 and 30.3) and slightly higher percentages of dry matter; these fruits resemble cherry or cocktail tomato varieties, traditionally higher in vitamin C. The titratable acidity levels shown in **Figure 5D** are not correlated to vitamin C (ascorbic acid levels). Indeed, there are other acids in tomato fruits that contribute more than vitamin C to total acidity, such as citric acid, which accounts for 80% of tomato acids, and the contribution of ascorbic acid is negligible. **Figure 5E** shows the correlation between fruit firmness and vitamin C levels, and although there might be a slight negative correlation between these traits, the lowest and highest vitamin C fruits also have

the firmest fruits and the difficulty in measuring this trait is shown by the relatively large error on the control fruits. Finally, the redox state of the vitamin C pool, which is constant in control plants, varies between the lines (**Figure 5F**), especially in the case of the plant 10.4. Also, the high vitamin C lines have slightly higher redox states on average.

DISCUSSION

A simple and rapid method for the assay of vitamin C levels (ascorbic acid and dehydroascorbate) in tomato is presented, which is suitable for other fruits and tissues and of use in both fundamental and applied research. The technique has been validated by comparison with a reference technique for vitamin C assays in tomato. The advantage of the new method is its low cost and its requirement for neither large quantities of plant material nor complicated equipment. The only disadvantage of the technique is the errors introduced on the small volumes required; however, because of the practicality of the method, multiple assays can be carried out easily, for minimal further cost or inconvenience, to improve accuracy. Also, improved pipetting methods (for example robots) can be used to overcome the major error introduced by pipetting of samples. Furthermore, the error is smaller than the differences that we routinely look for between tomato lines or varieties.

When working with vitamin C, the rapidity and ease of the assay method are of utmost importance as the molecule is easily degraded, particularly in the absence of an acid buffer or following freezing and thawing or, as shown here, by lyophilization. The homogeneity of the ground powder is also an important criterion for any assay technique and is therefore the major calculated source of error for the spectrophotometric technique.

The vitamin C levels of 118 lines grown in the field in July 2004 have been analyzed using the two techniques presented, and these show a good correlation ($R^2 = 0.7$). This analysis has enabled the selection of six high vitamin C lines and four low vitamin C lines for further phenotypic characterization. Within the 118 lines, vitamin C content values ranged from 6 to 30 mg/100 g with the parent plants containing 16−19 mg/100 g. Therefore, random mutation of the genome can nearly double and nearly halve the vitamin C content of tomatoes at the red ripe stage. These tomato lines thus constitute an interesting source of genetic variation for this trait, in a similar way to another study performed in wheat (*14*). The correlations that often exist in tomato varieties between sugar and vitamin C content (positive correlation) or fruit weight and vitamin C content (negative correlation) do not always seem to be borne out in the lines selected for further analysis. For example, in two lines, the sugar−vitamin C correlation has been uncoupled showing that, although these traits are often correlated, other factors play a role in their absolute levels. This observation is of interest as it may be possible to uncouple the genetics of fruit sugar and vitamin C levels: Fruits could be enriched in one trait without automatically being enriched in the other, and this may have repercussions for traditional fruit breeding programmes or QTL analysis. From this point of view, the uncoupling of the antagonistic relationship traditionally found between vitamin C (or sugar) content and fruit size would be advantageous.

In conclusion, a rapid and simple method for assaying vitamin C in tomato fruit has been developed and applied to a mutant collection. The assay is suitable for use with other fruits and has revealed a range of variation in vitamin C levels in the mutant lines from 6 to 30 mg/100 g as compared to wild-type plants, which contained 16−19 mg/100 g. Further characterization of a few high or low content vitamin C lines has revealed that correlations found in tomato varieties between sugar or dry matter and vitamin C are not always maintained.

ABBREVIATIONS USED

DTT, dithiothreitol; NEM, N-ethyl maleimide; TCA, trichloroacetic acid.

ACKNOWLEDGMENT

We acknowledge D. Zamir, Israel, for providing the mutant tomato lines and the experimental team at INRA, St. Maurice, for plant management.

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Received for review May 3, 2006. Revised manuscript received June 27, 2006. Accepted June 27, 2006.

JF061241E