Food Chemistry 115 (2009) 1108–1113

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Use of canonical variate analysis to differentiate onion cultivars by mineral content as measured by ICP-AES

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ARTICLE INFO

Article history:
Received 4 June 2008
Received in revised form 4 December 2008
Accepted 29 December 2008

Keywords:
Allium cepa
Calcium
Multivariate analysis
Sulphur

ABSTRACT

Three onion cultivars *viz.* Renate, Ailsa Craig and SS1 were characterised according to their mineral content. The concentrations of the macronutrients phosphorus, potassium, calcium, manganese and sulphur and the micronutrients iron, boron, manganese, copper and zinc were analysed in freshly harvested and stored onion bulbs using ICP-AES (Inductively coupled plasma-atomic emission spectroscopy). Onions were treated pre-harvest with additional sulphur (100 kg ha^{-1}) and/or calcium (300 kg ha^{-1}) applied in four combinations at the time of seed drilling, however these treatments did not affect the total concentrations of sulphur or calcium in the harvested bulbs. The data were subjected to canonical variate analysis in order to determine the most appropriate variate to discriminate between cultivars. Two canonical variates were sufficient to differentiate between the three cultivars, with the first canonical variate describing differences in micronutrients between the genotypes and the second separating the cultivars by differences in sulphur concentration.

1. Introduction

The elemental composition of the edible portion of some *Allium* species has been used as a method for defining geographic origin (Ariyama, Horita, & Yasui, 2004; Ariyama & Yasui, 2006; Ariyama et al., 2007). In addition, analysis of 63 major and trace elements was shown to allow differentiation between conventionally and organically grown onions cv. Hysam from Denmark (Gunderson, Bechmann, Behrens, & Strup, 2000). However, apart from these works and others (Alvarez, Marcó, Arroyo, Greaves, & Rivas, 2003; Bibak, Behrens, Stürup, Knudsen, & Gundersen, 1998; Rodríguez Galdón, Oropeza González, Rodríguez Rodríguez, & Díaz Romero, 2008) there has been little research concerning the elemental composition of bulb onions. Most work concerning manipulation of mineral nutrition of onions has concentrated on the effect on quality parameters, particularly the effects on pungency (enzymatically produced pyruvate after maceration), flavour precursors and firmness. Only a few studies have performed analysis to determine whether the changes in mineral nutrition resulted in an effect on total mineral concentrations in the bulb. Field, hydroponic and tissue culture trials worldwide have shown that attempting to manipulate the sulphur content of onions by varying the sulphur supply during growth has had mixed results and is highly dependent on a range of factors. These factors include cultivar, the extent of variation of sulphur supply, and other seasonal and environmental influences including climactic conditions and soil type (Coolong, Kopsell, Kopsell, & Randle, 2004; Hamilton, Yoo, & Pike, 1998; Kopsell, Randle, & Eiteman, 1999; Lancaster, Farrant, & Shaw, 2001; O'Donoghue et al., 2004; Randle, 1992; Randle, Bussard, & Warnock, 1993). In general, increasing the sulphur supply increases firmness, pungency, alk(en)yl cysteine sulphoxides (ACSOs) and dry matter when the treatment is applied constantly (e.g. in a hydroponic system), or when applied as a field treatment towards the end of the growing season when bulbing has been initiated. The effect of the addition of calcium chloride to the crop has also been investigated in the USA (Coolong & Randle, 2008). Additional calcium nutrition resulted in increased firmness of bulbs at harvest, although this effect did not persist throughout storage. Reduced pyruvate concentration was observed in $CaCl_2$-treated onions, but this effect only occurred in one out of two growing seasons. Work carried out on UK-grown onions was unable to replicate these results (Smith & Crowther, personal communication, 2005).

Given previous findings, the aim of the research presented herein was to determine the effects of field application of additional calcium and/or sulphur treatments on the mineral content of onion bulbs grown from seed, and to describe genotypic differences in the mineral content of onion bulbs of short, intermediate and long-storing onion cultivars *viz.* SS1, Ailsa Craig and Renate respectively using canonical variate analysis.

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doi:10.1016/j.foodchem.2008.12.090

2. Experimental

2.1. Plant material and storage regime

Short, intermediate and long-storing onion (*Allium cepa* L.) cvs.; SS1, Ailsa Craig and Renate, respectively, were spring-drilled on 18 March 2003 in four rows per bed using a tape seeder at a rate of 35 seeds m^{-2} and grown in a sandy loam soil field at FB Parrish & Son (Beds., UK) using standard agronomic practices. Two treatments of additional sulphur and/or calcium at rates of 100 kg ha^{-1} of sulphur and 300 kg ha^{-1} of calcium were applied in four combinations including a negative control (plus Ca minus S, plus Ca and S, minus Ca plus S and minus Ca and S). Sulphur, in the form of agricultural gypsum, was applied uniformly over the plot area at the time of drilling. Calcium was applied evenly by hand in the form of 77% calcium chloride flakes (Kemira, Cheshire, UK). Onion bulbs were harvested (cv. SS1 on 19 August 2003; cvs. Renate and Ailsa Craig on 2 September 2003) into standard 25 kg plastic nets and dried in bin driers with ambient air for five weeks (cv. SS1) and three weeks (cvs. Ailsa Craig and Renate) as per standard practice in the UK. The dry aerial parts and roots were removed, and any diseased or damaged bulbs discarded prior to storage. Bulbs were held under industry standard controlled atmosphere (CA) conditions (3 kPa CO_2 and 5 kPa O_2; Smittle, 1988) using an Oxystat 2 CA system, attached to an Oxystat 2002 Controller, and Type 770 fruit store analyser (David Bishop Instruments, Sussex, UK). This system was self-calibrating every 24 h against 5% CO_2 in N_2 (British Oxygen Co., Surrey, UK) as previously described by Chope, Terry, and White (2007). Bulbs were stored at 2 ± 1 °C inside two rigid polypropylene fumigation chambers (88 × 59 × 59 cm). Relative humidity was not measured.

2.2. Experimental design

The experiment was conducted as a completely randomised design with the assumption that the storage containers were identical and the samples were taken randomly. Bulbs were divided equally between the two storage containers. Bulbs were removed from storage at regular intervals. Samples of cv. Renate were taken after days 0, 40 and 230, cv. Ailsa Craig after days 0, 81 and 129, and cv. SS1 after days 0, 40 and 81. At each sampling date (except time 0) for each cultivar, three bulbs from each treatment were sampled from each storage chamber. At sampling time 0, four sets of six bulbs were sampled. Samples from each set of three bulbs from each sampling time and treatment were combined and lyophilised prior to mineral analysis (except at time 0 where sets of 6 bulbs were combined); therefore, values represent the mean of the replicates for each sampling date and cultivar.

2.3. Sample preparation

The dry outer skins were removed, and then a vertical wedge of tissue was taken from the basal section of the bulb. Each sample was weighed and immediately snap-frozen in liquid nitrogen, and stored at −40 °C until it was lyophilised. Dry weight measurements were made on lyophilised samples.

2.4. Mineral analysis

Lyophilised bulb tissue ($n = 60$) was ashed and then digested with 1 ml concentrated nitric acid (ADAS, 1985). The intensity of ion response was measured by ICP-AES (Inductively coupled plasma-atomic emission spectroscopy; Ultima 2, Jobin Yvon, London, UK). Results were expressed as total ion concentrations of boron, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, sulphur and zinc by comparison with external standards.

2.5. Statistical analysis

The effect of the application of additional calcium and sulphur to onion plants in the field on the concentration of each mineral analysed was assessed using analysis of variance (ANOVA). The calcium and sulphur treatments did not affect total bulb sulphur or calcium content at a probability level of 0.05. Therefore, the data sets for all calcium and sulphur treatments were pooled. A main effect of cultivar was identified by univariate ANOVA. The groups were further defined using canonical variate analysis (CVA) (Gardner, Gower, & Le Roux, 2006), which bases the analysis on grouping the data so that the variance between groups (cultivars) is maximised and the variance within groups is minimised. The circular 95% confidence limits represent the confidence interval for the population rather than the population mean and have radii of 2.448. The basic radius value is the square root of the chi-square distribution value at the 5% significance level, for a distribution with 2 degrees of freedom (as displaying the CVA in 2 dimensions). All statistical analyses were carried out using Genstat for Windows Version 7.1.0.198 (VSN International Ltd., Herts., UK).

3. Results and discussion

The variation in mineral content between cultivars was greater than the variation caused by storage time (Fig. 1). Bulbs of each cultivar were stored for different lengths of time due to differences in storage life; however, the sampling points represent the beginning, middle and end of the storage period for each.

To enable comparison with other published data on the elemental composition of onions, the data was calculated on a fresh weight basis using the mean dry weights of each cultivar (Table 1).

Free mineral content should not be expected to change on a dry weight basis during storage yet mineral content will change as a proportion of fresh weight due to weight loss. Therefore, the different concentrations recorded at various storage durations are more likely to represent the natural variation within the population rather than real changes in mineral concentration. Onion cv. Ailsa Craig bulbs showed the greatest variability over time (Fig. 1), and this may be attributed to the fact that it is a not a commercial cultivar and maintenance of the breeding line is poor (Smith, personal communication, 2004). Previously published data (Hansen, Wyse, & Sorensen, 1979; Holland, Unwin, & Buss, 1991; US Department of Agriculture, 2004) is comparable with that recorded in this investigation (Table 1); however, onion cv. Renate bulbs contained approximately 2-fold the concentration of calcium, copper, iron, potassium and sodium than the values cited by the literature (US Department of Agriculture, 2004) for unspecified cultivars. The data are, however, within the same range as that reported for calcium, copper, iron, manganese, potassium and zinc in cvs. 438 Granex and Yellow Granex, using a technique based on total reflection X-ray fluorescence and ultrasound-based extraction procedure (Alvarez et al., 2003), and for those reported for boron, copper, manganese, potassium and sodium in onion cv. Hysam measured using high resolution ICP-MS (Bibak et al., 1998). In addition, the data reported here are similar to those reported for copper, iron, manganese and zinc in the fresh leaves of other *Allium* L. species (*viz. A. schoenoprasum* L., *A. nutans* L., *A. angulosum* L., *A. fistulosum* L., *A. montanum* Schmidt, *A. odorum* L. and *A. flavescens* Bess.) measured using ICP-MS (Golubev, Golubkina, & Gorbunov, 2003). The reason for differences occurring between reported values for mineral concentrations in onion is likely to be due to a combination of factors including the preharvest growing conditions, postharvest treatment, analytical methods, and, as has been demonstrated here, genotype.

Onion cv. SS1 bulbs had a greater concentration of total sulphur per gram dry weight than cv. Ailsa Craig and Renate (Fig. 1). This is

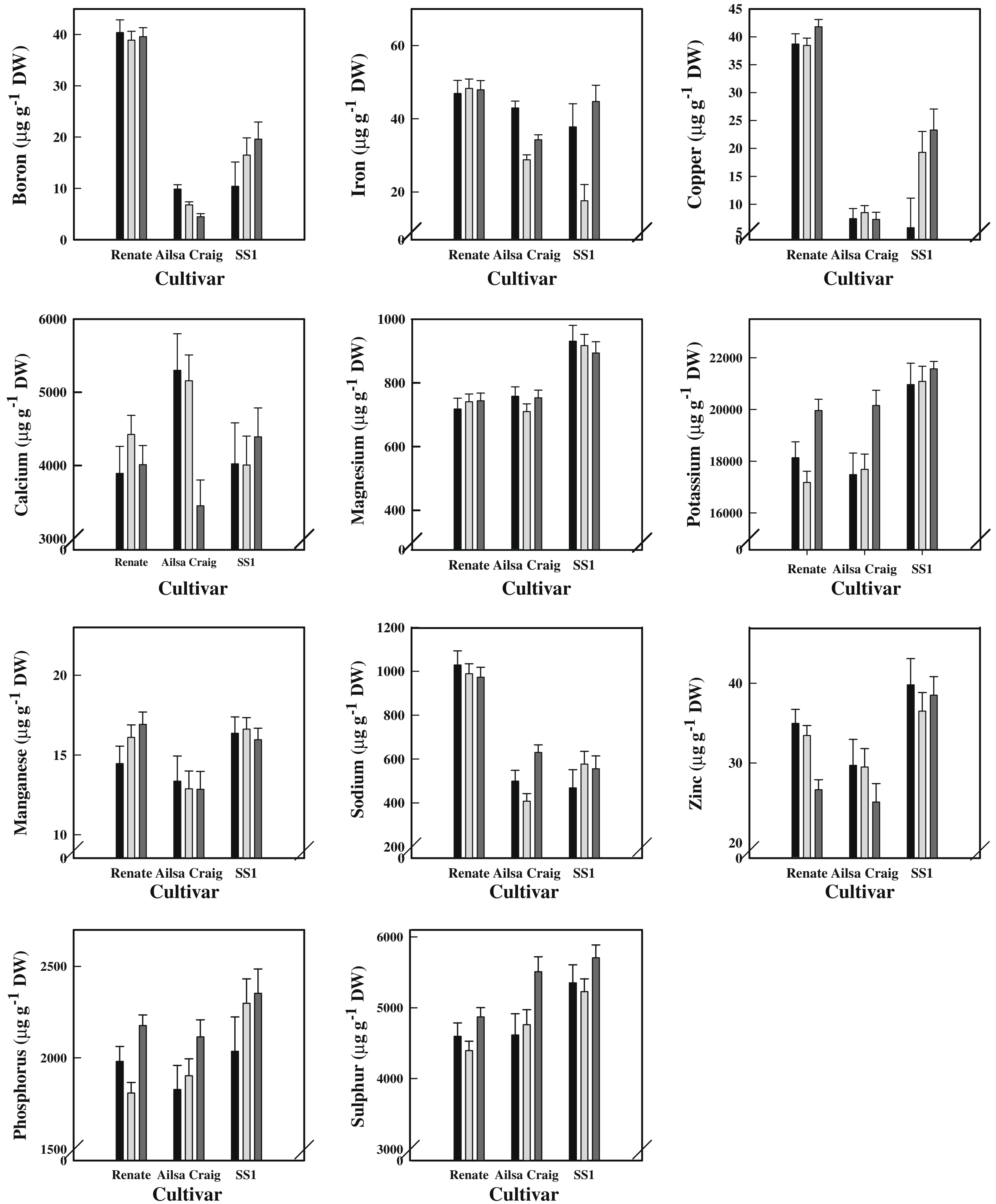

Fig. 1. Elemental content of onion cvs. Renate, Ailsa Craig and SS1 bulbs after postharvest curing (day 0, black bars, $n = 4$), and at early (light grey bars, $n = 8$) and late (dark grey bars, $n = 8$) time points during controlled atmosphere storage (cv. Renate: days 40 and 230, cv. Ailsa Craig: days 81 and 129, and cv. SS1: days 40 and 81). Standard error bars are shown.

Table 1
The mean elemental composition of onion cvs. Renate, Ailsa Craig and SS1 bulbs ($n = 20$) compared with data for raw and sweet onions from the USDA database (US Department of Agriculture, 2004) compared to the RDA and RNI values. Values are presented as mg 100 g^{-1} unless otherwise stated).

Element	Renate	Ailsa Craig	SS1	Raw onion[a]	Sweet onion[a]	Raw onion[b]	Raw onion[c]	RDA[d] (mg)	RNI[e] (mg)
B	0.58	0.07	0.12	NS	NS	NS	NS	NS	NS
Ca	60.34	45.34	29.22	22.00	20.00	25.00	27.06	1000–1200	700
Cu	0.58	0.08	0.13	0.04	0.06	0.05	NS	0.9	1.2
Fe	0.70	0.34	0.31	0.19	0.26	0.30	0.53	5.0–18.0	11.4
K	270.85	187.64	149.24	144.00	119.00	160.00	157.06	1600–3500	3500
Mg	10.81	7.42	6.39	10.00	9.00	4.00	NS	310–420	300
Mn	0.23	0.13	0.11	0.13	0.08	0.10	NS	1.8–2.3	>1.4
Na	14.57	5.19	3.84	3.00	8.00	3.00	NS	500–2400	1600
P	29.25	19.86	15.92	27.00	27.00	30.00	35.88	700	550
S	67.97	50.66	38.21	NS	NS	51.00	NS	NS	NS
Zn	0.45	0.28	0.27	0.16	0.13	0.20	NS	8.0–11.0	9.5

NS – not specified.
[a] Data from USDA database (US Department of Agriculture, 2004) cvs. not specified.
[b] Data from Holland et al. (1991) cv. not specified.
[c] Hansen et al. (1979) cv. not specified.
[d] RDA = Recommended daily allowance (US) (White & Broadley, 2005).
[e] RNI = Reference nutrient intake (UK) (White & Broadley, 2005).

perhaps surprising as cv. SS1 was the least pungent of the three cultivars (Chope, Terry, & White, 2006), and pungency is determined by the availability of sulphur-containing flavour precursors (ACSOs). Pungency is calculated on a fresh weight basis to reflect the edible product. However, all mineral analyses were performed on lyophilised tissue, and when the concentrations were calculated on a fresh weight basis (using the mean dry weight percentage), free sulphur concentration was least in cv. SS1 bulbs (Table 1). In addition, it has been shown that there are genotypic differences in the partitioning of sulphur accumulated by the onion plant. Randle, Kopsell, Kopsell, and Snyder (1999), showed that total bulb sulphur accumulation was poorly correlated with pungency in three onion cultivars. They concluded that a key contributing factor in determination of the pungency of an onion depends upon the capability of the plant to partition sulphur as sulphate in the vacuole, thus reducing the amount of sulphur incorporated into the ACSO biosynthetic pathway.

All elements measured (except for calcium) were present in significantly different ($P < 0.005$) concentrations in onion cvs. Renate, Ailsa Craig and SS1 bulbs (Fig. 1). There was no consistent pattern according to which cultivar had the highest and lowest concentration of each element, although cv. Ailsa Craig did not have the highest concentration of any of the elements measured. However, since ANOVA is a univariate technique which allows comparisons to be made between cultivars based on a single variate it does not give an insight into how the cultivars are grouped or which is the most important element in defining groups. Canonical variate analysis (CVA) allows these types of conclusions to be drawn, and has previously been used to discriminate between cultivars of other crop species (Cole & Phelps, 1979). Another type of multivariate analysis, principal component analysis (PCA) has been recently applied to data on mineral and trace elements in onion bulbs of six different cultivars from various seed origins cultivated under the same agronomic, climactic and soil conditions (Rodríguez Galdón et al., 2008). Significant differences in mineral composition were reported between cultivars, although relatively high genotypic overlap was evident.

Good separation of the cultivars was obtained using CVA. The majority of the variation, 89.23%, was explained by the first canonical variate (CV1), which separated Renate from cvs. Alisa Craig and SS1, whilst 10.77% of the variation was explained by the second canonical variate. Onion cv. Renate bulbs differed from cvs. Ailsa Craig and SS1, as shown by the high positive score for Renate in CV1 (5.901), versus negative scores for Ailsa Craig (−2.702) and SS1 (−2.134), respectively. Canonical variate 2 (CV2) discriminated between cv. SS1 and cvs. Ailsa Craig and Renate, as shown by the positive score for SS1 for CV2 (1.536), versus negative scores for Ailsa Craig (−1.424) and Renate (−0.118), respectively. In the case of CV1 there was less variation between the cultivars in terms of the macronutrients measured (*viz.* phosphorus, potassium, calcium, manganese and sulphur) than for the micronutrients measured (*viz.* iron, boron, manganese, copper and zinc), as demonstrated by the higher scores for CV1 for the macronutrients compared to the micronutrients (Table 2).

A plot of the means of the data relative to the first two canonical variates (Fig. 2) showed that there was minimal overlapping between the 95% confidence limits for each cultivar. The highest loading for CV1 was assigned to boron, and onions cv. Renate had a high positive value for CV1, signifying a high boron concentration compared to cvs. Ailsa Craig and SS1. The highest loading for CV2 was assigned to sulphur, indicating that onion bulbs cv. SS1 contained a higher concentration of sulphur per dry weight than cvs. Renate and Ailsa Craig, which was also demonstrated using ANOVA.

The sulphur and calcium treatments, applied at the time of seed drilling, did not affect bulb sulphur or calcium content. Similarly, fertilisation with calcium (in the form of calcium carbonate) or magnesium (in the form of magnesium sulphate) a few days prior to planting did not affect the concentration of magnesium or calcium in onion cv. Super-Kitamomjii bulbs grown in Japan (Ariyama, Nishida, Noda, Kadokura, & Yasui, 2006). Conversely, Coolong and Randle (2008) recently reported that application of calcium chloride to low pungency Vidalia cv. Georgia Boy onions increased bulb calcium concentration in each of two growing seasons. The final rate of application was 115 and 230 kg ha^{-1}, applied

Table 2
The mean scores for the first two canonical variates of the minerals analysed.

Variate	Canonical variate 1	Canonical variate 2
Boron	0.16033	0.04664
Calcium	−0.00049	−0.00047
Copper	0.03610	0.01124
Iron	0.02923	−0.03678
Potassium	−0.00011	−0.00010
Magnesium	−0.00540	0.01503
Manganese	0.13895	0.01173
Sodium	0.00185	−0.00143
Phosphorus	−0.00499	−0.00149
Sulphur	0.00105	−0.09950
Zinc	−0.01398	0.03573

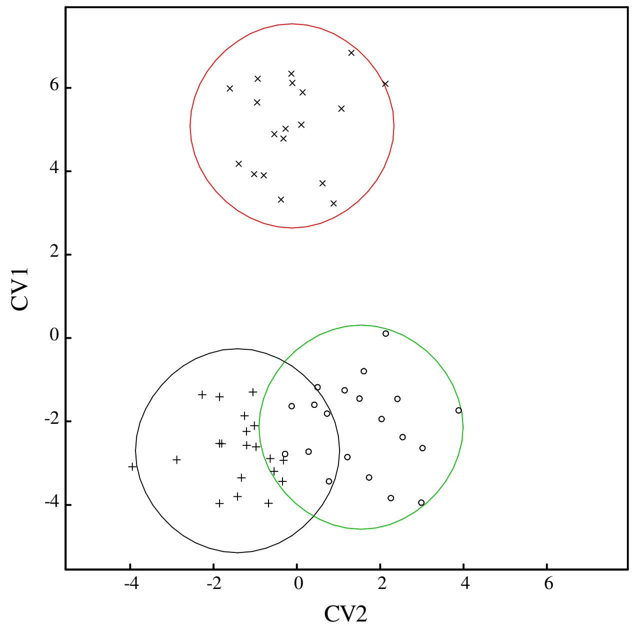

Fig. 2. The scores for the first two canonical variates of the three cultivars. The lines around the groups represent the 95% confidence interval for each group. ○ = cv. SS1, *x* = cv. Renate, + = cv. Ailsa Craig.

at 8, 12, 16 and 20 weeks after transplant, ensuring a regular supply throughout the growing season. It may be that this staggered approach to application was the reason why mineral content was affected. It is also possible that the method of cultivation i.e. the use of transplants rather than direct drilling could have affected the results. However, sulphur concentration was only increased in one growing season following application of 155 or 230 kg ha^{-1} ammonium sulphate staggered at 6 and 10 weeks after transplant. It is evident from the results presented herein that the sulphur and calcium applied was not present in the soil in a sufficiently available form, or was applied too early to the crop. There is also the possibility that the sulphur and calcium were taken up early on in the growth period, and retained in the upper part of the plant, however, it has been shown that ACSOs are translocated from the leaves to the storage scales during bulbing (Mallor & Thomas, 2008). In addition, it may have been that the soil conditions supplied sufficient sulphur and calcium nutrition to plants so that the applied treatments did not have a measurable effect.

4. Conclusion

The work presented herein has shown that it is possible to separate the three cultivars in this experiment on the basis of mineral content using just two canonical variates. It was also apparent that when onions of different cultivars are grown on the same site (i.e. same soil and climatic conditions), there are still significant differences in the mineral composition of the bulbs. It is therefore likely that the source of this variation is genotypic. Additional calcium and sulphur fertilisation applied at the time of drilling had no significant effect on the concentrations of these minerals in the bulbs. Therefore, it is important to consider the mode and timing of application of these treatments, and it should not be assumed that mere application to the soil results in greater bioavailability.

Acknowledgements

This work was performed as part of a project funded by the Horticultural Development Council; 'Understanding the mechanisms behind onion bulb dormancy in relation to the potential for improved onion storage' (CP20). Bulb material was supplied from HortLink project HL0164LFV (Defining quality assurance for sweet onions with rapid biosensor analysis) funded by the UK Government (Department for Environment, Food and Rural Affairs; Defra) and UK industry representatives. Thanks are given to both Brian Smith and David O'Connor. Mineral analysis was carried out at Warwick HRI (Warks., UK).

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