Changes in the pyruvic acid content correlates with phenotype traits in onion clones

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Abstract

Consumer demand is oriented toward onion cultivars with lower pungency, and the pyruvic acid content of onions is highly correlated with their pungency. Therefore, breeding programmes might be based on the pyruvic acid content of the parental bulbs. The present study was performed using 97 onion clones with the aim of correlating the pyruvic acid levels with phenotype traits in the different genotypes used in breeding programmes. The onion bulbs were provided from the breeding company with blind labels, and four bulbs were individually analysed for each clone. A comparison between the spectrophotometric and HPLC determination of pyruvic acid was also carried out and showed an excellent linear regression with an $R^2 = 0.951$. The pyruvic acid was studied in correlation with tunic colours, pungency and precocity, and the highest correlation coefficient was found for red skinned onions. The lowest pyruvate content was found in white onions, with 6.5 μmol g^{-1} FW, whereas higher values (8.4-8.5 μmol g^{-1} FW) were observed in yellow and red onions. The chemical analyses were compared with panel taste assessments. The results confirmed that the pungency and pyruvic acid content were positively and significantly correlated (P<0.0001),even when the coefficient resulted in a relatively low value ($r = 0.515$). The precocity trait was associated with pyruvate content: precocious onions, in particular, showed lower pungency.

Keywords: breeding, pungency, stringency.
Abbreviations: 2,4-dinitrophenylhydrazine (DNPH); sodium hydroxide (NaOH).

Introduction

The consumer perception of onion pungency depends on the presence of organosulphur compounds that are synthesised from enzymatic reactions involving S-alk (en) yl-L-cysteine S-oxide decomposition (Lancaster et al., 2000). This enzymatic decomposition leads to the formation of flavour precursors, such as 1-propenyl cysteine sulphoxide, methyl cysteine sulphoxide and propyl cysteine sulphoxide, and these compounds are well compartmentalised in onion cells. However, when onion bulbs are sliced open, the cells are disrupted, and these organosulphur compounds are released. These compounds then come into contact with the alliinase enzyme, which is normally isolated in the vacuole (Schwimmer and Mazelis, 1963; Lancaster and Collin, 1981), and the enzyme activity is almost instantaneous when the cell membranes are broken. The compounds produced by alliinase are pyruvate, ammonia and sulphenic acid, and the pungency of onions is absolutely correlated with the pyruvate production (Schwimmer and Weston, 1961). Because pyruvic acid content reacts with 2,4-dinitrophenylhydrazine (DNPH) in the presence of sodium hydroxide (NaOH), the pyruvic acid content can be quantified by measuring the colour development spectrophotometrically at 420 or 515 nm.

Recently, consumer demand has been oriented toward onion cultivars with low pungency (mild cultivars), and growers and breeders must address these new market demands. The accumulation of organosulphur compounds in onions depends upon many factors, especially sulphur-based fertilisation, the environment, and the genotype of the cultivars (Yoo et al., 2006; Chope et al., 2007). However, sulphur-based fertilisation and onion pungency are not always positively correlated, and contrary results have been reported (Randle 1992; Randle and Bussard, 1993). Although the pyruvic acid content is influenced by environmental growing conditions, clones and storage (Yoo et al., 2006), the genetic background seems to be the most important factor because different cultivars have different abilities to control sulphur uptake and assimilation in the biosynthesis pathway that results in the flavour (Randle, 1992; Randle et al., 1994). The aims of this work were the following: i) to compare the pyruvate content in bulbs with the pungency values assigned by the breeder following a taste evaluation by a panel and ii) to describe the variability of a wide onion clone sample set in relation to the bulb tunic colours and the precocity of the bulb.
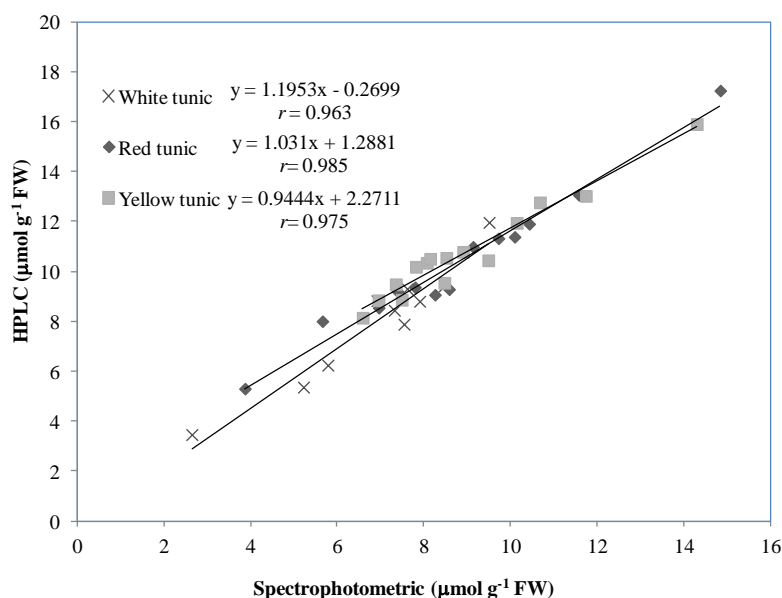
Results

Pyruvic acid and tunic colour

The pyruvic acid content was determined using both a spectrophotometric method and HPLC. In a preliminary approach, we explored the variability of the pyruvate content in the extracts of a sub-set of clones in relation to both the contribution of the native pyruvate content of cells, as

Table 1. Correlation coefficients and linear regression between bulb pyruvate content and onion traits.

Onion colour	n	Pyruvate vs. pungency	Pyruvate vs. precocity	Pyruvate vs. harvest date
		r	R^2	R^2
White	26	0.692***	0.092 n.s.	0.144 n.s.
Yellow	41	0.471**	0.138*	0.279*
Red	30	0.329 n.s.	0.382***	0.311**

Asterisks indicate significant differences, ***P<0.001, **P<0.01, *P<0.05

Fig 1. Correlation analyses between spectrophotometric and HPLC determinations of the pyruvic acid level in onion bulbs differentiated into three groups of tunic colour.

determined using heated samples, and the individual bulb characteristics. The native bulb pyruvate content, as determined in bulbs heated to inhibit enzymes, was very low, at approximately 10% of that produced after slicing. Therefore, the native pyruvate can be negligible (data not shown). The colorimetric results and HPLC measurements were found to be correlated, with a correlation coefficient of 0.976 (r). The correlation coefficients were also calculated for each group of onion clones with different tunic colours, and the correlation coefficient value was found to increase with the intensity of the tunic colour. The white bulbs showed a correlation coefficient of 0.963, whereas the yellow and red bulbs had r values of 0.975 and 0.985, respectively (Fig. 1). The average pyruvate concentration for each colour group was lower in the white tunic bulbs, at 6.93 μmol g^{-1} FW; the yellow and red tunic bulbs had higher values that, on average, were 8.9 and 8.8 μmol g^{-1} FW, respectively (Fig. 2). The analyses of frequency distribution in the different pyruvate/pungency classes considering the tunic colour showed that the white cultivars had the lowest pyruvate content. The highest number of white onion clones was found in the range of 6-8 μmol g^{-1} FW, and, in the yellow onion clones, the highest distribution was found within the 8-10 μmol g^{-1} FW range, similar to in the red onions (Fig. 3). Therefore, because pyruvate is related to pungency, the white clones were milder than the yellow and red clones. The white onions had a pyruvate content that ranged from 4.3 to 7.8 μmol g^{-1} FW, the yellow cultivars covered a range from 4.9 to 11.7 μmol g^{-1} FW, and the red tunic bulbs had a pyruvate content that ranged from 4.6 to 10.9 μmol g^{-1} FW.

Pyruvate content and precocity classes

The precocity of the clones significantly affected the bulb pyruvate content in the yellow and red clones (Fig. 4). In particular, the precocious onions were, on average, milder compared with the other clones, yet, the precocity did not influence the pyruvate content in the white onions (Fig. 4). In the yellow onions, those of precocity class 1 were significantly milder than the other two. The red clones showed an increase of pyruvate content, with higher pungency classes (Fig. 4). The frequency analyses showed that the class 1 onions had a low pyruvate content, ranging from 4.3 to 10.3 μmol g^{-1} FW, and the highest frequency of precocity class 1 onions was found in the range 6-8 μmol g^{-1} FW. Onion clones belonging to precocity classes 2 and 3 showed a similar range of pyruvate contents: from 5.8 to 11.75 and from 5.8 to 11.64 μmol g^{-1} FW, respectively, for classes 2 and 3. However, the highest distribution was found within the 8-10 μmol g^{-1} FW range for precocity class 2 onions and 6-8 μmol g^{-1} FW for those in class 3. The linear regression between the bulb pyruvate level and the harvest date for the three onion colours showed an increase of R^2 values from white to yellow to red (Table 1). The correlation analysis between the bulb pyruvate content and degree of pungency was significant (P<0.0001) even when r was not high, such as 0.515 (Fig. 5). The linear regression coefficient calculated for the different tunic colours showed higher values for the lower colours. In fact, the white onions had the highest R^2 value (0.47), whereas the lowest was found for the red onions (Table 1).

Discussion

Breeding programmes seek mild onion cultivars to satisfy new consumer demands. A pungency evaluation by a panel evaluation is laborious and may not give reproducible results; furthermore, the results depend on the training and abilities of the panel. Therefore, the analytical parameter of the bulb pyruvate content has been identified and has proven to be a good parameter for pungency evaluations (Randle, 1992; Randle et al., 1994). Our results confirmed the relationship between the pyruvic acid and pungency even when the correlation analysis was significant but not high. The linear correlation coefficients found for the white onions may lead to the hypothesis that bulb pigments may affect the taste assessment of the panel. Moreover, a low linear regression coefficient value may be due to the limited pungency classes (only three classes) used by the trained panel. The pyruvate concentration in onion bulbs ranges from 1.6 $\mu mol\ g^{-1}$ FW in mild to 13 $\mu mol\ g^{-1}$ FW in pungent cultivars (Randle, 1992). Considering this range, the clones used in our work may be considered as having a medium pungency for the white bulbs and a moderately high pungency for the yellow and red bulbs. The bulb pigments also appear to be correlated with the pyruvate content: darker pigments are associated with higher pungency. These results agree with previous experiments performed on white and red onion bulbs (Rodrigues et al., 2003), even though white bulbs can have a very strong pungency.The precocity trait of onions appeared to be negatively linked with the pyruvate content. It has been reported that the pyruvic acid content increases with the maturity of the bulb (Platenius and Knott, 1935), although contrary results were found by Hamilton et al. (1998) who measured pyruvate at a very immature stage. This result might be explained by the higher dry matter content of the bulb in early bulb developmental stages. In our study, the pyruvate content of the bulbs was affected by a shorter biological cycle of the onions (precocity). In conclusion, the pyruvic acid content in the bulb can be used as an objective parameter for screening purposes in breeding programmes. The pyruvic acid level in bulbs is affected by both the tunic colour and precocity. This study showed that the early clones were less pungent and had accumulated less pyruvate in their bulbs.

Materials and methods

Description of the sample set

The onion (*Allium cepa* L.) bulbs were grown under field conditions (Supplementary data 1) in experimental plots at the ISI seed company (Fidenza, Parma, Italy) during the spring and summer of 2005. The soil characteristics (a soil depth of 0-30 cm was measured in March before the beginning of the experiments according to MIPAF methods, Angeli, 2000) were as follows: pH 7.8; 27.9 g kg^{-1} $CaCO_3$; 231 g kg^{-1} sand; 397 g kg^{-1} silt; 372 g kg^{-1} clay; clay-loam soil texture; 14.7 g kg^{-1} organic matter; 1.25 g kg^{-1} total N; 7.79 mg kg^{-1} P; and 73.2 mg kg^{-1} K. Each plot contained 220 bulbs (2 plots with a total of 440 bulbs). After harvesting and drying on the field, the bulbs were stored under dry conditions at room temperature until September when they were delivered to the laboratory for analysis at the plant production department of the University of Milan. The sample set consisted of 26 white, 41 yellow and 30 red or
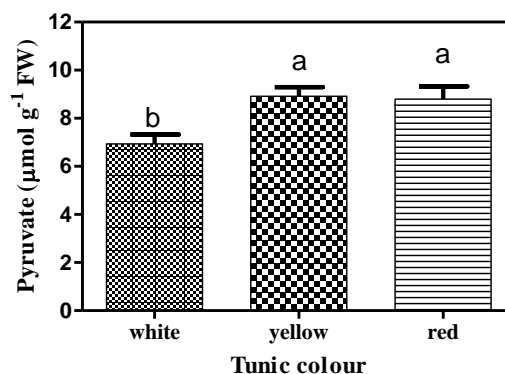

Fig 2. Pyruvate content in onion bulbs with different tunic colours. The values are the means with standard errors (n=4). The data were subjected to one-way analyses of variance (ANOVA). Different letters indicate statistically significant differences at P<0.05.
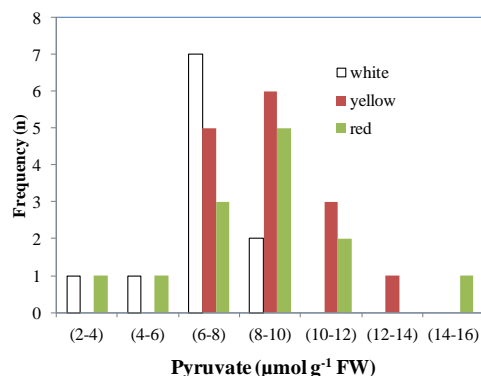

Fig 3. Number of bulb clones distributed in different pyruvate classes based on the tunic colour.
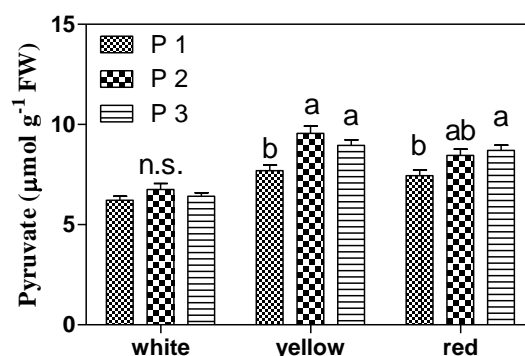

Fig 4. Pyruvate content in onion bulbs with different tunic colours in relation to the different precocity classes. The values are the means with standard errors (n=4). The data were subjected to two-way analyses of variance (ANOVA). Different letters indicate statistically significant differences at P<0.05.
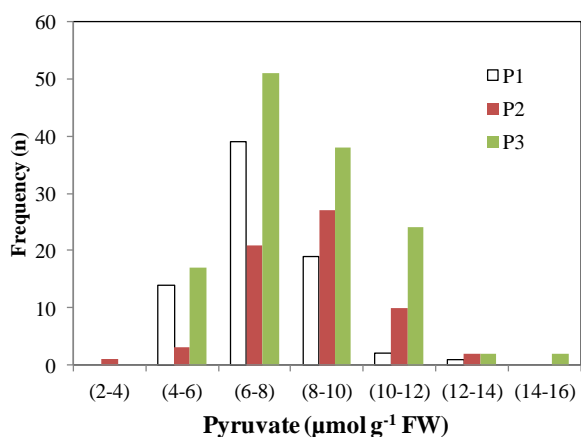
Fig 5. Distribution of onion clones in different pyruvate classes, as based on the precocity of the clones.

brown open-pollinated populations referred to as phenotypes (Table 1). These phenotypes were classified into the three precocity classes based on the criterion that a bulb reaches maturity when 80% of the plants in the plot showed senesced top leaves: 38 in class 1 (bulb maturity before 15 July); 21 in class 2 (bulb maturity between 16 and 23 July); and 36 in class 3 (bulb maturity after 23 July).

Extraction of pyruvate

Samples consisting of one-half of a bulb were homogenised by an Ultraturrax® homogeniser, filtered through two layers of cheesecloth and centrifuged for three minutes at 12000 rpm. The clear extracts were then used for the pyruvate assay. To evaluate the incidence of the native pyruvate content on the total pyruvate content recovered after homogenisation, analyses of heated half-bulbs were also performed on a sub-set of the samples. The analyses were performed on four bulbs per genotype, and the pyruvic acid assay was performed twice for each extract.

Spectrophotometric and HPLC pyruvic acid determination

The spectrophotometric assay was performed using 25 μL of clear extract in a test tube with 1 mL of water and 1 mL of 250 mg L^{-1} dinitrophenylhydrazine (1 M HCl solution DNPH, Sigma, Italy). The samples were placed in a water bath at 37 °C for 10 minutes to allow the reaction to proceed. After incubation, 1 mL of 1.5 M NaOH was added. The colour developed was measured using a spectrophotometer at 515 nm, as described by Anthon and Barrett (2003). The standard curve was constructed using 0, 2, 4, 6 and 8 mM sodium pyruvate (Sigma, Italy). The chromatographic reverse-phase method adopted was a slightly modified version of the method proposed by Evans et al. (1983) and modified by Perez et al. (1997). A Shimadzu LC-10 AD-VP HPLC pump with a Shimadzu SPD 10Avp UV-VIS detector was used. Integration and data storage were performed with Shimadzu EZ START 7.3 software. The pyruvic acid was eluted isocratically using a 250 x 4.6 mm Purosphere C_{18} column (Merck, Germany). The mobile phase (0.01 N H_2SO_4) was filtered through a 0.45 μm Millipore membrane-filter (HAWP Millipore Co., Bedford) and degassed in an

ultrasonic bath; the mobile phase was used at a flow rate of 0.8 ml min^{-1}. The analyses were performed on 0.45 μm filtered homogenate, and the organic acids were detected at 210 nm. The standard curve was generated using the same standard concentrations used in the spectrophotometric method.

Pungency evaluation

The phenotypes were divided into three classes of increasing pungency (mild = 1, pungent = 2 and very pungent = 3) based on an organoleptic evaluation made by the breeder immediately after harvest. Three people were trained for a test panel evaluation following the procedure reported by Wall and Corgan (1992). The data in fig. 2 and 4 were subjected to ANOVA, and the differences among the means were determined using Bonferroni's post-test (P<0.05).

Statistical analysis

The data reported in fig. 3 are the means with standard errors (*n*=4). The data were subjected to a one-way analysis of variance, and the differences among the treatments were analysed by a Bonferroni post-test (P<0.05). The correlation and linear regression analyses were performed using Excel statistical tools (Microsoft software).

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