Genetic variation for bulb size, soluble solids content and pungency in the Spanish sweet onion variety Fuentes de Ebro. Response to selection for low pungency

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Received April 15, 2009/Accepted October 16, 2009 Communicated by M. Havey

Abstract

The cultivar 'Fuentes de Ebro' is a long-day onion grown in the northeast of Spain, which is characterized by its succulence and low pungency. However, to match the market demand the size, pungency, and storability need to be improved. We have evaluated these quality-related bulb traits in 15 grower's open-pollinated lines of this cultivar. Phenotypic variation observed for bulb weight, size and soluble solids content was significantly affected by location, growing season and line, while pungency depended on line and plot location. We found higher levels of genetic variation for bulb size and pungency than for soluble solids content, and significant phenotypic correlations indicated that milder onion tend to show larger size and lower soluble solids content. After one cycle of selection, we have obtained progeny with significantly lower pungency levels, and therefore, we estimated a realized heritability of 0.67. As conclusion, it is feasible to obtain a sweet cultivar after some selection cycles in the 'Fuentes de Ebro' onion, although considering indirect consequences in size and soluble solids content.

Key words: Allium cepa — breeding — flavour — heritability — long-day onion

Onion (*Allium cepa* L.) is the second most valuable vegetable in the world, following only tomato. It is one of the oldest cultivated vegetables, being primarily consumed because of their unique flavour or for their ability to enhance the flavour of other foods, since onion bulbs do not have a highly significant nutritional value (Randle 1997). Onions are also recognized for their health-giving properties such as providing no digestible dietary fibre from fructans, antiplatelet activity from organosulfur compounds and antioxidant activity from flavonoids like quercetin (Galmarini et al. 2001).

Selection by growers and breeders has produced cultivars that vary widely in adaptation, sweetness, storability and processing quality. Bulb composition, namely its content in carbohydrates (glucose, fructose, sucrose and fructans) and sulphur derivatives (1-propenyl, methyl- and propyl-cysteine sulfoxides; Randle and Lancaster 2002), determines its quality for an intended use. Varieties known as 'storage onions' have high dry matter content (15–20%) and relatively high amounts of fructans but lower levels of reducing sugars, besides relatively high rates of organosulfur compounds. They have an intense, pungent flavour and desirable characteristics for cooking or for industrial processing. 'Fresh onions' are consumed directly in salads as they do not store well because of its low dry matter content (5–9%), particularly in fructans. They have a mild or sweet flavour, as they contain low levels of organosulfur compounds which are related to pungency. Less pungent onion varieties with higher content of reducing sugars, known as 'sweet onions' have become preferred for fresh consumption, especially in the USA, Japan and many countries of Europe. The repeating demand of the consumer has generated a large niche market (Pike 1997, Phaff 2007).

Breeders involved in the development of sweet onions have first to choose cultivars with low pungency, since pungent flavour can mask a high level of sugars, so that the onion is not perceived as sweet. The genetic basis of pungency has been studied for more than 50 years and heritability estimates of 0.4-0.7, with predominantly additive effects, have been reported for this quantitatively inherited trait (Lin et al. 1995, Simon 1995, Wall et al. 1996, Galmarini et al. 2001). More recently, molecular markers have contributed to find in the large genome of this plant species regions regulating bulb quality traits. Two quantitative trait loci have been found to exert pleiotropic effects on pungency, dry matter and soluble solids content (Galmarini et al. 2001, McCallum et al. 2007). These findings constitute genetic evidence for experimental data, since dry matter content, soluble solids content and pungency show significant positive phenotypic correlations (Galmarini et al. 2001). Development of sweet onions with good storage ability (high dry matter content) is therefore a difficult challenge for breeders. Dry matter content is usually estimated by soluble solids content, a character that shows high heritability rates (0.6–0.8). Quantitative trait loci affecting fructans and reducing sugar contents have also been reported (Havey et al. 2004, McCallum et al. 2006).

Although onion flavour has a genetic component, environmental factors such as water supply, temperature, and especially nitrogen and sulphur fertility can affect flavour intensity (Randle 1997, Hamilton et al. 1998, McCallum et al. 2005, Yoo et al. 2006). Pungency can be determined indirectly using a colorimetric test for pyruvic acid concentration, since this compound is formed in the reaction that produces the volatile sulphur compounds that are responsible for onion flavour. The National Onion Labs Inc. (NOL) certificates in the USA sweet onions according to its pyruvic acid content: varieties are described as 'very mild' or 'extra-sweet' when pyruvic acid content is lower than 3.5 µmol/g FW and as 'mild' or 'sweet' when this content is $3.6-5.5 \mu mol/g$ FW. Besides, they consider bulbs 'of moderate pungency' when pyruvic acid content is 5.6–7.5 µmol/g FW and 'pungent' when this content is higher than 7.5 μ mol/g FW, respectively.

Soluble solids content is also related to onion flavour, apart of its effect on bulb texture and storability (McCallum et al. 2006, and references therein). About 80% of soluble solids content depends on the accumulation of fructans, fructose, glucose and sucrose. While fructans probably do not make much of a contribution to taste, as they function as storage carbohydrates, sucrose and reducing sugars are the compounds that mainly determine onion sweetness.

The onion 'Fuentes de Ebro' is a very popular open pollinated cultivar, originary from the Aragón region in the northeast of Spain, known for their mild, sweet flavour (Mallor et al. 2007). Because of the increasing interest in developing sweet onion cultivars, particularly long-day onions adapted to temperate areas, a breeding programme has been initiated with this plant material defective on several traits as bulb size, uniformity and storability. Nowadays, in the traditional growing area (about 150 ha in the province of Zaragoza) seed production depends on each grower's selection criterion. This results on the coexistence of different lines within the 'Fuentes de Ebro' cultivar. In this work, we first report the characterization of this plant material in order to determine the extent of genetic variation for three important attributes of onion: pungency, soluble solids and bulb size. Secondly, we studied the response to selection for low pungency.

Materials and Methods

Plant material: Onion seeds were supplied by local growers through the Onion Fuentes de Ebro Association. Fifteen different and representative lines were cultivated in four plots located in the traditional growing area (latitude 41°39'N), where soils have mid-lime texture, basic pH and relatively high salt contents. Crops were cultivated in two plots during two consecutive seasons (2006 and 2007), by using the horticultural practices conventionally applied to this variety: (i) direct sowing in February, each line in one 70 m long eight-row bed 2.1 m wide and with a distance of 6.5 cm between seeds, which finally resulted in approximately 60 plants/m^2 , the 15 beds were 0.5 m apart; (ii) border irrigation and (iii) fertilization by applying 50 : 100 : 100 kg/ha of N, P_2O_5 and K_2O at seeding, 90 kg/ha of N in April and 35 kg/ha of N and 110 kg/ha of K2O in June. Harvest was performed on the 3rd week of August. For each line, plot and season, three samples of 10 bulbs were obtained and analysed for weight, size (maximum diameter), soluble solids and pyruvic acid content.

From the 2006 pyruvic acid analyses, bulbs were grouped according to the NOL classification. Five bulbs from each group were planted in isolation cages (2007) and seeds were harvested from each plant individually. In 2008, a plot with four replicates of these 20 half-sib families was established in the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) in Montañana, Zaragoza. The crops were initiated in April with seedlings cultivated in greenhouse from January, that were transplanted in double-row beds 0.8 m apart where plants were set at 10×15 cm. Plants were drip-irrigated and bulbs harvest was performed at the 2nd week of August. A sample of 10 onions from each family and replicate was analysed for weight, size, and pyruvic acid and soluble solids content. Three samples were also obtained from sub-plots established with seeds from unselected bulbs, and were used as a control for comparative purposes.

Bulb analyses: Pyruvic acid content was determined in onion juice samples obtained from equator-transverse sections 1 cm thick, following the Schwimmer and Weston (1961) method adapted for microplates (Boyhan et al. 1999) with slight modifications. Soluble solids content assay was performed using a hand-held refractometer (Euromex RF532, Model 53015C; TR, Forli, Italy).

Data analysis: A one-way analysis of variance (ANOVA, $\alpha = 0.05$) was performed on data from each year and plot for testing equality of means of bulb's weight, diameter, soluble solids content and pungency, among 15 grower's lines. A *post hoc* Tukey-*b* test was performed to construct homogeneous groups. For these ANOVA-based methods, spss 16.0.1 (SPSS Inc., Chicago, IL, USA) for Windows was employed.

The response to selection (R) for low pungency was calculated as the difference between the mean pungency of the unselected progeny and the mean pungency of the five half-sib families of group 1. The selection differential (S) was the difference between the mean pungency of the 2006 unselected population and the mean pungency of the selected five bulbs of group 1. Realized heritability after one selection cycle or broad sense heritability in the 'Fuentes de Ebro' cultivar was estimated as H = R/S (Falconer 1981).

Results

Effect of year, location and line on bulb traits

The 15 'Fuentes de Ebro' grower's lines produced bulbs which varied widely in weight and size, and these traits were also affected by environmental conditions, since the ANOVA showed significant (P < 0.005) effects for year, location and line (Table 1). Larger bulbs were obtained, on average, in 2007 from plot 2. Weight means separation among lines depended on plot and year, implying that the different genetic response of the 15 lines was as well highly influenced by environmental factors. Bulb weights ranged from 181 \pm 37 g to 422 \pm 103 g (line 13, plot 1 in 2006, and line 3, plot 4 in 2007, respectively). When averaged through year and plot, bulbs weight ranged among lines from 223 \pm 57 g in line 13 to 323 \pm 33 g in line 8. Bulb size, determined as maximum diameter, ranged from $75 \pm 8 \text{ mm}$ (line 12, plot 1 in 2006) to 100 $\pm 9 \text{ mm}$ (line 3, plot 4 in 2007). When averaged through plots and years, bulb sizes ranged among lines from 80 ± 5 (lines 12 and 13) to 89 ± 5 (lines 5, 8 and 9). These values corresponded to large onion sizes (Kopsell and Randle 1997).

The ANOVA variance for soluble solids content revealed significant effects of year, location and line (P < 0.005), except for the line × plot interaction (P = 0.033). However, significant differences among lines were not observed in 2006 for plot 1. Mean soluble solids contents of each line were low (Table 2), ranging from 6.4 \pm 0.9 (line 2) to 7.4 \pm 0.1 (lines 12 and 13), which is expected for a fresh-consumption onion cultivar.

The results of the ANOVA for bulb pungency data (Table 3) showed significant effects of location and line (P < 0.005) but not for year and year × line interaction (P = 0.119 and P = 0.067, respectively). Values for pungency averaged through year and plot varied from 5.4 ± 0.2 in line 5 to $6.8 \pm 0.1 \,\mu$ mol/g FW in line 13. Significant negative correlation was found between bulb weight and pungency except for plot 4 (r = -0.165, -0.181 and -0.122 for plot 1, 2 and 3, respectively). Bulb weight also correlated negatively with soluble solids content (SSC), except for plot 3 (r = -0.164, -0.181 and -0.149 for plot 1, 2 and 4, respectively). These results indicate a trend in larger onions to contain lower rates of both organosulfur derivatives and carbohydrates, therefore, suggesting that bulb size increase was because of higher water content.

Pungency and SSC showed also significant positive correlations, except for bulbs sampled in plot 3 in 2007. Pearson's correlation coefficient ranged from 0.221 in plot 4 (2007) to 0.371 in plot 1 (2006), indicating that onions with higher carbohydrate contents also presented higher levels of organosulfur compounds and therefore higher pungency rates.

Table 1: Bulb weight and size in 15 'F	Fuentes de Ebro' onion lines
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	Bulb weight (g)				Bulb diameter (mm)			
	2006		2007		2006		2007	
Line	P1	P2	Р3	P4	P1	P2	P3	P4
1 2 3 4 5 6 7 8 9 10 11 12 13 14	$\begin{array}{r} 243 \pm 46cd \\ 239 \pm 44bcd \\ 220 \pm 51abc \\ 238 \pm 49bcd \\ 221 \pm 45abc \\ 222 \pm 48abc \\ 222 \pm 56abc \\ 286 \pm 138d \\ 279 \pm 74d \\ 258 \pm 51cd \\ 219 \pm 44abc \\ 186 \pm 53a \\ 181 \pm 37a \\ 195 \pm 30ab \end{array}$	$\begin{array}{r} 277 \ \pm \ 47bc\\ 278 \ \pm \ 77bc\\ 246 \ \pm \ 85abc\\ 257 \ \pm \ 50abc\\ 304 \ \pm \ 76c\\ 221 \ \pm \ 91ab\\ 200 \ \pm \ 68a\\ 309 \ \pm \ 87c\\ 292 \ \pm \ 57c\\ 288 \ \pm \ 58c\\ 283 \ \pm \ 59bc\\ 217 \ \pm \ 59ab\\ 200 \ \pm \ 57a\\ 295 \ \pm \ 48c \end{array}$	$\begin{array}{r} 285 \pm 63ab\\ 288 \pm 80ab\\ 342 \pm 76bc\\ 325 \pm 66abc\\ 320 \pm 65abc\\ 302 \pm 67abc\\ 317 \pm 91abc\\ 362 \pm 99c\\ 320 \pm 70abc\\ 271 \pm 54a\\ 288 \pm 69ab\\ 302 \pm 53abc\\ 288 \pm 37ab\\ 294 \pm 75ab \end{array}$	$\begin{array}{r} 366 \ \pm \ 79abcd \\ 412 \ \pm \ 72cd \\ 422 \ \pm \ 104d \\ 387 \ \pm \ 81abcd \\ 385 \ \pm \ 91abcd \\ 359 \ \pm \ 77abcd \\ 389 \ \pm \ 133abcd \\ 336 \ \pm \ 71abc \\ 384 \ \pm \ 110abcd \\ 393 \ \pm \ 77bcd \\ 308 \ \pm \ 57a \\ Nd \\ Nd \\ 328 \ \pm \ 74ab \end{array}$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{c} 86 \pm 6abc \\ 85 \pm 8ab \\ 90 \pm 7c \\ 88 \pm 5abc \\ 89 \pm 5abc \\ 88 \pm 6abc \\ 89 \pm 7abc \\ 92 \pm 9c \\ 88 \pm 8abc \\ 83 \pm 5a \\ 85 \pm 7ab \\ 86 \pm 7abc \\ 86 \pm 6abc \\ 85 \pm 10ab \end{array}$	$\begin{array}{c} 96 \ \pm \ 8bc \\ 99 \ \pm \ 7bc \\ 100 \ \pm \ 9c \\ 96 \ \pm \ 7bc \\ 98 \ \pm \ 7bc \\ 98 \ \pm \ 7bc \\ 93 \ \pm \ 6abc \\ 93 \ \pm \ 6abc \\ 93 \ \pm \ 6abc \\ 95 \ \pm \ 7abc \\ 90 \ \pm \ 7abc \\ 90 \ \pm \ 8a \\ Nd \\ 91 \ \pm \ 7ab \end{array}$
15 Mean CV%	$192 \pm 43a$ 227 ± 59 25.9	$266 \pm 50bc$ 262 ± 65 24.6	$276 \pm 42ab$ 305 ± 67 22.0	$354 \pm 85abcd$ 371 ± 85 23.0	$\begin{array}{rrr} 77 \ \pm \ 6ab \\ 80.6 \ \pm \ 6.6 \\ 8.2 \end{array}$	$85 \pm 7bc$ 83.9 ± 7.9 9.4	$84 \pm 5ab \\ 86.9 \pm 6.9 \\ 7.9$	$94 \pm 9abc$ 95.4 ± 7.9 8.3

Values are mean and standard deviation of 30 bulbs.

Mean separation within columns by Tukey's multiple range test, P = 0.005. Values followed by the same letter are not significantly different. Nd, no determined; CV, coefficient of variation.

Table 2: Soluble solids content in 15 'Fuentes de Ebro' onion lines

Line	2	2006	2007		
	Plot 1	Plot 2	Plot 3	Plot 4	
1	$7.0 \pm 0.6a$	6.8 ± 0.7 abc	7.7 ± 0.8 cd	$5.8 \pm 0.7 c$	
2	$7.2 \pm 1.1a$	$6.5 \pm 0.9a$	$6.8 \pm 1.0a$	$5.1 \pm 0.8ab$	
3	$7.2 \pm 0.7a$	$6.4 \pm 0.9a$	$7.9 \pm 0.8d$	5.5 ± 0.8 abo	
4	$7.1 \pm 0.7a$	6.7 ± 0.7 abc	$7.0 \pm 0.6 bc$	5.5 ± 0.9 abo	
5	$7.0 \pm 0.8a$	$6.5 \pm 0.8 ab$	$6.5 \pm 1.3a$	$4.9 \pm 0.5a$	
6	$7.5 \pm 0.8a$	$7.3 \pm 0.9 \mathrm{bc}$	$7.7 \pm 0.8 \text{bcd}$	5.5 ± 1.0 abo	
7	$7.5 \pm 0.6a$	$7.1 \pm 0.7 abc$	7.4 ± 0.8 abcd	5.3 ± 0.5 abo	
8	$7.3 \pm 0.6a$	6.9 ± 0.9 abc	7.2 ± 0.8 abc	$5.8 \pm 0.6c$	
9	$7.0 \pm 0.9a$	$6.6 \pm 0.8 ab$	6.9 ± 0.9 ab	5.5 ± 0.4 abo	
10	$7.6 \pm 0.6a$	6.7 ± 1.0 abc	7.3 ± 0.9 abcd	$5.7 \pm 0.7 bc$	
11	$7.1 \pm 0.7a$	$6.8 \pm 0.7 \mathrm{abc}$	7.4 ± 0.9 abcd	$5.7 \pm 0.6 bc$	
12	$7.5 \pm 0.6a$	7.5 ± 1.0	7.3 ± 0.6 abcd	Nd	
13	$7.3 \pm 0.5a$	$7.3 \pm 0.9b$	7.6 ± 0.6 abcd	Nd	
14	$7.2 \pm 0.6a$	6.5 ± 0.6	$6.8 \pm 0.9a$	5.3 ± 0.5 abo	
15	$7.3 \pm 0.6a$	7.1 ± 0.4 abc	7.5 ± 0.8 abcd	5.4 ± 0.5 abo	
Mean	7.3 ± 0.68	$6.8~\pm~0.81$	7.3 ± 0.83	$5.5~\pm~0.65$	
CV %	9.3	12.3	11.1	11.8	

Values are mean and standard deviation of 30 bulbs.

Mean separation within columns by Tukey's multiple range test, P = 0.005. Values followed by the same letter are not significantly different.

CV, coefficient of variation.

Response to selection for low pungency

Lowest pungency group was obtained from the cross-pollination of five bulbs with an average pyruvic acid content of 3.0 μ mol/g FW. The five half-sib families of group 2 were also derived from mild bulbs (average pungency 4.5 μ mol/g FW), while groups 3 and 4 were progeny from five pungent bulbs (average pungency rates 6.5 and 8.8 μ mol/g FW, respectively).

Significant differences among groups were found when weight, size, and pyruvic acid and soluble solids content of bulbs were analysed (Table 4). Plants obtained from bulbs with the lowest pungency rates (group 1) formed onions with significantly lower pyruvic acid content. Progeny from groups 2, 3 and 4 formed bulbs significantly more pungent than those from unselected plants. Group 1 onions were also significantly heavier than those of families from groups 2 and 4. However, largest bulbs were obtained in the unselected population, which showed also the lowest soluble solids content. Onions derived from bulbs with the highest pungency rates (group 4) showed soluble solids contents significantly higher than that of progeny from bulbs with lower pungency levels (Table 4). Significant differences were still found among families within groups for the analysed bulb parameters, while large variation within family was observed for bulb weight (data not shown).

Corroborating this, low Pearson's correlation coefficients were obtained through the 20 half-sib families between pyruvic acid content of bulbs and their weight and size (r = -0.189, P < 0.001 and r = -0.181 P < 0.001, respectively). Correla-

Line	200	06	2007		
	Plot 1	Plot 2	Plot 3	Plot 4	
1	5.8 ± 1.4abc	5.9 ± 1.4ab	5.9 ± 2.0a	4.5 ± 1.4a	
2	5.9 ± 1.3 abcd	$5.6 \pm 1.4ab$	$6.6 \pm 2.1 ab$	4.7 ± 1.4ab	
3	6.0 ± 1.2 abcd	$6.0 \pm 1.1 ab$	$5.5 \pm 2.1a$	5.4 ± 1.4 abc	
4	6.2 ± 1.7 abcd	$6.2 \pm 1.6ab$	$6.9 \pm 2.6ab$	5.5 ± 1.6abc	
5	$5.3 \pm 1.4a$	5.7 ± 1.0 ab	$5.2 \pm 1.7a$	5.3 ± 1.6 abc	
6	6.0 ± 1.3 abcd	$6.3 \pm 1.4 ab$	$6.2 \pm 2.3 ab$	5.4 ± 1.9 abc	
7	6.2 ± 1.4 abcd	$5.8 \pm 1.3 ab$	$6.9 \pm 2.7 \mathrm{ab}$	$4.3 \pm 1.8a$	
8	5.8 ± 0.9 abc	$5.6 \pm 1.1 ab$	$6.1 \pm 1.8 ab$	$6.4 \pm 2.1c$	
9	6.2 ± 0.9 abcd	$5.7 \pm 1.1 ab$	$5.1 \pm 2.1a$	$6.3 \pm 1.7 bc$	
10	6.6 ± 1.5 bcd	$5.9 \pm 0.8ab$	$8.1 \pm 3.0b$	5.6 ± 1.6 abc	
11	$5.6 \pm 1.0 ab$	$5.3 \pm 1.2a$	$6.5 \pm 2.0 ab$	5.5 ± 1.9abc	
12	$7.1 \pm 1.2d$	$6.1 \pm 1.1 ab$	$4.6 \pm 1.2a$	Nd	
13	6.9 ± 1.2 cd	$6.7 \pm 1.4b$	$6.7 \pm 1.2 ab$	Nd	
14	6.0 ± 1.3 abcd	$5.3 \pm 1.3a$	5.4 ± 1.7a	5.2 ± 1.7 abc	
15	5.8 ± 1.2 abc	5.8 ± 1.6ab	$5.0 \pm 1.8a$	5.5 ± 2.0 abc	
Mean	6.1 ± 1.2	5.9 ± 1.3	6.0 ± 2.0	5.4 ± 1.7	
CV %	20.3	21.2	33.5	31.5	

Table 3: Pyruvic acid content (μmol/g FW) in 15 'Fuentes de Ebro' onion lines

Values are mean and standard deviation of 30 bulbs.

Mean separation within columns by Tukey's multiple range test, P = 0.005. Values followed by the same letter are not significantly different.

CV, coefficient of variation.

Group	Weight (g)	Size (mm)	Soluble solids content (B)	Pyruvic acid (µmol/g FW)
1 2 3 4 Unselected	$511 \pm 198b 437 \pm 183c 483 \pm 188bc 422 \pm 172c 578 \pm 135a$	$\begin{array}{r} 101 \ \pm \ 17b \\ 97 \ \pm \ 17bc \\ 101 \ \pm \ 16b \\ 93 \ \pm \ 18c \\ 109 \ \pm \ 13a \end{array}$	$7.7 \pm 1.2b 7.8 \pm 1.2b 7.9 \pm 1.4b 8.4 \pm 1.5a 7.3 \pm 1.1c$	$\begin{array}{c} 2.6 \ \pm \ 1.6c \\ 5.2 \ \pm \ 3.0a \\ 5.8 \ \pm \ 3.4a \\ 5.8 \ \pm \ 3.2a \\ 3.9 \ \pm \ 1.7b \end{array}$

Table 4: Bulb traits analysed in progeny derived from the openpollination of onion plants grouped according to their pungency

Values for groups 1–4 are mean and standard deviation of 200 bulbs from five families (40 bulbs/family), while data for the unselected population are mean and standard deviation from 40 bulbs.

Mean separation within columns by Tukey's multiple range test, P = 0.005. Values followed by the same letter are not significantly different.

tion between pyruvic acid and soluble solids content of bulbs was also low but significant, r = 0.272 (P < 0.001). These results confirmed the trend found in the original 'Fuentes de Ebro' lines, where a low pungency rate was associated with larger bulb size and lower soluble solids content. However, no significant correlation was found between bulb soluble solids content and weight, while a very slight but significant correlation was observed between bulb soluble solids content and size (r = 0.08, P = 0.01).

Unselected progeny also cultivated in the Montañana 2008 experiment produced onions with average pyruvic acid content of 3.9 μ mol/g FW. Therefore the response to selection of group 1 was -1.3μ mol/g FW. Since the selection differential in 2006 bulbs was $S = -2.0 \mu$ mol/g FW, heritability in broad sense for low pungency in the 'Fuentes de Ebro' cultivar can be estimated as H = 0.67.

Discussion

Organosulfur compounds dominate the organoleptic experience in fresh consumed onion by producing the pungency and heat sensations (Randle 1997). According to this, onion pungency, estimated as pyruvic acid content, is the main criterion used for sweet onion certification, mild or sweet onions containing $< 5.5 \mu mol/g$ FW. The great variation found in our experiments for 15 'Fuentes de Ebro' lines indicated that this plant material frequently produces bulbs of moderate pungency, since only three lines in 2006 and 16 lines in 2007 showed mean pyruvic acid contents that satisfied the NOL criterion. However, we found pungency rates lower than those reported for other mild onions as 'Granex 33', 'Walla Walla Sweet' and 'Sweet Sandwich' (Kopsell and Randle 1997), although higher than those of 'Sweet Vidalia' and 'Pegasus' (Randle et al. 1998). Promising plant material could be found in grower's selections 5 and 14, since these two lines produced mild onions in three out of four plots.

Environmental influence on onion pungency has been reported by several authors (Randle 1997, Hamilton et al. 1998, McCallum et al. 2005, Yoo et al. 2006) which referred different relative relevance as compared with genetic factors. In our experimental conditions, mean temperature trends during the 2006 and 2007 crop seasons very similar, and also precipitation levels did not differ substantially (123 and 137 mm, respectively). We found pungency rates that did not depend on year, while significant differences were found for the other bulb traits depending on season. When averaging among the 15 lines, variation found in bulb traits derived mainly from the plot 4 results, where we obtained larger onion bulbs with lower soluble solids and pyruvic acid contents. These results could be explained by a slightly lower clay content (11% front to a 31% on average) that would allow higher bulb size. Our estimates of realized heritability for low pungency in the 'Fuentes de Ebro' onion, although they might be inflated as were derived from a reduced

number of half-sib families, are similar to the higher values referred for other onion populations. While Simon (1995) and Li et al. (1995) estimated the broad sense heritability of pungency ranging from 0.48 to 0.7, Wall et al. (1996) reported realized heritability values ranging from -0.10 to 0.51, and narrow-sense heritability estimates that varied from 0.25 to 0.53 in three breeding populations. Selection in the 'Fuentes de Ebro' onion significantly reduced bulb pungency and increased uniformity, since 93% of onions from group 1 progeny showed pyruvic acid contents lower than 5.5 µmol/g FW. Furthermore, 78% of onions from this group could be described as 'extra sweet', as they showed pyruvic acid contents lower than 3.5 µmol/g FW. The mean low pungency levels obtained in the 2008 experiment were probably consequence of favouring crop conditions (especially drip irrigation), but indicated that it is possible to obtain a longday sweet onion variety adapted to temperate areas after few selection cycles in the 'Fuentes de Ebro' plant material.

Our results suggest that selecting for low pungency would indirectly increase onion size and reduce soluble solid contents, as has been already described for other onion varieties (McCollum 1968, Sinclair et al. 1995, Debaene et al. 1999, Galmarini et al. 2001). These two effects are undesirable, since firstly in Spain there is a consumer preference for medium-size fresh onions and secondly low soluble solids content could limit bulb storage. Therefore, these two traits should be considered when breeding the 'Fuentes de Ebro' onion for low pungency.

Among the 15 lines initially screened, highest mean CV was found for pungency (26.6%), followed by weight (23.9%) and soluble solids content (11.1%). These coefficients of variation are similar to values previously reported for open-pollinated onion varieties (Yoo et al. 2006). The 'Fuentes de Ebro' cultivar showed therefore lower variability for soluble solids content than for pungency or size. This information is highly relevant for breeding purposes, in order to obtain a sweet onion with appropriate size and uniformity, and good storage characteristics. Low coefficients of variation are needed in developing uniform varieties, while the limited genetic variability for soluble solids content found in the 'Fuentes de Ebro' plant material could condition obtaining a variety with good storage ability. With these premises, breeding populations have been developed and are under evaluation for these bulb traits, as well as for firmness and sugar content.

Acknowledgements

This work forms part of the RTA2007-00080 project funded by the National Institute for Agriculture and Food Research and Technology (INIA). Authors thanks M. Gutiérrez and A. Llamazares from the Centro de Transferencia Agroalimentaria and the ACEFUENTES association for their helpful cooperation.

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