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EXPLORING GENETIC DIVERSITY AND QUALITY TRAITS IN A COLLECTION OF ONION (*Allium cepa* L) LANDRACES FROM NORTH-WEST SPAIN

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Seventeen onion landraces from North-West Spain were evaluated using microsatellites markers. Eleven polymorphic markers identified 32 alleles in the whole collection, with an average of 2.9 alleles per locus. High values of observed (mean of 0.45) and expected heterozigosity (mean of 0.51) were detected for the majority of loci. Wright's fixation index confirmed an excess of heterozygotes and a low level of inbreeding within the collection. Multivariate analyses revealed that Oimbra was the most distinctive genotype. The remaining 16 onion genotypes were in part assorted according to some morphological traits of bulbs. Pungency and solid soluble content highly varied among landraces and bulbs. Five landraces were classified as sweet, whereas 9 possessed medium pungency and 3 were recorded as pungent. This onion collection represents a useful source of genetic heterogeneity that might be exploited in breeding programs for the generation of new onion varieties that satisfy consumer demands.

Key words: onion, *Allium cepa*, landrace, genetic diversity, pyruvic acid, solid soluble content

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INTRODUCTION

Onion (*Allium cepa* L.) is one of the most widely cultivated vegetables around the world (FAOSTAT 2011). The economic value of this crop derives from its culinary usages, nutritional benefits and health–giving properties (BENKEBLIA 2005). In spite of its worldwide significance, the availability of new onion genetic resources and the knowledge and exploitation of those are still very limited. Therefore, the characterization of new sources of interesting and commercially important traits would be beneficial for breeders, growers, markets and processing industries.

In Spain, onion represents the third most important vegetable crop. Around 5% of the total cultivated area is located in Galicia (Northwest Spain) (MAGRAMA 2013). In this region, onion cultivation is mainly based on several traditional local landraces with excellent agronomic performance. Seeds of these landraces were collected in 1998 at the main productive regions of Galicia and they are maintained at the Centro de Investigaciones Agrarias (A Coruña, Spain). As happened with other onion landraces all over the world, Galician accessions are well differentiated according to morphological traits (RIVERA-MARTÍNEZ *et al.*, 2005). However, the characterization of their genetic diversity and the assessment of some chemical parameters related to onion quality, such as soluble solids content (SSC) and pungency have not been performed on these lines yet.

Genetic diversity assessment on the basis of molecular markers should be preferred compared to morphological traits because of their neutral behavior, independency on the environment, their easy access, assay and exchange of data between laboratories (JOSHI *et al.*, 1999). Molecular characterization constitutes the first essential step towards the successful exploitation of the potentially new genetic variability, as it allows us to gain knowledge on the genetic relationship among lines, the level of fixation or heterozygosity and the development of genetic profiles for variety identification and protection of breeders and grower´s rights (TSUKAZAKI *et al.*, 2010, KHAR *et al.*, 2011). Genetic background studies in onion have used different types of molecular markers to characterize the variability inside germplasm collections (TANIKAWA *et al.*, 2002, JAKSE *et al.*, 2005, ARAKI *et al.*, 2010). However, microsatellite or Simple Sequence Repeat (SSR) markers have lately become the markers of choice because they are highly informative, co–dominant, PCR–based and locus–specific (POWELL *et al.*, 1996).

Pungency and solid soluble content (SSC) are two important traits of onion bulbs in terms of processing and storage. SSC, consisting mostly of fiber, starch and sugars (LIN *et al.*, 1995) contributes to the flavor, texture and storability of onions. Pungency in onions is derived from a number of volatile sulphur compounds released after cell disruption. This parameter can be determined indirectly using a colorimetric test for pyruvic acid concentration, which is formed in the reaction that produces the volatile compounds (WALL and CORIGAN 1992). Over the last years, there is an increasing consumer preference for less pungent onion varieties ("sweet onions"), which has generated a large differentiated market (PHAFF 2007).

The main goal of the present study was to evaluate the genetic diversity and quality traits (pungency and SSC) in a collection of onion landraces from North-West Spain. The results obtained in this work will complement the previous morphological assessment, providing new data of pivotal importance for the management and conservation of this potentially valuable genetic resource.

MATERIALS AND METHODS

Plant material

Seeds from the 17 onion landraces were supplied by the Centro de Investigaciones Agrarias de Mabegondo (A Coruña, Spain) (Table 1). The morphological descriptions of these lines were previously reported by RIVERA-MARTÍNEZ *et al.,* (2005). The landraces were sown under greenhouse conditions at Mabegondo (43º 15´N, 8º 18´N), the seedlings were transplanted to the field in May and the harvest took place in September. The experimental design was a complete randomized block design with three replications for a total of 84 plants per plot (0.25×0.15 m). At the end of the experiment, all bulbs were pooled and 10 bulbs per landrace were randomly selected and used for the analysis of pungency and soluble solids content (SSC).

Table 1 Name, origin and bulb morphological description of the 17 landraces from North-West Spain used in this study.

Line	**Origin**	**Bulb shape[1]**	**Skin colour[1]**	**Type (subgroup)[2]**
Ameixenda	A Coruña	TE	Yellow-brown	Red storage (4.3.2)
Baldaio	A Coruña	TNE	Yellow-brown	Red storage (4.3.2)
Betanzos1	A Coruña	-	-	-
Betanzos2	A Coruña	TNE	Pale-Yellow	Other
Chata Miño	A Coruña	TNE	Brown	Red storage (4.3.2)
Outes	A Coruña	TE-R	Yellow-brown	Conical (4.4.1)
Mondoñedo	Lugo	TNE	Yellow-brown	Red storage (4.3.2)
Ribadeo1	Lugo	R	Brown	Conical (4.4.1)
Ribadeo2	Lugo	R	Brown	Conical (4.4.1)
San Julián	Lugo	TNE	Brown-Red	Red storage (4.3.4)
Cea	Ourense	TE-R	Yellow-brown	Conical (4.4.1)
Oimbra	Ourense	R	Yellow-brown	Conical (4.4.1)
A Guarda	Pontevedra	TNE	Yellow-brown	Red storage (4.3.2)
Bordóns	Pontevedra	-	-	-
Ponteareas	Pontevedra	TNE	Yellow-brown	Red storage (4.3.2)
Vilagarcía1	Pontevedra	R	Pale-Yellow	Other
Vilagarcía2	Pontevedra	TE-R	Pale-Yellow	Other

[1]Morphological characteristics reported by RIVERA-MARTÍNEZ *et al.*, (2005)

[2] Onion types following CASTELL AND DÍEZ (2000), as reported by RIVERA-MARTÍNEZ *et al.*, (2005)

Bulb analysis

Pungency of the bulbs was evaluated by quantifying the pyruvic acid produced after cell disruption, using the method proposed by SCHWIMMER and WESTON (1961) as modified by boyhan *et al.,* (1999). Onion juice samples were obtained from equator–transverse sections 1 cm thick. Results for pungency were expressed in micromoles of enzymatically formed pyruvic acid per gram of fresh weight (μmol g^{-1} FW). The soluble solids content (SSC) of onion juice samples was measured using a hand refractometer (Shibuya Optical Co., LTD). Data were expressed as equivalent ºBrix.

Microsatellite analysis

Total DNA was extracted from young leaves following a CTAB–based method (GARCÉS–CLAVER *et al.,* 2007). For SSR analysis, 12 EST–based SSRs (ACM004, ACM006, ACM045, ACM101, ACM119, ACM124, ACM134, ACM138, ACM146, ACM187, ACM235, ACM300) (MCCALLUM *et al.,* 2008) and 5 genomic SSRs (ACM373, ACM443, ACM446, ACM449 and ACM520) (BALDWIN *et al.,* 2012) were used. SSR markers were selected for their high polymorphism, according to the studies quoted above. PCR amplification was carried out in a final volume of 20 μl, which contained 50–100 ng of genomic DNA, 1× PCR Buffer (NZYTech), 2.5 mM $MgCl_2$ (NZYTech), 0.05 pmol of the M13 tailed forward primer and 0.5 pmol of the reverse, dNTPs (Fermentas) at 0.2 mM each, DMSO 2% and 1 U of Taq DNA Polymerase (NZYTech). For SSR amplification, 0.5 pmol of M13 primer were used (5´–CACGACGTTGTAAAACGAC–3´) labeled with 5´fluorescent dyes (6–FAM, HEX or NED). Two PCR programs were used. For the majority of markers the PCR conditions were as follows: 94 ºC for 5 min; followed by touchdown PCR with 12 cycles of 30 s at 94 ºC, 45 s at 62 ºC, 45 s at 72 ºC; and then 40 cycles with 30 s at 94 ºC, 45 s at 56ºC, 45 s at 72 ºC, and a final extension at 72 ºC for 10 min. Genomic SSRs were amplified according to Balwin et al. (2012), except that primer ratios were maintained as above. PCR products were separated on a capillary electrophoresis system (ABI3130xl, Applied Biosystems, USA), using GeneScan™ 500 ROX™ Size Standard (P/N 4322683) (Applied Biosystems) as the internal lane size standard. Raw data were analyzed using Genemapper v. 3.7 software (Applied Biosystems, USA).

Genetic data analysis

The number of observed alleles per locus, observed heterozygosity (H_o=number of heterozygous individuals/number of individuals scored), expected heterozygosity ($H_e=1-\Sigma\rho i^2$, where ρi is the frequency of the ith allele) (NEI 1973), Wright's fixation index ($F_{is}=1-H_o/H_e$), and the Polymorphic Information Content (PIC) (BOTSTEIN *et al.,* 1980) were calculated using GenAlex v. 6.5 software (PEAKALL and SMOUSE 2012) and the Microsatellite Toolkit (PARK 2001). The marker data were used to generate a 0/1 matrix (presence/absence of allele in heterozygosis or homozygosis at the marker locus) that was employed to estimate the genetic distance (GD) between landraces. Genetic similarities were calculated using the Dice coefficient (NEI and LI 1979), and a dendrogram depicting relationships of the collection was built from the GD matrix based on the Unweighted Pair Group Method Average (UPGMA) cluster analysis in NTSYS–pc v. 2.1 (ROHLF 2000). The cophenetic correlation coefficient (r) was calculated to measure goodness of fit between the similarity and cophenetic matrices. The significance of the cophenetic coefficient was determined by 1000 random permutations. Principal coordinate

analysis (PCoA) based on genetic similarity matrices was performed with the DCENTER and EIGEN options of the NTSYS–pc version 2.1 to identify the number of groups based on eigenvectors. The PCoA result was displayed by a 3D- plot constructed with SigmaPlot v. 11.0 software (Systat Software, Inc., Hounslow, London, UK).

RESULTS

Three EST-SSRs and two genomic SSRs did not amplify and the marker ACM520 did not exhibit clear peak resolutions on the electropherograms. The remaining 11 markers were polymorphic and generated a total of 32 alleles in a range of 118–299 bp (Table 2). The number of alleles per locus ranged from 2 (ACM119, ACM146, ACM187, ACM300) to 6 (ACM134) with a mean of 2.9. The number and size of alleles identified in the Galician genotypes were compared with those obtained recently by Mallor et al. (2014) with a nearly identical group of markers in a collection of Spanish onion landraces (Table 2). In general, the number of alleles per locus detected in the Spanish onions was higher, which was expected considering that a larger number of landraces were screened. Common alleles were observed for markers ACM146 and ACM235. Certain alleles were specific to the Galician group in some loci. Thus, the marker ACM134 amplified 2 specific fragments (190 bp and 192 bp) and markers ACM138 and ACM300 gave particular alleles (231 bp and 169 bp, respectively) in the Galician genotypes (Table 2). None of these unique alleles were considered as rare alleles (those with a frequency ≤ 5%) (data not shown).

Table 2 Allele size and SSR amplicons observed in 17 onion landraces from North-West Spain and comparison with those observed in previous reports with a similar set of SSR markers.

Marker	**Repeat motif**	**Observed size**	**Allele number observed**	**Expected size (bp)**		**Allele number reported**	
				MCCALLUM *et al.* (2008)	MALLOR *et al*. (2014)	MCCALLUM *et al.* (2008)	MALLOR *et al*. (2014)
ACM045	$(TC)_6$	260-264	3	226-275	255-280	5	7
ACM101	$(TCC)_5$	230-239	3	227-248	227-239	8	5
ACM119	$(AAT)_8$	246-255	2	241-259	242-260	3	5
ACM134	$(GA)_8$	190-206	6	198-212	192-206	5	5
ACM138	$(CTGC)_1$	231-243	3	242-286	242-274	1	6
ACM146	$(ACA)_5$	230-233	2	239-242	230-233	2	2
ACM187	$(GT)_6$	262-264	2	225-262	228-266	5	4
ACM235	$(TTTG)_4$	287-299	3	292-304	288-299	4	2
ACM300	$(GCA)_7$	169-172	2	170-177	172-181	3	4
				BALDWIN *et al.* (2012)	MALLOR *et al*. (2014)	BALDWIN *et al.* (2012)	MALLOR *et al*. (2014)
ACM446	$(TA)_8$	118-122	3	122-124	-	12	-
ACM449	$(GT)_7$	132-142	3	133-148	-	13	-

PIC value was calculated to determine the effectiveness of markers in distinguishing among genotypes (Table 3). The genomic SSR ACM134 was the best at discriminating between two random individuals (PIC=0.69), whereas the less informative marker was

ACM235 (PIC=0.12). Four EST-SSRs and one genomic SSR gave PIC values higher than 0.5. The PIC per locus showed a significant, positive correlation with the number of alleles per locus for all genotypes (r = 0.61, $p < 0.05$). Parameters of genetic diversity were calculated for the markers (Table 3).The marker ACM235 revealed the lowest (0.13) observed heterozigosity, whereas the highest (0.75) was produced by ACM300. The expected heterozigosity ranged from 0.12 (ACM235) to 0.72 (ACM134)), leading to large values of Nei´s gene diversity index (0.51) in the collection (Table 3). Wright´s fixation index fluctuated from negative (-0.23) to positive (0.62) among markers, with an average value of 0.08 (Table 3), confirming the excess of heterozygotes within the collection.

Table 3 Polymorphic information content (PIC), observed heterozygosity (H_o), expected heterozygosity (H_e) and Wright's fixation index (F_{is}) for 11 SSR markers in 17 Galician onion landraces.

Marker	**PIC**	**H_o**	**H_e**	**F_{is}**
ACM045	0.58	0.25	0.66	0.62
ACM101	0.59	0.44	0.66	0.34
ACM119	0.32	0.38	0.43	0.13
ACM134	0.69	0.60	0.72	0.17
ACM138	0.58	0.33	0.62	0.46
ACM146	0.26	0.38	0.30	-0.23
ACM187	0.37	0.47	0.50	0.07
ACM235	0.12	0.13	0.12	-0.05
ACM300	0.35	0.75	0.47	-0.60
ACM446	0.54	0.56	0.62	0.09
ACM449	0.47	0.63	0.54	-0.16
Mean	0.44	0.45	0.51	0.08
SD	0.16	0.17	0.17	0.32

The genetic distance between each pair of genotypes ranged from 0.40 to 0.88 with an average of 0.61 (data not shown). The highest genetic dissimilarity was found between Vilagarcía2 and Ameixenda, whereas the minimum genetic distance was observed between Cea and Mondoñedo (Fig. 1). The dendrogram derived from SSR data using distance–based UPGMA cluster analysis clearly separated Oimbra from all the other lines that constitute the tree. The remaining 16 genotypes were assorted into 2 groups at a coefficient of similarity of 0.60. The largest group (I) consists of 11 genotypes, while the smaller (II) comprised only 5 individuals (Fig. 1). At a similarity level of ca. 0.70, the group I was further divided into sub–groups $I_{a,}$ (comprising 6 genotypes) and $I_{b,}$ (2 genotypes), whereas Outes, Ameixenda and Vilagarcia 1 remained independent (Fig. 1). Similarly, the group II was further divided into one sub–group ($II_{a,}$) holding 3 different individuals, and 2 separate genotypes (Betanzos 1 and Chata-Miño) (Fig.

1). To test the dendrogram goodness of fit, the cophenetic correlation between the similarity matrix and the corresponding cophenetic matrix was calculated. The cophenetic correlation coefficient value, r = 0.71 (approximate Mantel t test: t=5.5651; probability random Z < observations Z: P = 1.0000) suggested a good fit between the dendrogram and the similarity matrix from which it was derived based on the finding of SNEATH and SOKAL (1973). The onion genotypes were grouped irrespective of their geographical origin and the clustering pattern seems to be partly in agreement with morphological traits of bulbs. Thus, group I clustered mainly onions of the "Red Storage type" (CASTELL and DÍEZ 2000) with yellow–brown colors, transverse narrow elliptic bulb shape and low weights, The group II comprised mixed genotypes, which were previously classified as "other types" according to CASTELl and DÍEZ (2000) criteria (Table 1) (for more clear morphological descriptions see also RIVERA–MARTÍNEZ *et al.*, 2005).

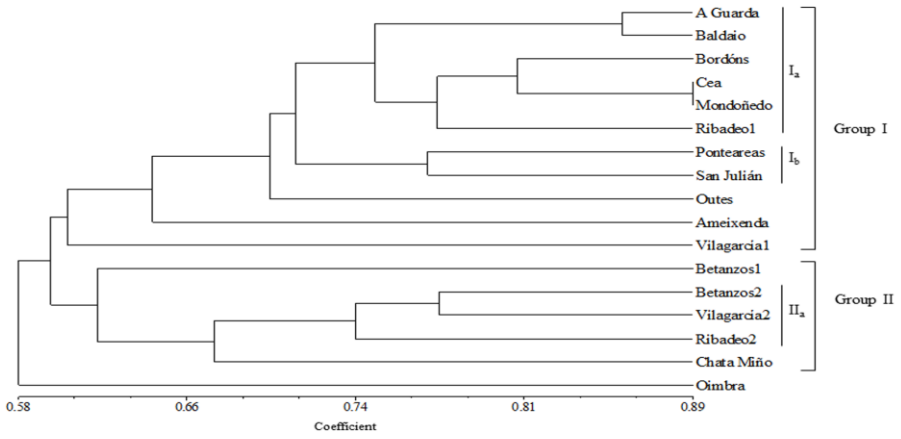

Figure 1 Dendrogram of genetic relationships among 17 Galician onion landraces obtained from the UPGMA cluster analysis, using the Dice´s coefficient after amplification with 11 microsatellite markers.

The grouping of genotypes was investigated further with a principal coordinate analysis, which allowed the visualization of onion genotypes in a three dimensional space, independently of their hierarchical relationships (Fig. 2). Principal coordinate 1 (PCo1) accounted for 19.3 % and mainly separated groups I and II. Principal coordinates (PCo) 2 and 3 (accounting for 13.23% and 10.74%, respectively) provided further differentiation (Fig. 2). Principal coordinate analysis was in part consistent with results from the cluster analysis, supporting the occurrence of the sub-groups I_a (A Guarda, Baldaio, Cea and Mondoñedo) and II_a (Betanzos 2, Vilagarcía 2 and Ribadeo 2), the individuality of the other genotypes and Oimbra as the most distinguished line (Fig. 2).

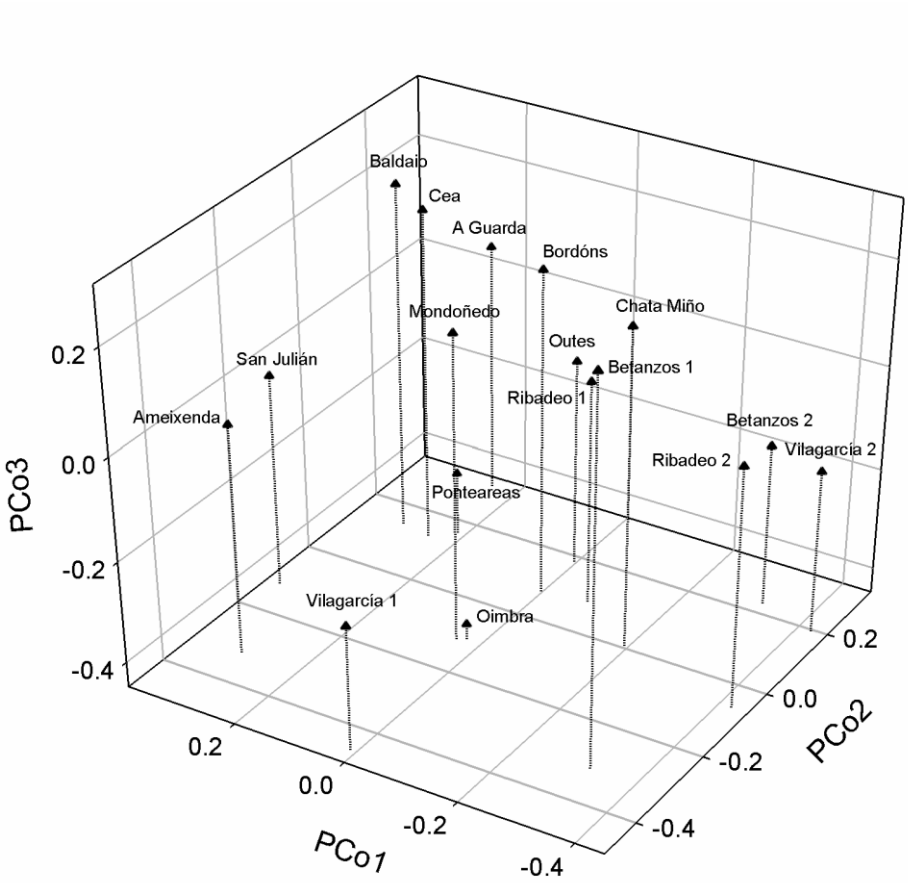

Figure 2 Three-dimensional plot of principal coordinate analysis with 11 SSR markers and 17 onion landraces.

Onion pungency was estimated by measuring the pyruvic acid content (Table 4). The results show a high degree of variability in pungency, not only among the Galician landraces, but also among the bulbs obtained from each accession. Pyruvic acid ranged from 1.16 to 8.35 μmol g–1 FW, with an average value of 4.47 μmol g–1 FW. Oimbra showed the highest value followed by Chata–Miño and Ribadeo. The lowest levels of pyruvate were detected in Bordóns and Outes. The coefficients of variation fluctuated between 23.2 % (Chata–Miño) and 83.7% (Outes), with a mean value of 56.4%. Considering low pungency or mild onions as those with a pyruvate concentration smaller than 5 μmol g^{-1} FW (Abayomi and Terry 2009), 58.8 % of Galician onions could be considered as mild onions. According to the sweet onion industry guidelines, onions are

classified on the basis of pungency as low pungency/sweet (0–3 μmol g^{-1} FW), medium pungency (3–7 μmol g^{-1} FW), and high pungency (above 7 μmol g^{-1} FW) (Dhumal et al. 2007). As per this classification, 5 (29.4 %), 9 (52.9%) and 3 (17.6%) accessions of the Galician collection should be classified as landraces with low, medium and high pungency, respectively.

Table 4 Pyruvic acid and soluble solid content in bulbs of 17 Galician onion landraces.

	Pungency (μmol g^{-1} FW)		**Soluble solid content (°Brix)**	
Line	**Mean**	**CV[1] (%)**	**Mean**	**CV[1] (%)**
Ameixenda	2.90	63.25	4.68	22.63
Baldaio	5.40	42.95	4.70	25.71
Betanzos1	5.66	58.91	7.10	24.42
Betanzos2	5.42	39.49	4.78	30.33
Chata Miño	7.54	23.21	5.06	17.68
Outes	1.70	83.72	4.84	35.54
Mondoñedo	7.31	47.68	3.74	16.35
Ribadeo1	3.77	70.01	5.26	27.36
Ribadeo2	3.10	40.23	4.86	35.46
San Julián	3.65	77.57	4.18	19.72
Cea	6.48	48.60	8.56	19.39
Oimbra	8.35	57.40	6.88	18.29
A Guarda	4.30	49.75	6.36	22.47
Bordóns	1.15	83.58	4.06	26.58
Ponteareas	3.00	60.13	5.92	37.46
Vilagarcía1	2.54	59.38	5.24	58.13
Vilagarcía2	3.78	53.20	7.12	15.11
Mean	4.47	56.42	5.49	26.63

[1]Coefficient of variation

SSC was also variable among and within landraces. SSC values ranged from 3.74 to 8.56 ºBrix, with a mean value of 5.49 ºBrix. The accessions with the highest content of SSC were Cea (8.56) and Vilagarcía2 (7.12), followed by Betanzos 1 (7.10) (Table 4). Mondoñedo, Bordóns and San Julián showed the lowest value for this parameter (3.74, 4.06 and 4.18, respectively). The mean CV of SSC (26.6%) was lower than that obtained with the pyruvate measures (Table 4).
No correspondence was found between the traits evaluated in this research (pungency and SSC) and the clustering patterns obtained after multivariate analyses on SSR data.

DISCUSSION

Onions have probably been cultivated on the Iberian Peninsula for more than 2000 years, and many landraces have been well documented (CASTELL and DÍEZ 2000, CARRAVEDO and MALLOR

2007, SIMÓ *et al.,* 2014). During these centuries of cultivation, farmers greatly contributed to the diversification of this crop by selecting ecotypes adapted to the specific agro-climatic conditions of the different cultivation areas. Traditional onion landraces are still produced in certain regions of North-West Spain due to their high quality and acceptance in local markets. A collection of these local lines, maintained at the Centro de Investigaciones Agrarias de Mabegondo (A Coruña, Spain) was previously evaluated for morphological traits (RIVERA–MARTÍNEZ *et al.,* 2005). The aim of this study was to characterize this collection at the DNA level, using SSR markers, as well as to assess two significant traits (pungency and SSC) associated to the organoleptic quality of bulbs.

Genetic background studies in onion have used different types of molecular markers to characterize the variability inside germplasm collections (TANIKAWA *et al.,* 2002, JAKSE *et al.,* 2005, ARAKI *et al.,* 2010). In this work, we selected microsatellites because of their stability, capacity of multi-allelic detection, ease of application and excellent sensitivity (POWELL *et al.,* 1996, VARSHNEY *et al.,* 2005). Twelve out of 17 SSR markers (9 EST-SSRs and 3 genomic SSRs) were able to amplify in Galician onion genotypes. The rate of amplification was higher for EST-SSRs (75%) than for genomic SSRs (60%), which confirm earlier results by other authors (KHAR *et al.,* 2011, MALLOR *et al.,* 2014). The failure of some genomic SSRs suggests that further optimization should be performed for these primers pairs (JAKSE *et al.,* 2005, SANTOS *et al.,* 2010, BALDWIN *et al.,* 2012). The percentage of amplification obtained for EST-SSRs was larger than those previously reported by santos *et al.,* (2010) and KHAR *et al.,* (2011) but equal to that observed by MALLOR *et al.,* (2014) with a similar set of markers. Eleven SSRs were recorded as polymorphic and marker ACM520 was discarded after the capillary electrophoresis because the electropherograms were not easily interpreted. The rate of polymorphism detected in our work (91.6%) was high in comparison to other onion collections evaluated with an equivalent set of markers (KHAR *et al.,* 2011, MALLOR *et al.,* 2014). The average number of alleles per locus (2.9) was similar to that obtained by KHAR *et al.,* (2011) (2.84 alleles) but lower than those observed in other reports (3.59, MCCALLUM *et al.,* 2008; 7.9, BALDWIN *et al.,* 2012; 3.9, MALLOR *et al.,* 2014).). Although some markers are in common among these studies and ours, they surveyed larger samples than that of the current research, which could explained the lower values recorded for the Galician collection. The comparison of alleles detected in the Galician group with those identified by MALLOR *et al.,* (2014) in a collection of Spanish onion landraces, revealed the presence of unique alleles in the genotypes coming from North-West Spain. These alleles were detected in various Galician genotypes (they are not rare alleles) and they could point the existence of substantial diversity arisen from the adaptation of these genotypes to local constraints.

The set of 11 microsatellite markers unveiled a relatively high level of diversity within the collection, with a Nei´s gene diversity index of 0.51. Such level of variability could be attributed to a high frequency of heterozygosity among genotypes. Indeed, all genotypes exhibited a moderately high percentage of heterozygous loci, ranging from 18.2 to 72.3 % (data not shown) and the majority of markers showed a large value of observed heterozygosity (H_o). The observations of high heterozigosity but low allele number were also reported in previous works on onion (BARK and HAVEY 1995, MCCALLUM *et al.,* 2008). It is likely that these results reflect the effects of outbreeding derived from open pollinations and continuous flux of genes in the relatively small geographical region where these landraces have undergone differentiation. Such hypothesis has been also proposed for other Spanish onion landrace collections (SIMÓ *et al.,* 2014)

as well as for other naturally out–crossing species, such as maize (PRESSOIR and BERTHAUD 2003, VIGOROUX *et al.,* 2008). In fact, the inbreeding coefficient (F_{is}) obtained in our work was much lower than that reported in those studies.

Multivariate analyses revealed that grouping of genotypes was not on the basis of their geographical origin, as landraces from the same province (A Coruña, Lugo, Ourense or Pontevedra) were not clustered together. Though there was not a straightforward correspondence between the morphological and chemical characteristics of Galician genotypes and their group placement, certain trends could be observed. Thus, genotypes with the highest genetic similarity, Cea and Mondoñedo, have a pyruvic content near to 7 μmol g^{-1} FW, a weight around 122 g and a yellow–brown color. Similarly, A Guarda and Baldaio are sweet onions with medium weight, similar bulb height and diameter and identical shape and color. The most distinctive genotype defined by the multivariate analyses was Oimbra. This genotype possesses unique morphological characteristics (the most pungent and biggest bulbs, a rhombic bulb shape and the total absence of axes) which might explain its position on the dendrogram and the 3D plot. Interestingly, genotypes from the same locations, Ribadeo 1 and 2 or Vilagarcía 1 and 2, were assorted across different groups. This could be also explained using morphological traits, since those genotypes with common geographical origins (Ribadeo in Lugo and Vilagarcía in Pontevedra) displayed various distinguishing features (RIVERA–MARTÍNEZ *et al.,* 2005). It is not unusual that a hundred percent of agreement between morphological groups previously established and current clusters based on molecular data was not observed. This is simple to explain considering that agro–morphological attributes are normally coded by few genes that affect a few easily identifiable traits and are not necessary linked to the molecular markers employed. Comparable results were reported by KHAR *et al.,* (2011) for Indian onions in which the clustering pattern was not made on the geographical origins or relevant onion characteristics. Similarly, MALLOR *et al.,* (2014) did not found that the branches that grouped Spanish onions after the cluster analysis were clearly defined by pungency, skin color or day length requirements.

Estimation of pungency in any onion line has become necessary as the popularity of low pungency onions has increased (DHUMAL *et al.,* 2007). Onion pungency can be accurately determined by measuring of biochemical components such as pyruvic acid (LIN *et al.,* 1995). Pyruvate values have been significantly correlated to sensory ratings, indicating that the estimation of this compound could be used as a reliable selection technique for breeding programs (PINEDA *et al.,* 2004). Our results showed a high degree of variability in pungency among and within the Galician onion landraces. Such level of variability was expected if we consider the high proportion of heterozigosity detected in the collection and the fact that more than 80% of onion pungency is determined by genetic factors (YOO *et al.,* 2006). Obviously, this assumption is based on data obtained from only 11 SSRs, which are not representative of the entire genome. The mean CV of pungency found in this study (56.8%) was similar to that reported by MALLOR *et al.,* (2011) for eighty–six onion landraces from Spain. Such high CV should be expected in landrace collections that have never been under selection pressure (YOO *et al.,* 2006). Despite the variability, 5 landraces could be classified as sweet (pyruvate content <3μmol g^{-1}FW) and 4 of them as almost sweet (pyruvate content <4μmol g^{-1}FW). This number of milder landraces is higher than that found in other collections of Spanish onions landraces (MALLOR *et al.,* 2011), and they appear promising for their incorporation to breeding programs focus on the development of new cultivars with low pungency. Indeed, MALLOR and SALES (2012) have already obtained progenies with lower and more uniform levels of pungency after two cycles of selection on the

Spanish traditional cultivar "Fuentes de Ebro". The most pungent landraces, such as Oimbra, Chata Miño and Mondoñedo, could be exploited for the industries of sauces, canned soups, extracts and dehydrated products (GALMARINI *et al.,* 2001).

The SSC in the Galician collection ranged from 3.7 to 8.6 ºBrix, which is consistent with results obtained in other reports dealing with onion landraces (RODRÍGUEZ–GALDÓN *et al.,* 2009, MALLOR *et al.,* 2011). SSC is an indirect estimator of the dry matter content (DMC), which determines in part the end use and storage life of bulbs (PORTA *et al.,* 2014). Thus, the storability normally increases with the percentage of DMC (GALMARINI *et al.,* 2001, MCCALLUM *et al.,* 2007). Our results confirm the excellent storage quality of some Galician landraces, as previously attributed by RIVERA-MARTÍNEZ *et al.,* (2005). It is common that pungency and SSC appear negatively correlated with bulb weight (YOO *et al.,* 2006, MALLOR *et al.,* 2011). No clear correlations were established for the Galician landraces, since previous and current data could not be compared. However, comparison between the level of pungency and SSC with previous results (RIVERA–MARTÍNEZ *et al.,* 2005) does not seem to point to a relationship between all these parameters. Indeed, the most pungent landrace Oimbra is also the one with the highest bulb weights, and Mondoñedo, with the lowest SSC value, has the smallest bulbs.

Genetic characterization and analysis of organoleptic traits revealed that onion landraces from North-West Spain are very heterogeneous among them. The heterogeneity is an inherent characteristic of landrace materials, which are commonly composed of different selections (LOUETTE 2000, NEGRI *et al.,* 2009). Such heterogeneity makes Galician onions a useful source of genetic variability that could be exploited in breeding programs. For this purpose, lines with low coefficients of variation for the interesting traits should be selected in order to guarantee uniformity of the future cultivars and consolidate conservation approaches (MALLOR *et al.,* 2011, CEBOLLA–CORNEJO *et al.,* 2013). In this context, the knowledge of the genetic diversity and relationships among landraces in the collection, based on molecular markers, result compulsory to accelerate breeding strategies. The research presented here has contributed new valuable data on the diversity and organoleptic traits preserved in a collection of onion landraces originating from North-West Spain. This study served to complement the previous morphological assessment, reinforcing the distinctiveness of Galician landraces and their potential value for researches and breeders, as a novel genetic resource.

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PROUČAVANJE GENETSKE DIVERGENTNOSTI I OSOBINA KVALITETA U KOLEKCIJI LOKALNIH POPULACIJA CRNOG LUKA (*Allium cepa* L) POREKLOM IZ SEVERNO-ZAPADNE ŠPANIJE

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Izvod

Sedamnaest lokalnih populacija crnog luka poreklom iz severnozapadne Španije je proučavano korišćenjem mikrosatelitskih markera. Jedanaest polimorfnih markera detektovalo je ukupno 32 alela u celoj kolekciji sa prosečnim brojem od 2.9 alela po lokusu. Visoke vrednosti uočene (srednja od 0.45) i očekivane heterozigotnosti (0.51) su zabelezene kod najvećeg broja lokusa. Wrightov indeks fiksacije je potvrdio povećanu heterozigotnost i niske vrednosti samooplodnje u okviru kolekcije. Multivariaciona analiza je pokazala da je Oimbra genotip koji je najviše udaljen od ostalih. Preostalih šesnaest genotipova su delimično grupisani na osnovu morfoloških osobina lukovica. Oporost i rastvorljivost čvrstih komponenti su jako varirali izmedju lokalnih populacija i lukovica. Pet populacija je klasifikovano kao slatke, devet ih je bilo osrednje oporosti dok su tri populacije grupisane kao opore. Ova kolekcija crnih lukova predstavlja koristan izvor genetičke varijabilnosti koja moze biti iskorištena u oplemenjivačkim programima za stvaranje novih sorata koje mogu zadovoljiti zahteve potrosača.

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