Advance Journal of Food Science and Technology 4(4): 182-188, 2012
ISSN: 2042-4876

Submitted: February 27, 2012 Accepted: March 16, 2012 Published: August 20, 2012

Induced Mutations for Improved Lycopene, Total Antioxidant Properties and Other Quality Factors in Wild Tomato (*Solanum pimpinellifolium*L)

[1]Emmanuel K. Quartey, [1,3]Harry M. Amoatey, [2]Daniel G. Achel, [3]George Y. P. Klu and [2]Rudolf A. Mba
[1]Nuclear Agriculture Centre, Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, P.O. Box LG 80, Legon-Accra, Ghana
[2]Applied Radiation Biology Centre, Radiological and Medical Sciences Research Institute, Ghana Atomic Energy Commission, P.O. Box LG 80, Legon-Accra, Ghana
[3]Department of Nuclear Agriculture and Radiation Processing, Graduate School of Nuclear and Allied Sciences, University of Ghana, P.O. Box AE 1, Atomic Energy-Accra, Ghana

Abstract: The objective of the study was to determine biochemical changes in (5) gamma radiation induced variant lines of wild tomato after mutation induction. Five gamma radiation-induced variant lines of wild tomato (*Solanum pimpinellifolium* L.), selected in the M_3 generation following mutagenic treatment of seeds using three doses (150, 300 and 450 Gy, respectively) of gamma radiation from a Co-60 gamma source, were analysed for their lycopene content, total antioxidant properties, total and soluble solids as well as pH. Fruits of variant line BV-21 (deep red fruits) recorded higher lycopene contents of 146.1 mg/kg on fresh weight basis and 156.7 mg/kg on dry weight basis compared to 136 and 152 mg/kg, respectively for the control. They also recorded higher total antioxidant properties compared to the control. Fruits of variant line BV-40 (yellow fruits) recorded higher total solids of 17.9% and the lowest pH value of 4.17 compared to 12.8% and 4.36 for fruits from un-irradiated plants. The highest amounts of total soluble solids (7%) were contained in fruits harvested from variant lines BV-27 (light red fruits) and BV-23 (deep red fruits) as against 5.6% for fruits from control plants. The study indicates that wild tomato has immense nutritional properties which can be further improved through mutation breeding.

Key words: Gamm radiation, lycopene, mutation, pH, *Solanum pimpinellifolium* L., soluble solids, total antioxidant activity

INTRODUCTION

Increasing interest in antioxidants is as a result of their capability in preventing deleterious effects of free radicals in the human body and the deterioration of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources (Abdalla and Roozen, 1999). Different methods are available for estimating the efficiency of antioxidants (Schwarz *et al*., 2001). One such method is based upon the use of the stable free radical diphenylpicrylhydrazyl (DPPH), according to Brand-Williams *et al.* (1995).

Lycopene has been shown to have strong antioxidant activity and exhibits high physical quenching rate constant with singlet oxygen (DiMascio *et al*., 1989). On the average, lycopene constitutes about 80-90% of the total carotenoid content found in tomato (Shi and Le Maguer, 2000). Cultivars vary in lycopene content depending on genotype and environmental conditions (Perkins-Veazie *et al*., 2001). Spectrophotometric methods and High-Pressure Liquid Chromatography (HPLC) are used most commonly in the quantitative estimation of total lycopene in food and biological lines. Lycopene is quantified from HPLC profile by using purified lycopene standard available from several commercial sources.

Rao *et al.* (2006) compared the spectrophotometric and HPLC methods and found the results to be in good agreement. The spectrophotometric method offers a convenient, fast and less expensive method for the detection of total lycopene compared to the HPLC procedure. A large number of lines can be analysed by this method in a relatively short period of time without compromising the accuracy. HPLC, on the other hand, potentially affords separation and identification of

Corresponding Author: Emmanuel K. Quartey, Nuclear Agriculture Centre, Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, P.O. Box LG 80, Legon-Accra, Ghana, Tel.: +233-24-9981220

individual carotenoids as well as their quantification (Fish *et al*., 2002). However, HPLC may be subject to the vagaries of line extraction/preparation, many sources of column-and solvent-generated artifacts and a paucity of pure carotenoid standards (Kimura and Rodriguez-Amaya, 1999). Furthermore, HPLC is too expensive and too slow for the routine screening of fruits from many plants produced in breeding programmes designed to develop new cultivars.

Total Solids (TS) content is an important quality factor in fruit assessment. For years, efforts have been directed at breeding for tomatoes with high total solids content for the processing industry. Some of these efforts have produced new varieties containing high solids content (Stevens and Rick, 1986). Wild species such as *Solanum pimpinellifolium* have high solids content and have thus been used in crosses to transfer this trait into modern cultivated cultivars.

Total Soluble Solids content (TSS) is one of the most important quality factors in tomato fruits. In general, the tomato fruit is 94-95% water and 5-6% organic compounds (solids). The solids comprise various components. Sugars (fructose, glucose, sucrose which are found primarily in the fruit wall) make up about 50% of the solids. Pectins, cellulose, proteins, polysaccharide (alcohol in-soluble solids) compose 25% of the solids. Organic acids such as citrate and malate make-up 12% of the solids and the remainder of the solids consists of carotenoids, volatile compounds, aminoacids and inorganic compounds (Jones, 1999).

pH is also an important quality factor in fruit processing as it determines the safety of the product. In the processing industry cultivars with high acidic content in the fruits (i.e., pH lower than 4.6) are not suitable for processing (Jones, 1999).

The main objective of the study was to determine biochemical changes in (5) gamma radiation induced variant lines of wild tomato. Specific objectives were to determine variations in amount of lycopene (on dry weight and fresh weight basis), total solids, total soluble solids, pH and total antioxidant activity potential of fruits of the variant lines generated through mutation induction.

MATERIALS AND METHODS

Study area: The study was carried out at the Biotechnology and Nuclear Agriculture Research Institute and the Radiological and Medical Sciences Research Institute of the Ghana Atomic Energy Commission between January and March 2010. Dried seeds of wild tomato (*Solanum pimpinellifolium* L.) were acutely irradiated at 150, 300 and 450 Gy, respectively using a cobalt-60 gamma source. For each dose of irradiation approximately 1000 M_1 seedlings were transferred from the nursery to the field for study from which M_2 (800) and M_3 (500) populations were generated. Selection was done on single plant basis using plant architecture, fruit size and fruit colour. Harvested fruits of five promising variants in the M_3 population along with the control were used for this study.

Sample preparation: Whole fruits of the different variant lines, comprising the skin (pericarp), pulp and seeds, were homogenized in a stainless steel blender. Blended materials were further ground into fine paste with a laboratory mortar and pestle. Liquid nitrogen was added to aid the grinding process.

Determination of lycopene content: Fine homogenates of the tomato lines were vacuum-air dried into fine powder with a rotary evaporator [(5-240 rpm; 20-100ºC water bath R-480, BüchiLabortechnik, Flawil, Switzerland) connected to a vacuum pump with absolute minimum pressure of 10 mbar. The extraction method was modified after Fish *et al*. (2002) and Ravelo-Perez *et al*. (2008). (0.5 g) of the homogenized samples (paste and powder) were weighed into screw top vials wrapped with aluminium foil and 5.0 mL of 0.05% (w/v) Gallic acid in acetone, 5.0 mL of ethanol and 10.0 mL of hexane were added. The hexane was delivered with a volumetric burette. Each puree was assayed in triplicate. Weights of the samples were determined to the nearest 0.001 g. Vials were introduced in ice and stirred on an orbital shaker to mix at 200 rpm for 15 min.

After shaking, 3 mL of deionised water was added to each vial and the lines shaken for another 5 min on ice. Vials were left at room temperature for 5 min to allow for phase separation. The absorbance of the hexane (upper) layer was measured in a 1 cm path length quartz cuvette at 503 nm blanked with hexane solvent using a Shimadzu UV-Spectrophotometer. The absorbance of the supernatant (hexane layer) containing lycopene was read three times for consistency and the lycopene content estimated by the equation according to Fish *et al*. (2002):

Lycopene (mg/kg tissue):

$$= \frac{A_{503}}{17.2 \times 10^4 / M \times cm} \times \frac{536.9g}{mole} \times \frac{1L}{10^3 mL} \times \frac{10^3 mg}{1g} \times \frac{10.0mL}{kg\,tissue}$$

$$= \frac{A_{503} \times 0.0312}{kg\,tissue}$$

$$= \frac{A_{503} \times 31.2}{g\,tissue}$$

where, the molar extinction coefficient of 17.2×10^4/ M×cm is that reported by Zechmeister *et al*. (1943) for lycopene in hexane and A_{503} is the absorbance at 503 nm. Values of lycopene were expressed in terms of mg/kg for ease of data processing and in conformity with the unit of concentration commonly used in literature (Fish *et al*., 2002; Ravelo-Perez *et al*., 2008).

Determination of total antioxidant activity: In the presence of an antioxidant, DPPH radical obtains one more electrons and the absorbance decreases. The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability, using stable radical DPPH. A 0.1 μM solution of DPPH in methanol was prepared by dissolving 0.004 g of DPPH in 100 mL methanol. 1.0 mL of this solution was added to 3.0 mL of control (without the test compound, but with an equivalent amount of methanol), i.e., gallic acid at different concentrations (25, 50, 75 and 100 μL/mL, respectively) and the test solutions at the same concentrations as the gallic acid in test tubes. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm which is induced by antioxidants after 30 min incubation in the dark. Methanol solvent was used as the blank. The absorbance was read 3 times for each sample.

The activity of the test samples was determined in terms of the percentage reduction of the DPPH, Q, (sometimes referred to as "inhibition" or "quenching"), which is defined by:

$$Q = 100(A_o - A_c)/A_o$$

where, A_o is the initial absorbance and A_c is the value for added line concentration (Brand-Williams *et al*., 1995).

Determination of total solids: Total solids in tomato samples (pulp + seeds) were determined by a gravimetric method. 10 g of homogenate blended samples were weighed into glass petri-dishes and dried overnight in an oven at 60ºC AOAC (1985). The dishes were kept in a desiccator to allow for cooling before recording their final weights. All measurements were done in triplicate and the means of these were recorded. Results were expressed as percentages.

Determination of total soluble solids: Ten g of homogenized tomato samples were weighed into 50 mL centrifuge vials and span at 3000 rpm for 10 min. Two mL of the supernatant was weighed into pre-weighed glass petri-dishes and the weight taken before drying in an oven at a temperature of 60ºC overnight (~17 h). Samples were weighed after oven drying and results expressed in percentages and mg/mL. All measurements were done in triplicate.

Determination of pH: pH of homogenized samples was determined by using a microprocessor pH metre. There were (3) replicates per tomato line.

Data analysis: Comparison between treatments was made using One-way ANOVA. Data were analyzed using Minitab Statistical Analysis Software, version 15. At least three replications for each material were used for these analyses. Graphical presentations were prepared based on the mean values using Microsoft Excel (2007 version).

RESULTS AND DISCUSSION

Lycopene content: Determining and reporting lycopene content on dry and wet weight basis has been the subject of some controversy. Advocates for lycopene determination on fresh weight basis argue that tomato is normally eaten in the fresh state and hence it is prudent to calculate lycopene levels based on the fresh state (Sharma and Le Maguer, 1996). However, Goula and Adamopoulos (2003) argued that water constitutes about 60-70% of tomato and thus calculations based on dry weight give precise and accurate amounts of lycopene ingestion since in the drying process all moisture is eliminated and there is no loss of lycopene as little (or no heat) is applied. In our study, lycopene content of the tomato lines was determined both on the wet and dry basis.

The amount of lycopene (in the wet and dry phase) in samples is as shown in Fig. 1(a). Lycopene content in the wet phase varied between 6.0-148.1 mg/kg tissue. Fruits of line BV-21 (deep red fruits) had the highest lycopene content while fruits of line BV-40 had the lowest lycopene content. In the dry phase, lycopene content varied between 75.3 mg/kg in fruits of line BV-40 and 156.7 mg/kg in fruits of line BV-21. Significant differences ($p \leq 0.05$) existed in the lycopene content among fruits of all lines in the wet and dry phases.

Lycopene values reported for fruits of the control and other variants lines [especially line BV-21 (deep red fruits)], on fresh and dry weight basis, were far above most values reported by other researches. Javanmardi and Kubota (2006) reported the lycopene content of tomatoes (*S. lycopersicon* cv. Clermon) stored under cold temperatures to be between 40-68 mg/kg of fresh weight. Sharma and Le Maguer (1996) determined the lycopene content in tomatoes obtained from Canada to be 54.0 mg/kg on fresh weight basisbasis. Toor *et al*. (2006) reported the lycopene levels of 27-47 mg/kg FW in three (3) commercially produced varieties of tomato (*S. lycopersicon*). Martínez-Valverde *et al*. (2002) reported lycopene values of 1.8-6.5 mg/kg FW in different commercial Spanish tomato varieties.

In general, lycopene content was higher in the dry phase for all lines compared to the wet phase. Similar observations have been reported by George *et al*. (2004), who studied lycopene levels on fresh and dry weight basis in different varieties of tomato (*S. lycopersicon*) and found out that lycopene levels increased about (8) to(9) fold in the dry state. They attributed this to the concentration of lycopene in the dry phase.

In this study, lycopene value reported for fruits of variant line BV-40 (yellow fruits) was the least (6.0 mg/kg on FW basis) and in conformity with results of

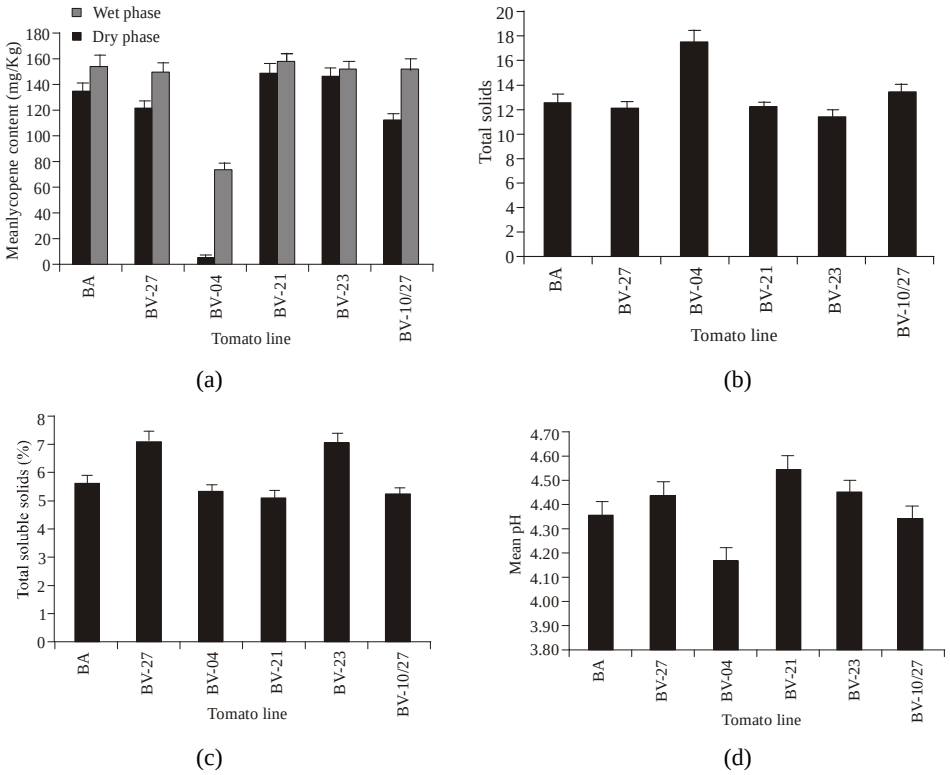

Fig. 1(a): Lycopene content in fruits of variant tomato lines; (b) Total solids content in fruits of variant tomato lines; (c) Total soluble solids in fruits of variant tomato lines; (d) pH of fruits of variant tomato lines, Note: Error bars represent standard error of the mean

analysesof yellow tomato fruits reported by other researchers (Bramley, 1997; Do Rêgo, 1999; Tadmor *et al*., 2005).

Total antioxidant activity: Table 1 shows the free radical scavenging potential of the tomato lines used in the study. At all four concentrations of gallic acid (used as standard), scavenging potential was above 90% inhibition. Different concentrations of gallic acid, therefore, did not have any effects on scavenging ability as there were no differences in their DPPH inhibition. At sample concentration of 25 μg/kg, fruits of BV-21 (light red fruits) exhibited the highest inhibition of DPPH of 54.3%±0.551 as against 50.8%±0.24 in the control and 24.1%±0.40 in BV-10/27, which recorded the lowest. Similarly, fruits of tomato variant line BV-21 recorded the highest DPPH inhibition of 88.4%±0.52, 91.1%±0.40 and 90.6%±0.78 at sample concentrations of 50, 75 and 100 μg/kg, respectively.

Significant differences existed ($p \leq 0.05$) in total antioxidant potentials among fruits of all the lines. In general scavenging abilities of lines BV-21 and BV-27 exceeded that of the control (parental line) across all concentrations. Variant line BV-10/27 exhibited the least scavenging ability across all concentrations.

Tomato lines investigated in this study produced assorted fruit colours ranging from yellow through light red to red and deep red. Line BV-21 which produced deep red fruits also exhibited the highest scavenging ability. However, BV-40 which produced yellow fruits surprisingly exhibited quite high scavenging ability across all sample concentrations, which were higher than BV-10/27 and BV-23 both of which produced red fruits.

Though lycopene is the main carotenoid responsible for the final red colour of the tomato (Zeb and Mehmood, 2004), violaxanthin, neoxanthin, lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, α-carotene, β-carotene, γ-carotene, ζ-carotene, neurosporene, phytoene, phytofluene, cyclolycopene and β-carotene 5,6-epoxide are other carotenoids commonly cited in tomato and tomato-derived products (Burns *et al*., 2003; Khachik *et al*., 2002; Paetau *et al*., 1998; Fraser *et al*., 1994). Among these, α-carotene, β-carotene and β-cryptoxanthin have pro-vitamin A activity, since they are converted to vitamin A by mammals (Burns *et al*., 2003).

It is probable that these compounds, besides lycopene, were involved in the antioxidant activity of most of the lines tested, especially high antioxidant-possessing lines like BV-27 (light red fruits) and BV-21 (deep red fruits). Long *et al*. (2006), through mutagenesis,

Table 1: Free radical scavenging potential of fruits of tomato lines

Line	Colour description	Concentration of dissolved samples (ug/mL)	DPPH radical inhibition (%) +S. E.
Gallic acid	Pure antioxidant	25	96.2±0.384[a]
		50	95.0±0.285[a]
		75	95.5±0.284[a]
		100	95.6±0.384[a]
Parental line	Red fruits	25	50.8±0.240[a]
		50	64.9±0.441[b]
		75	74.8±0.410[c]
		100	84.4±0.608[d]
BV-27	Light red fruits	25	54.3±0.551[a]
		50	78.8±0.635[b]
		75	81.1±0.404[c]
		100	89.6±0.874[c]
BV-40	Yellow fruits	25	31.3±0.664[a]
		50	55.7±0.520[b]
		75	76.4±0.551[c]
		100	88.2±0.639[d]
BV-21	Deep red fruits	25	50.5±0.436[a]
		50	88.4±0.520[b]
		75	91.1±0.404[c]
		100	90.6±0.874[c]
BV-23	Red fruits	25	51.9±0.333[a]
		50	56.9±0.333[b]
		75	70.6±0.333[c]
		100	78.0±0.577[d]
BV-10/27	Red fruits	25	24.9±0.240[a]
		50	41.9±0.328[b]
		75	59.5±0.448[c]
		100	63.3±0.285[d]

Means with same letters in a column are not significantly different ($p \geq 0.05$) from each other according to Tukey's test

identified a high antioxidant mutant line of tomato after subjecting various mutant lines to chemical analysis.

Total solids: Figure 1(b) displays the amount of total solids present in the tomato lines analyzed. Total solids content ranged between 11.6 % in fruits of line BV-23 (red fruits) and 17.9% in BV-40 (yellow fruits). Significant differences ($p \leq 0.05$) were generally observed among all lines analyzed. In general, commercial tomato varieties have total solids of between 6.8 and 12% (Agong, 2001). Fruits of line BV-40 were found to possess total solids of 17.9% and this was significantly higher ($p \leq 0.05$) than in fruits of all other lines as well as the control.

Total soluble solids: Total soluble solids content ranged between 5.1 and 7.1% (Fig. 1(c)) with lines BV-23 (light red fruits) and BV-27 (red fruits) recording the highest total soluble solids content while line BV-21 (deep red fruits) recorded the least value. There were significant differences ($p \leq 0.05$) among tomato lines for total soluble solids.

Hewitt and Garrey (1992) reported that standard tomato cultivars generally have total soluble solids content of between 5.1 and 7.1%. In general however, total soluble solids content of 4.80-8.80% are accepted as good indicators of quality tomato (Kumar *et al*., 1993). All tomato lines tested in this study were within this range. Although fruits of line BV-40 (red fruits) had the highest total solids content, their soluble contents were low. Also fruits of line BV-21, which recorded the highest values of lycopene both on fresh and dry weight basis, recorded the lowest total soluble content.

Efforts at breeding for higher fruit solids have not been successful because of a negative correlation between yield and solids content. However, fruits of *S. pimpinellifolium* have been observed with higher total solids as well as soluble contents than their domesticated counterparts (Foolad, 2009).

pH: The pH of the fruit samples of the lines is shown in Fig. 1(d). pH values ranged between 4.17 in fruits of line BV-40 (yellow fruits) and 4.55 in fruits of line BV-21 (deep red fruits). Generally, there were significant differences in the pH of the fruit samples from the tomato lines used for this study. Fruits of line BV-21 which had the highest lycopenecontent also had the highest pH value of 4.55 while fruits of BV-40 had the lowest pH of 4.17, which was significantly different ($p \leq 0.05$) from the rest.

Tomatoes are generally considered as acidic fruits whose pH values must preferably fall within the range 4.0 to 4.5 (Jones, 1999). Also there is not much variation in pH of yellow, white or red fruited varieties (Sorellina, 2006), as observed in this study. However, new varieties, over-mature tomatoes, tomatoes from dead or frost-killed vines and tomatoes harvested late in season often have been known to have a pH greater than 4.6 (Burtness, 2005).

In general, the lower the pH, the greater is the so-called "tartness," a factor by which some consumers judge the quality of the tomato fruit. Moneruzzaman *et al*. (2008) however, reported a wide range of variation of pH content from 3.6 to 4.6 in several commercial tomato varieties. The relationship between the pH and solids content (mainly sugars) of the tomato fruit is also a significant factor in its perceived flavour.

CONCLUSION

Lycopene content in fruits of the tomato lines ranged from 6.0-148.1 and 75.3-156.7 mg/kg in the wet and dry phases respectively. Tomato line BV-21 (deep red fruits) gave the highest lycopne content at the wet and dry phase while BV-40 had the lowest lycopene content at both phases. In the dry phase, contrary results were obtained. With respect to pH of the fruit samples, tomato line BV-40 recorded the lowest value (4.17; most preferred) while BV-21 recorded the highest (4.55; least preferred), both within acceptable range for direct consumption as well as for canning. Similarly, fruits of tomato line BV-21 exhibited the highest percent DPPH inhibition values of 88.4%±0.520, 91.1%±0.404 and 90.6%±0.874 for sample concentrations of 50, 75 and 100 µg/kg, respectively. However, at sample concentration of 25 mg/kg, tomato

line BV-27 exhibited the highest percent DPPH value of 54.3%±0.551. Tomato variant line BV-40 yielded the highest total solids of 17.9% while BV-23 yielded the lowest of 11.6%. Total soluble solids content ranged 5.1 to 7.1%, with lines BV-23 and BV-27 recording the highest.

The parental line (control; BA) was surpassed by one variant or the other in all fruit quality characteristics investigated. No single variant emerged superior with respect to all five quality characteristics studied, implying the need to combine the variants in a breeding programme to produce a synthetic variety acceptable in three or more characteristics.

ACKNOWLEDGMENT

The authors would like to acknowledge the assistance of the Government of Ghana through the Ghana Atomic Energy Commission for providing financial assistance during the research which formed part of Emmanuel K. Quartey's project work for the Master of Philosophy degree.

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