Communications in Soil Science and Plant Analysis, 36: 649–660, 2005

ISSN 0010-3624 print/1532-2416 online
DOI: 10.1081/CSS-200043307

Micronutrient Composition and Quality Characteristics of Traditional Tomato Cultivars in Southeast Spain

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Abstract: Several traditional tomato cultivars still survive in the orchards in southeastern Spain, which are highly esteemed due to their excellent quality. However, modern tomato hybrid varieties used in intensive agriculture mean that these types of local cultivars are being gradually phased out. This study was conducted to characterize the diversity of tomato micronutrient composition and fruit quality parameters of several traditional cultivars. The experiment was carried out under field conditions using traditional cultivars of the "Muchamiel" and "De la Pera" type and commercial F1 hybrids. Micronutrient fruit concentration (Fe, Cu, Mn, and Zn) was determined by atomic absorption spectroscopy. Fruit quality parameters analyzed were titratable acidity, soluble solid content, and color parameters. Strong differences have been found among the analyzed cultivars, both in terms of micronutrients and also quality parameters, suggesting that there are considerable

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levels of genetic diversity. Not a single "superior" variety could be identified, because it would depend on type of use, consumers' and growers' preferences. These traditional cultivars are frequently consumed at the breaker maturity stage, but we have found low differences between red and breaker fruits regarding micronutrient concentrations. Through relatively simple chemical analyses, we have detected significant differences among tomato genotypes that we are still not able to detect with molecular tools. Cultivars could be clearly differentiated on the basis of a multivariate data analysis of selected fruit parameters. This knowledge could aid in the efficient conservation of an important part of the agricultural biodiversity in southeastern of Spain. These results are also potentially useful for tomato breeders working on the development of new varieties.

INTRODUCTION

The tomato (*Lycopersicon esculentum*) was probably domesticated in Mexico, but the first transfer of varieties to Europe was made by Spanish explorers (1, 2). Spain and Italy were the first European countries where the tomato acquired commercial importance. After its introduction, a wide range of local cultivars was developed, and organoleptic quality was one of the main selection criteria. But modern tomato hybrid varieties used in modern intensive agriculture have almost completely taken over, meaning that these traditional cultivars are becoming extinct. With the availability of tomatoes all year round and with the spread of long shelf life varieties, consumers began to complain about fresh-market tomato quality, and they frequently associate later varieties with a lack of flavor (3). Although such an association has not been proven, some authors believe that poor flavor quality in tomato appears to be a result of breeding practices that do no select for flavor (because of lack of information). Indeed, information on flavor for use by breeders and molecular biologist is lacking (4).

Tomato is the main vegetable crop in Spain, and it is the horticultural crop with the highest value (5). Southeastern Spain is the most important area of fresh market tomato production in the country. Although this production is almost exclusively based on modern hybrid varieties, there are still several traditional tomato landraces that are renowned for high quality. In fact, in local markets, traditional cultivars sell for 3–5 times the price of the hybrid varieties.

Although cultivated tomato has a very narrow genetic base (6), there is a huge diversity of cultivars, which greatly differ in characteristics such as shape, firmness, solid soluble contents, aroma volatiles, etc. For example, Gomez et al. (7) have found important differences for some colorimetric and physicochemical parameters among some closely related Spanish local cultivars, and other authors have found strong effects of tomato genotype on

foliar micronutrient concentrations (8). Modern genetic and genomic tools have been intensively applied to the tomato, but these techniques are still not of much use for characterizing phenotypic differences among closely related cultivars. For example, Nesbitt and Tanksley (2) obtained a near absence of polymorphism among four cultivars (after sequencing about 7000 nucleotides in each cultivar). This lack of genetic variation in cultivated tomato is consistent with previous surveys, which determined levels of polymorphism to be extremely low (9). Using chemical analyses techniques, we can detect striking differences among tomato cultivars that we probably are still not able to detect by using modern molecular tools.

The aim of this study was to characterize the micronutrient composition diversity of different forms of two types of traditional cultivars, the "Muchamiel" and the "De la pera" type, and to evaluate several parameters related to fruit quality. These cultivars are often consumed at the breaker maturity stage ($<10\%$ of the fruit surface showing red color). For this reason, analysis was performed at two different maturity stages.

MATERIALS AND METHODS

Plant Material and Experiment Design

Six currently cultivated cultivars from the province of Alicante, corresponding to the "Muchamiel" (MUCH4, MUCH18, and MUCH30) and the "De la pera" (PERA5, PERA16, and PERA25) types were used. In addition, two commercial F1 hybrids (BOND and ULISES) were used as controls.

Each local cultivar and hybrid were grown in the open air in three randomly distributed blocks of 10 plants per block at an experimental field located in Orihuela (Alicante, Spain). The characteristics of the soil are specified in Table 1. Fruits were harvested at two ripening stages, breaker ($<10\%$ of the surface showing red colour) and red ($>90\%$ showing red color). A total of 96 tomato fruits were individually analyzed (8 genotypes $\times$ 2 ripening stages $\times$ 6 fruits per block).

Analytical Determinations

External color was measured at three points in each fruit by a Minolta chromameter, which resulted in three parameters: lightness (L) and chromaticity coordinates ("a," a green-to-red scale, and "b," a blue-to-yellow scale). From these values, the a : b ratio, the chroma $[C = (a^2 + b^2)^{0.5}]$, the hue angle $[\tan^{-1}(b/a)]$, and the Tomato Color Index $[TCI = a(L(a^2 + b^2)^{0.5})^{-1}]$ were calculated. These parameters are used for classifying tomato fruits according to color, but they have no optimal ranges, because desirable

Table 1. Characteristics of the soil (dry weight basis) of the experimental field plot

Parameter	Orihuela soil
Texture	Clay loam
Equivalent $CaCO_3$ (%)	36.1
Equivalent active $CaCO_3$ (%)	10.1
EC, water extract (1 : 5) dS/cm	0.701
pH, water extract (1 : 5)	8.61
Organic carbon ($g\,kg^{-1}$)	9.23
N Kjeldahl ($g\,kg^{-1}$)	0.97
P ($g\,kg^{-1}$)	0.037
K ($g\,kg^{-1}$)	3.8
Na ($g\,kg^{-1}$)	6.3
Ca ($g\,kg^{-1}$)	4.0
Mg ($g\,kg^{-1}$)	0.55
Fe ($mg\,kg^{-1}$)	3.1
Mn ($mg\,kg^{-1}$)	6.1
Cu ($mg\,kg^{-1}$)	2.3
Zn ($mg\,kg^{-1}$)	1.1

values would depend on type of use, consumers, etc. Two opposite slices from each fruit were homogenized, and the filtered juice was used for soluble-solids content (SSC) and titratable acidity (TA) determination. SSC was estimated by a digital refractometer (Atago Co. Ltd., Tokyo); the results were expressed in °Brix, and (TA) was measured by titration with 0.1 N NaOH and presented as $g\,kg^{-1}$ of citric acid. A maturity index (MI) was calculated as the ratio SSC : TA. The rest of each fruit sample was dried and mineralized by microwave acid digestion using HNO3, according to Kalra et al. (10). Fe, Cu, Mn, and Zn were measured by absorption atomic spectrometry using Zeeman background correction with SPECTRAA-220FS of Varian.

Data Analysis

The data were first subjected to a variance components analysis (ANOVA with a nested or hierarchical design) to estimate the amount of variability provided by each of the three factors in the experiment (variability among cultivars, between maturity stages within each cultivar, and among fruits of the same cultivar and maturity stage). A multifactor analysis of variance was also performed to identify the significant factors and interactions between the factors. Finally, the data were analyzed by using multivariate statistical analysis to group the data as a function of representative factors, linear

combinations of the original parameters. A principal component analysis was performed by using standardized quantitative data to obtain a graphical representation of the relationship structure of the analyzed samples. Computations were done by using the procedures in the NTSYS pc 2.0 statistical package.

RESULTS AND DISCUSSION

Micronutrient Content

The contribution of each factor to the total variability for the different micronutrients is presented in Table 2. The variance component associated with the "among fruits" level was very low (3.3–14.5%), which shows that the variation among the fruits of the same cultivar and maturity stage was low and the analytical technique was accurate. This result could be useful for future diversity surveys among our tomato genotypes, allowing small numbers of fruit to be sampled. The maturity stage had little effect in the Mn and Zn fruit concentration, but it was important for Fe and Cu. For all the analyzed micronutrients, the factor that contributes most variance is "cultivar," ranging from 55.3% in Fe to 88.2% for Zn, clearly showing that there are very strong differences among cultivars in their micronutrient composition. This was confirmed by the multifactor ANOVA analysis, which showed that the effect of the genotype (cultivar) was highly significant ($P < 0.001$) for all the micronutrients. Because the interactions cultivar × maturity stage were also highly significant in all cases, the micronutrient concentration data are presented in a graphical representation form (Fig. 1).

For Fe concentration, there were huge differences among cultivars. At the "red" maturity stage, Duncan's multiple-range tests detected significant differences for almost all cultivars, ranging from as low as 9.1 mg kg^{-1} for PERA25 to 77.5 mg kg^{-1} for MUCH30. At the "breaker" stage, PERA25 is also the cultivar with the lowest Fe concentration (14.5 mg kg^{-1}), but a cultivar of

Table 2. Micronutrients variance components (%) for each level of variation, calculated from an ANOVA with a hierarchical design

Source of variation	Degree of freedom	Fe (%)	Cu (%)	Mn (%)	Zn (%)
Among cultivars	7	55.3	60.8	85.6	88.2
Among maturity stages	8	40.3	24.7	8.9	8.5
Among fruits	80	4.4	14.5	5.5	3.3
Total		100	100	100	100

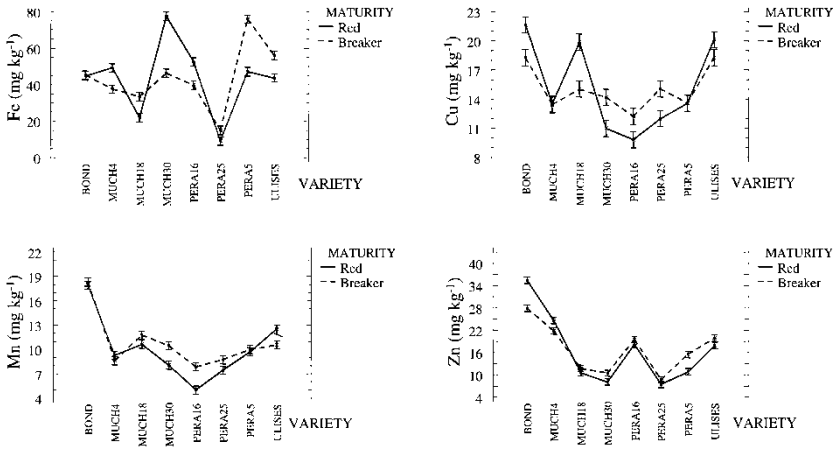

Figure 1. Fe, Cu, Mn, and Zn concentrations: interactions between variety and maturity stage. Vertical bars are LSD intervals at $p = 0.95$.

the same type, PERA5, showed the highest concentration (75.9 mg kg^{-1}). A strong effect of the tomato genotype on foliar concentration of Fe was previously reported (8). The maturity stage had a significant effect on Fe concentration for all cultivars except for the hybrid BOND, but the effect depended on the genotype. Red fruits showed the highest concentration in MUCH4, MUCH30, and PERA16, but in MUCH18, PERA25, PERA5, and ULISES, the breaker fruits had higher levels.

There were also important differences in Cu concentration. The two F1 hybrids, BOND and ULISES, had higher concentrations (a mean of 19 mg kg^{-1}) than the traditional cultivars, although the red fruits of MUCH18 reached values similar to the hybrids. The maturity stage also had a significant effect on Cu concentration for five of the eight cultivars, but the effect was again different depending on the cultivar. For example, red fruits of MUCH18 had a 30% higher Cu concentration than breaker fruits, but the effect was the opposite for MUCH30, PERA16, and PERA25.

In Mn concentration, important differences were also found among almost all cultivars. The hybrid BOND showed the highest concentration (18 mg kg^{-1}), followed by the other hybrid, ULISES, and MUCH18 (about 11 mg kg^{-1}), varying from the other cultivars between 6.4 and 9.9 mg kg^{-1}. Maturity stage had little effect on Mn concentration, being only significant for MUCH30, PERA16, and ULISES.

Zn concentration also varied among cultivars, showing the hybrid BOND 31.7 mg kg^{-1}, about 4 times the concentration of PERA25, the cultivar with the lowest Zn concentration. The effect of the maturity stage was significant for some cultivars, but the differences were not large.

If we use the sum of the four micronutrients as a rough indicator of the nutritional value of each cultivar, the best one would be the hybrid BOND (a mean of 115 mg kg^{-1} of micronutrients), followed by ULISES and PERA5 (about 99 mg kg^{-1}). The worst cultivar would be PERA25, with only 42 mg kg^{-1}.

Quality Parameters

For (SSC), TA, and colorimetric parameters, the variability among fruits was considerably higher than for micronutrient concentration (Table 3). The parameters "a," "L," ratio a : b, hue angle, and Tomato Color Index (TCI) show most of their variability between maturity stages, reaching values of about 86% of the total variability, with little variability among cultivars. However, the "b" and chroma parameters exhibited the most variation among cultivars.

The values for the analysed parameters and the calculated ratios and indices are presented in Table 4. The multifactor ANOVA shows a completely different situation than in the case of micronutrients because there were no significant interaction cultivars × maturity stages for any parameter or calculated indices. Only in the case of the Maturity Index ratio (SSS : TA), the effect of the interaction was within the limits of statistical significance ($P = 0.049$). Because it could be deduced from the previous variance component analysis, the effect of each of the cultivars was significant in SSC, TA, MI, "L," "b," and chroma, and the effects of the maturity stages were significant in "L," "a," chroma, TCI, and hue angle.

The SSC varied from 4.1 to 5.5 °Brix, the maximum corresponding to the hybrid ULISES and the local cultivar PERA25, and the minimum corresponding to MUCH18. This range of variation is similar to that found in 20 cultivars and hybrids tested by Gómez et al. (7). These results are in accordance with sensorial evaluations that we are conducting with these kinds of traditional

Table 3. Quality parameters variance components (%) for each level of variation, calculated from an ANOVA with a hierarchical design

Source of variation	DF	SSC (%)	TA (%)	L (%)	a (%)	b (%)	a : b (%)	Chroma (%)	TCI (%)	Hue angle (%)
Cultivars	7	30.9	68.0	0.0	0.0	52.7	0.0	48.5	0.0	0.0
Maturity stages	8	2.8	0.0	62.7	86.5	2.5	62.7	7.3	86.0	75.4
Fruits	75	66.3	32.0	37.3	13.5	44.8	37.3	44.2	14.0	24.6
Total		100	100	100	100	100	100	100	100	100

Table 4. Quality parameters and calculated ratios and indices for each cultivar and maturity stage: Solid soluble contents (SSC, °Brix), Titratable Acidity (TA, g kg^{-1} citric acid), Maturity Index (SSC : TA), color parameters "L," "a," "b," chroma [$C = (a^2 + b^2)^{0.5}$], hue angle [$\tan^{-1}(b : a)$], and tomato color index [$TCI = a(L(a^2 + b^2)^{0.5})^{-1}$]

Cultivars	Maturity	SSC	TA	M.I.	L	a	b	Chroma	TCI	Hue
BOND	Breaker	4.2	0.44	9.6	51.4	−2.5	28.1	28.4	−0.06	−0.44
	Red	4.8	0.47	10.2	46.6	14.9	31.4	35.4	0.37	1.14
MUCH4	Breaker	4.8	0.33	15.7	60.8	−0.8	44.8	45.1	−0.02	0.03
	Red	4.4	0.37	12.1	51.6	17.6	42.8	46.4	0.36	1.18
MUCH18	Breaker	4.2	0.29	14.3	62.0	−4.3	38.3	38.5	−0.09	−1.46
	Red	4.1	0.30	14.2	54.1	16.1	37.3	41.1	0.34	1.17
MUCH30	Breaker	4.9	0.33	15.5	57.2	−6.6	36.7	37.4	−0.14	−1.40
	Red	4.5	0.28	17.1	51.7	14.2	36.2	39.4	0.32	1.21
PERA16	Breaker	4.5	0.29	15.7	56.3	−5.6	40.8	41.5	−0.12	−0.80
	Red	4.9	0.34	14.7	47.6	14.4	36.9	39.9	0.34	1.19
PERA25	Breaker	5.1	0.28	18.5	51.9	−3.8	36.6	37.1	−0.08	−0.95
	Red	5.7	0.27	21.5	49.6	7.3	36.3	37.3	0.17	1.37
PERA5	Breaker	5.3	0.33	13.5	56.1	−3.1	42.5	42.8	−0.06	−0.25
	Red	4.9	0.28	16.7	48.7	15.7	38.2	41.7	0.36	1.18
ULISES	Breaker	5.5	0.55	10.1	57.3	−4.4	36.4	36.8	−0.10	−1.45
	Red	5.6	0.54	10.3	47.5	15.6	35.4	39.1	0.37	1.16
Significance										
Cultivars (C)		***	***	***	***	NS	***	***	NS	NS
Maturity (MS)		NS	NS	NS	***	***	NS	*	***	***
C × MS		NS	NS	*	NS	NS	NS	NS	NS	NS

NS, *, **, *** nonsignificant or significant at $p = 0.05$, 0.01, or 0.001, respectively.

cultivars (not published) and also with their type of use. Cultivars of the "Muchamiel" type used to be described by panelist as less "sweet" than the "De la pera" type, the former being used mainly for salads and the latter frequently having a dual purpose, for salads and for processing when the market price is low. The maturity stage had no significant effect on SSC, with the global mean for the red fruit just 0.1 °Brix higher than the mean of the breaker fruits. The same is true for the titratable acidity. BOND and ULISES were the cultivars with the highest acidity, and PERA25, one of the cultivars with the highest SSC, was the one with the lowest acidity. For this reason, PERA25 exhibited the highest SS : TA ratio. This index, which we have called the Maturity Index (MI), accentuated the differences among cultivars, thus being more useful for genotype characterization, but it did not differentiate between maturity stages. Considerable progress has been made in the identification of important flavor components in tomato and the determination of their concentration in fresh fruit, but additional information is required for the optimal ranges and ratios for sugar, acids and aromatics required for good flavour (4). The SS : TA ratio has been related to tomato overall taste intensity (11), but it is still very difficult to establish desirable or recommended levels, because they would strongly depend on consumers, type of use, varieties, etc.

From all the color parameters and calculated ratios and indices (a, b, L, a : b, chroma, hue angle, and Tomato Colour Index), only "L" and chroma were able to distinguish between both cultivars and maturity stages (Table 4). There were significant differences among cultivars for "L," "b," and chroma, but to better differentiate among cultivars, the best index was chroma, showing the traditional cultivars with the highest values and the lowest ones belonging to the two hybrids. All the parameters and indices could be used to differentiate between maturity stages, with the exception of "b," with the simplest one being parameter "a," which is in fact a green-to-red scale.

Correlation Analysis

Although Fe concentration showed no correlation with the other micronutrients, Cu, Mn, and Zn were positively correlated to each other (Table 5), with the maximum correlation between Mn and Zn ($r = 0.75$; $p < 0.001$). This correlation increased to 0.85 when considering the values of the fruits at the red stage only. It was previously reported that Cu, Mn, and Zn usually evolve in a correlated manner when tomato plants are subjected to stresses (12). The fruit concentration in Cu, Mn, and Zn was also positively correlated with the titratable acidity ($r = 0.56$, 0.48, and 0.43, respectively). Cu and Mn concentrations were negatively correlated with the color parameter "b," which measures the change from blue to

Table 5. Correlations between micronutrient concentrations and some quality parameters

	Fe	Cu	Mn	Zn	°Brix	Acidity	b
Fe	1	−0.16	0.01	0.19	−0.16	0.18	0.06
Cu		1	0.75***	0.45***	0.03	0.56***	−0.40***
Mn			1	0.62***	−0.15	0.48***	−0.54***
Zn				1	−0.03	0.54***	−0.19
°Brix					1	0.43***	−0.26*
Acidity						1	−0.01
b							1

*, **, *** Significant at $p = 0.05$, 0.01, or 0.001, respectively.

yellow ($r = -0.40$ and -0.54; $p < 0.001$). If we consider only the breaker fruits, these two values increase to -0.61 and -0.71. We have found no explanation for the correlations of the micronutrients with the acidity, and although the micronutrients Cu and Mn could have some influence on the color of the tomato surface, more data would be needed, because correlation analysis is a simple technique that should be further confirmed.

Multivariate Data Analysis

Using the values of Fe, Cu, Mn, Zn, and the parameters chroma, "L," and MI, which have shown to be the most useful variables for differentiating among cultivars, a principal component analysis were performed on red fruit data. The three principal components extracted accounted for 80% of the total variation. The first component was mainly correlated with Mn and Cu concentration and with MI. The parameters with the highest weights on the second component were chroma and Fe concentration, and L was the parameter with the highest loading value on the third component. Using these components as the axes of a three-dimensional representation of the original data, we can clearly differentiate the fruits from all the cultivars, except for MUCH30 and PERA16, which are not clearly separated (Fig. 2).

CONCLUSIONS

Strong differences have been found among the traditional tomato cultivars analyzed, both for micronutrients and for quality parameters, suggesting that there are considerable levels of genetic diversity among the cultivars grown in southeastern Spain. These cultivars are frequently consumed at the breaker maturity stage, and although we have found differences between

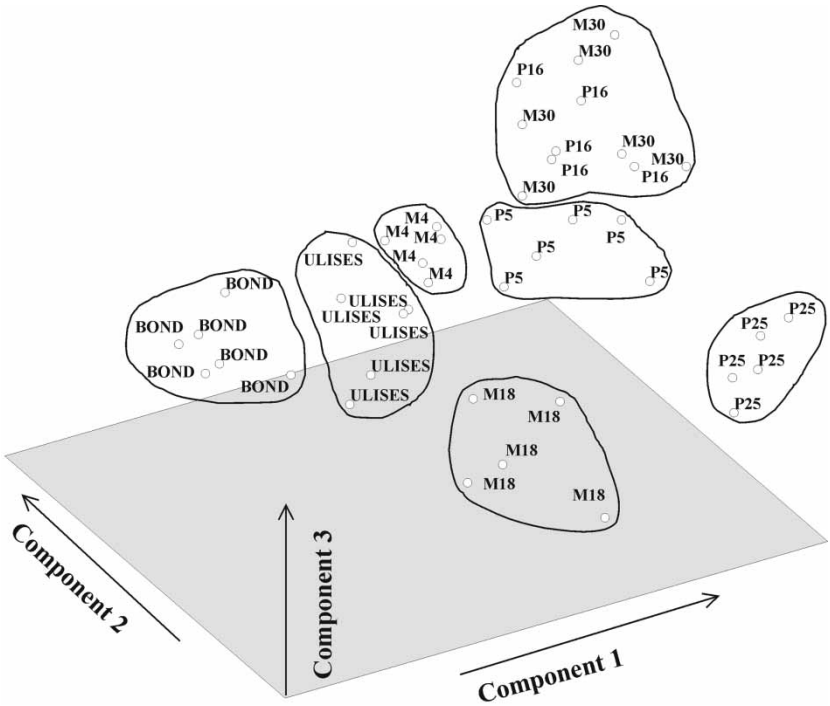

Figure 2. Representation of the red fruits from the eight cultivars based on the three principal components extracted from the values of Fe, Cu, Mn, and Zn content, Maturity Index (SSC : TA), chroma, and "L." The cultivars are two commercial hybrids (BOND and ULISES), three local cultivars of the "Muchamiel" type (MUCH4, MUCH18, MUCH30), and three local cultivars of the "De la pera" type (PERA5, PERA 16, and PERA25).

red and breaker fruits for micronutrients concentration, the effect depended on the cultivar and was of low importance in the majority of cultivars. We have been able to differentiate among genotypes of the same type by means of a principal component analysis performed on the most discriminating parameters. Presently, modern genetic and genomic tools are being intensively applied to the tomato, but these techniques are still not useful in characterizing phenotypic differences among closely related cultivars. By relatively simple chemical analyses, we can detect important differences among tomato genotypes that we are still not able to detect with molecular tools. This knowledge will aid with the efficient conservation of traditional tomato cultivars, which are an important part of the agricultural biodiversity in southeastern Spain. These results are also potentially useful for tomato breeders working on the development of new varieties.

ACKNOWLEDGMENTS

This research was supported in part by the *Generalitat Valenciana* and the *Comisión Interministerial de Ciencia y Tecnología* (CICYT) through the projects GV00-090-3 and AGL2002-03329. S. García-Martínez is a fellow of the *Ministerio de Educación, Cultura y Deporte* of Spain.

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