Potential of cultivar and crop management to optimise phytochemical content in winter-grown sprouting broccoli (*Brassica oleracea* L. var. *italica*)

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Running title: Purple_broccoli_JSFA

Kim Reilly, * [1,2] Juan Valverde, [1] Leo Finn, [2] Dilip K. Rai, [1] Nigel Brunton, [1,3] Jens C. Sorensen, [4] Hilmer Sorensen, [4] Michael Gaffney. [1]

[1] TFRCA, Ashtown, Dublin 15, Ireland; [2]Teagasc Kinsealy Research Centre, Dublin 17, Ireland; [3] School of Agriculture and Food Science, University College Dublin, Ireland. [4] Department of Food Science, Biochemistry and Bioprocessing, Faculty of Science, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark.

***Correspondence:** kim.reilly@teagasc.ie

ABSTRACT

BACKGROUND: Variety and crop management strategies affect the content of bioactive compounds (phenolics, flavonoids and glucosinolates) in green broccoli (calabrese) types, which are cultivated during summer and autumn in temperate European climates. Sprouting broccoli types are morphologically distinct and are grown over the winter season and harvested until early spring. Thus they show considerable potential for development as an import substitution crop for growers and consumers during the 'hungry gap' of early spring. The present study investigated the effect of variety and management practices on phytochemical content in a range of sprouting broccoli varieties.

RESULTS: Yields were significantly higher in white sprouting broccoli varieties. Levels of phenolics and flavonoids were in the range 81.6-270.4 and 16.9–104.8 mg 100g $^{-1}$ FW respectively depending on year and cultivar, and were highest in varieties TZ 5052, TZ 5055, Red Admiral and Improved White Sprouting. In-row spacing did not affect flavonoid content. Phenolic and flavonoid content generally increased with increasing floret maturity and levels were high in edible portions of the crop. Crop wastes (leaf and flower) contained 145.9-239.3 and 21.5–116.6 mg 100g $^{-1}$ FW total phenolics and flavonoids respectively depending on cultivar, tissue and year. Climatic factors had a significant effect on phenolic and flavonoid content. Levels of total and some individual glucosinolates were higher in sprouting broccoli than in the green broccoli variety Ironman.

CONCLUSION: Levels of total phenolics, flavonoids and glucosinolates are higher in sprouting than green broccoli types. Sprouting broccoli represents an excellent source of dietary bioactive compounds.

Keywords: phenolics, flavonoids, glucosinolates, bioactive compounds, sprouting broccoli.

INTRODUCTION

Over the last 20 years a range of epidemiological and other studies have shown that a diet rich in fruit and vegetables offers considerable health benefits. [1-10] Numerous studies have indicated a significant inverse relationship between fruit and vegetable consumption and the risk of developing many forms of cancer [1-4] as well as cardiovascular and other diseases. [5-10] These beneficial health effects are attributed to the presence of bioactive phytochemicals - defined as “non-nutrient chemicals found in

plants that have biological activity against chronic diseases". [11] Considerable research effort has been directed towards understanding the role of bioactive phytochemicals in human health; in promoting increased fruit and vegetable consumption and in increasing the levels of bioactive compounds in fruit and vegetables to produce functional (or "Super") foods. The World Health Organization has identified low fruit and vegetable intake as one of the top 10 risk factors contributing to mortality and has set guidelines for a minimum intake of 400g of fruit and vegetables per day (excluding potatoes and other starchy tubers) for the prevention of chronic non-communicable diseases such as heart disease, cancer, diabetes and obesity as well as for the prevention and alleviation of micronutrient deficiencies especially in less developed countries. [10]

Broccoli (*Brassica oleracea* L. var. *italica*) has been extensively studied due to the presence of both relatively high levels of dietary flavonoids (antioxidants) and due to the discovery of potent anti-cancer activity of the isothiocyanates, particularly sulforaphane [12] (the isothiocyanate breakdown product of the glucosinolate glucoraphanin), which is reported to occur at high levels in green broccoli. There is considerable diversity within the *B. oleracea* var. *italica* group in terms of i) seasonality and winter-hardiness (green broccoli types are harvested in summer and autumn depending on sowing date whilst sprouting broccoli types are hardy and overwintered for harvest in spring, ii) floret colour (which can be white, green or purple), and iii) floret morphology (which ranges from a single large, dense central floret as in green broccoli types, to multiple smaller secondary florets as in sprouting broccoli types). Green broccoli types are an important commercial crop and several studies have focussed on bioactive (glucosinolate and phenolic) content in varieties grown under uniform cultural conditions (reviewed in [13]) . Although a single purple sprouting variety Viola, [14, 15 16] or Purple Mountain [17] has been included in a small number of studies for

comparison purposes to our knowledge no previous studies have evaluated bioactive content in a range of sprouting broccoli varieties.

In the UK and Ireland sprouting broccoli currently occupies a small niche market. However its seasonality and potential bioactive content offer advantages for development as both as a fresh, seasonal, locally produced crop for consumers; and as an import substitution cash-generating crop for growers. In this study we carried out two-year field trials to evaluate agronomic performance and levels of phenolics and flavonoids in 24 sprouting broccoli varieties. Levels of total glucosinolates in a subset of 3 purple sprouting varieties (TZ 6002, TZ 5055 and Red Admiral) were compared to a commercially grown green broccoli type (Ironman) and a commercial white spouting type (TZ 4043). Two year trials were also carried out using the purple spouting variety TZ 6002 to determine the effect of in-row spacing; and finally levels of phenolics and flavonoids in different tissues and different floret maturity stages were evaluated in three varieties (TZ 6002, TZ 5055 and TZ 5052) to assess potential of crop wastes as asource of functional ingredients.

MATERIALS AND METHODS

Plant material and field trial experiments

All field trials were carried out at Teagasc, Kinsealy (53° 25’ N Lat 6° 10’ W), located in north county Dublin, Ireland. Soil type was characterised as a moderately well drained loam to clay loam belonging to the grey brown podzolic soil group (Altitude: 28 metres O.D., Slope: 1^{o}). Trials were repeated over two growing seasons in order to account for variability between years. Soil tests were carried out yearly in the spring and nitrogen (N), phosphorus (P) and potassium (K) were applied according to Teagasc recommendations for the crop. [18] Fertilizer was applied as calcium ammonium nitrate

(CAN), single super-phosphate and sulphate of potash. Weed and pest control treatments were in accordance with an Integrated Pest Management plan in keeping with commercial growing practises in North Dublin. [19] Plants were sown as modular transplants in 216 trays and (except for the spacing trial) were transplanted after 5-7 weeks at 40cm in-row spacing with 2 rows per 60inch (1 .52m) bed. Climatic conditions during the growing season (transplant date to harvest date) for trial years are shown in Table 1. For the variety trial, seeds of 24 broccoli (*Brassica oleracea* var. *italica)* varieties were obtained from commercial seed companies or from the Warwick HRI seed bank for heritage varieties. Variety trials were carried out in 2009 and 2010 and used a randomized complete block design (n=3). For year 1 (2009) seedlings were transplanted on 19th August. For year 2 (2010) seedlings were transplanted on 5th August. Primary and secondary florets were harvested at maturity between late December and May of the following year depending on variety. Yield was calculated as total weight of both primary and secondary florets (kg per plot). Primary florets were scored for susceptibility to white blister (*Albugo candida*) and bacterial wet rot (*Pseudemonas* spp.). Additional information on the field trials including variety photographs is available at http://www.ipfn.ie/publications/agronomic/. Experimental samples were composite samples comprising 100g healthy, disease free, secondary florets of marketable quality.

For the spacing trial purple sprouting variety TZ 6002 was transplanted on 28th July 2008 (year 1 trial) or 5th August 2010 (year 2 trial). The trial design was a randomised complete block (n=4) in both years. Plants were manually transplanted at 30cm, 45cm, 60cm, 75cm and 90cm in-row spacing with 2 rows per 60 inch (1 .52m) bed. Samples (secondary floret) were harvested on 23rd March 2009 (year 1 trial) and 24th March 2011 (year 2 trial).

For the maturity and tissue type trial commercial purple sprouting broccoli varieties TZ 5055, TZ 5052 and TZ 6002 were transplanted on 28th July 2008 or 19th August 2009 in a randomized complete block with 6 replicates in 2008 and 4 replicates in 2009. Samples (leaf, immature primary floret, mature primary floret, secondary floret, flower) were harvested between 23rd February and 21st April 2009 (year1 trial) and 18th March and 15th April 2010 (year 2 trial).

Samples for glucosinolate analysis of sprouting broccoli varieties (TZ 6002, TZ 5052, Red Admiral, TZ 4039) were transplanted on 23rd July 2008 and harvested in March 2009. Samples of the green broccoli variety Ironman were from a green broccoli trial transplanted on 23rd June 2008 and were harvested in September 2009. Both trials were a randomised complete block (n=4).

Quantification of total phenolics and total flavonoids

For the determination of total phenolics a modification of the Folin-Ciocalteau method was used. Briefly, broccoli samples were ground to a fine powder under liquid nitrogen. Frozen tissue (0.50g) was transferred to a Falcon tube and 5mL of 80% methanol in distilled water (v/v) was added. Tubes were vortexed thoroughly and allowed stand at RT for 20 minutes. Tubes were mixed by inversion and 1 .5mL aliquots of methanolic extract were transferred to a microfuge tube. Microfuge tubes were centrifuged at 12,000g for 5 minutes at 4 ^{o}C and the supernatant was transferred to a fresh tube. For each sample 150.iL of the methanolic extract, 150.iL 80% aqueous methanol, 150.iL Folin-Ciocalteau reagent and 1050.iL sodium carbonate solution (20% w/v) were pipetted into a microfuge tube, votexed and placed in the dark at RT for 20 minutes. Tubes were centrifuged at 12,000g for 3 minutes and the supernatant was transferred to a fresh tube. The absorbance at 725nm $_{(A725)}$ was determined relative to a blank

containing 80% aqueous methanol instead of extract, and the concentration was determined from a calibration curve using gallic acid. Results are expressed as gallic acid equivalents (GAE mg $100g^{-1}$ FW). Determination of flavonoids was according to Marinova *et al.* [20] The absorbance at 510nm $_{(A510)}$ was determined from methanolic sample extracts prepared as above, and flavonoid concentration was determined from a standard curve using catechin as a standard. Results are expressed as catechin equivalents (CE mg $100g^{-1}$ FW).

Quantification of glucosinolates

Glucosinolate profiles and total amounts in a subset of varieties were determined using Micellar Electrokinetic Capillary Chromatography (MECC) using standard procedures. [17,21] Glucosinolates were extracted from freeze-dried powdered broccoli. Samples were prepared in a large scale freeze-drier (*Frozen in Time Ltd.*) with the chamber below 0° C during the freeze-drying process. Once freeze-dried, samples were milled, vacuum packed in polypropylene bags and kept at -80°C until analysis. Freeze-dried broccoli powder, (500mg) was extracted (2-3 min) in a boiling 70% methanol solution (3mL) with addition of two internal standards (glucotropaeolin and sinalbin, 0.5 and 0.8μmol respectively, from the laboratory collection (Denmark)). The collected supernatant was dried under a constant N_2 flow and re-dissolved in deionised water (4mL). An aliquot (1mL) of this crude extract was applied to a DEAE Sephadex A-25 column (0.5mL) and the unbound material was removed by washing with deionised water (2 x 1mL) and sodium acetate buffer (2 x 0.5mL, 20 mM, pH 5.0). After washing, purified sulfatase (75μL, Type H-1 from *Helix pomatia*, Sigma, MO, USA) was added and the columns were incubated overnight at room temperature. After overnight incubation, the desulphoglucosinolates (dGLS) were eluted from the columns with deionised water (3 x

1ml). The collected eluate was dried under constant N_2 flow and re-dissolved in deionised water (200µL). Aliquots of 50 µL were used for the MECC analysis using a Hewlett-Packard HP^{3D} CE capillary electrophoresis system (Agilent, Waldbronn, Germany) equipped with diode array detector. All separations were performed on a fused silica capillary (Agilent, Stevens Creek, CA; 75 tm ID, 64.5cm total length, 56cm effective length). Samples were injected from the anodic end of the capillary (vacuum injection, 40 mbar, 1s). The separation buffer consisted of sodium cholate (250 mM) and boric acid (200 mM) at pH 8.5. The separation was carried out at 12 kV and at 60 °C. The capillary was used between each run sequentially with 1 .0 M NaOH (3 min), 0.1 M NaOH (1 min), water (1 min) and separation buffer (5 min). Detection was performed on column at 230 and 280 nm. The quantity of the dGLS was estimated as the average between the quantities calculated from the two internal standards, taking into account the relative response factors. [21]

Data analysis

Statistical analyses of data were carried out using SAS 9.3 (Cary, NC).Yield, disease susceptibility, phenolic, flavonoid and glucosinolate content data for the variety and spacing trials were analysed using an ANOVA linear model with Tukey adjustment. For the tissue type trial an ANOVA mixed model was used. Pearson correlation coefficients between total phenolics and total flavonoids; disease incidence and climatic conditions and between phenolics, flavonoids, and climatic conditions were determined. Parameters used were monthly mean or total values (T, Tm, TM, H, PP, RA) and total solar radiation (SR) from month of transplant to harvesting date of each variety.

RESULTS AND DISCUSSION

Sprouting broccoli variety trials

Twenty four sprouting broccoli varieties including commercial and landrace heritage varieties obtained through the HRI gene bank were grown during two harvest seasons (2009 and 2010). Yield was consistently higher in white sprouting types with Cardinal, TZ 6002 and TZ 5052 the highest yielding purple sprouting types (Table 2). Both variety and year had a significant effect on yield ($p<0.01$) with total yield of most varieties higher in 2009. Green broccoli crops at the trial site are commonly affected by white blister (*Albugo candida*) and bacterial wet rot (*Pseudemonas* and *Erwinia s*pp.). Bacterial wet rot in green broccoli has genetic and environmental components with some cultivars more susceptible. It is exacerbated under wet conditions and high N fertilization with average UK losses in a wet year of up to 29% of the crop. [22] In this study incidence of both diseases in sprouting broccoli varieties was low. Variety had no significant effect on susceptibility to white blister, however occurrence of wet rot was significantly affected by variety ($p<0.05$) with the Italian purple heading types Purple Cape, Sicilian Purple Lake and Sicilian Purple Early Autumn especially badly affected. Of the commercial purple sprouting varieties TZ 7035, TZ 5055, TZ 5052 and TZ 6002 performed well and would be suitable for successional harvesting from the same planting date. These varieties produce a reasonably large primary floret (20–1 50g) as well as smaller secondary florets which form the bulk of the harvest. Occurrence of wet rot but not white blister was weakly correlated with levels of total phenolics ($r =0.28$, $p<0.01$). This weak correlation may reflect increases in defence related phenolic compounds in response to bacterial infection. There were no significant correlations between disease incidence and flavonoid content, or between diseases incidence and any of the climatic variables in Table 1.

Beneficial health effects have been widely attributed to plant phenolic compounds, in particular flavonoids. [23,24] A number of studies on phenolic content in green broccoli types, in some cases including a purple sprouting type for comparison purposes have been carried out. Major phenolic compounds found in broccoli include flavonols such as quercetin and kaempferol glycosides, hydroxycinnamoyl derivatives and phenolic acids. Anthocyanins (cyanidin glycosides) are also found and accumulate to higher levels in purple broccoli. [14] In a study of six green broccoli varieties levels of total phenolics measured using the Folin Ciocalteau method ranged from 44.5 - 82.9 GAE mg 100g $^{-1}$ FW depending on variety. [25] Levels of flavonoids as measured by HPLC in 12 green broccoli varieties grown in Spain ranged from 1.23 - 6.54 mg 100g $^{-1}$ FW [26]; and 5.7 - 27.3 mg 100g $^{-1}$ FW in a Polish study which evaluated three commercial green varieties (Marathon, Lord, and Fiesta) over three years depending on cultivar and year .[27]

For this study levels of total phenolics and total flavonoids were determined in secondary florets (which comprise the bulk of the harvest) in 22 sprouting broccoli varieties. Two heritage varieties Sicilian Purple Lake and Sicilian Purple Early Autumn produced primary florets only and were excluded from the analysis. Data (Table 2) indicate that levels of total phenolics and total flavonoids are considerably higher in sprouting broccoli than published values for green broccoli and ranged from mean values of 117.3 - 243.3 GAE mg 100g $^{-1}$ FW for total phenolics and 41.7 - 104.6 CE mg 100g $^{-1}$ FW for total flavonoids depending on cultivar. Levels of phenolics and flavonoids in green broccoli varieties grown at the same trial site in 2011 were in the range 67.9 - 130.5 GAE mg 100g $^{-1}$ FW for total phenolics and 7.4 - 41.7 CE mg 100g $^{-1}$ FW for total flavonoids (data not shown). For sprouting broccoli varieties levels of both phenolics and flavonoids were highest in the commercial purple sprouting varieties

TZ 5052, TZ 5055 and Red Admiral. Lowest levels were found in the white sprouting heritage variety Early Broccoli. Interestingly another heritage white sprouting type Improved White Sprouting produced high levels of flavonoids and was ranked fourth after TZ 5052, TZ 5055 and Red Admiral with mean levels of 85.1 CE mg 100^{-1} FW (Table 2).

Levels of total phenolics and flavonoids showed a significant positive correlation ($r = 0.64$, $p<0.001$). Linear model ANOVA indicated that variety and year had a significant effect on total phenolic content in sprouting broccoli ($p<0.05$). For total flavonoids variety but not year was significant ($p<0.01$). A block effect was seen for the total flavonoid data. Where such block effects occur they indicate local variation (e.g. fertilizer, drainage or other gradients) between replicate blocks in the field trial. The source of variation is commonly as in this case not known, but is accounted for within the analysis by the block factor. Phenylpropanoid metabolism is commonly reported as responsive to environmental factors and variation in broccoli phenolic content has been attributed to climatic factors such as light and temperature. [27-29] Levels of total phenolics were higher for most varieties in the 2010 trial. Pearson correlations were determined for levels of total phenolics or total flavonoids with a range of climatic variables. Total phenolic content showed a very weak negative correlation ($r = -0.19$, $p<0.05$) with total solar radiation during the growing season. No other significant correlations with climate data were found for either phenolics or flavonoids in this trial.

In-row spacing

In green broccoli very high density planting (97,000 plants per ha) has been shown to increase levels of some glucosinolates [16] and manipulation of plant density has been explored as a method of increasing levels of bioactive phenolic compounds in some

crops. [30] In this study we measured levels of phenolics and flavonoids in the purple sprouting variety TZ 6002 in 2 growing seasons (2008 and 2010). Plants were transplanted as 2 rows on a 1 .52m bed at in-row spacing of 30cm (equivalent to 44,444 plants per ha); 45cm (29,630 plants per ha); 60cm (22,222 plants per ha); 75cm (17, 778 plants per ha); and 90cm (14,815 plants per ha). Spacing distances were bracketed around recommended in-row spacing for commercial production. Levels of total phenolics ranged from 118.0 - 333.2 GAE (mg 100g $^{-1}$ FW) in 2008 and 154.7 - 197.5 GAE (mg 100g $^{-1}$ FW) in 2010. Levels of flavonoids ranged from 64.0 - 94.8 CE (mg 100g $^{-1}$ FW) in 2008 and 29.15 - 61.5 CE (mg 100g $^{-1}$ FW) in 2010 (Figure 1). ANOVA showed a significant year x spacing interaction ($p<0.01$) on phenolic but not flavonoid content. Conversely year, but not spacing, had a significant effect on flavonoid but not total phenolic content. The growing season in 2008-2009 was considerably warmer and wetter than the 2010-2011 season – and the higher levels of flavonoids seen in the 2008 trial may be due to altered phenolic metabolism in response to climatic effects and/or associated disease pressure.

Tissue type and floret maturity

For many *Brassica* crops including broccoli, cauliflower and Brussels' sprouts less than 50% of the biomass is used for human consumption with the remainder either discarded or used for fodder. [31] Crop wastes therefore represent a potential source of functional ingredients. In this study levels of total phenolics were higher in leaf than in other tissue types of all three cultivars examined and levels of flavonoids were also high ranging from 72.8 – 78.2 mg 100g $^{-1}$ FW (Table 3). Thus broccoli harvest waste would represent a readily utilizable source of phenolic and flavonoid compounds.

Tissue type and maturity had a significant effect ($p<0.01$) on both phenolic and flavonoid content. In terms of dietary intake mature primary and secondary florets form the edible part of the crop with secondary florets forming the bulk of the harvest. Levels of both phenolics and flavonoids were relatively high in secondary floret tissue, with highest levels of flavonoids in TZ 6002 and TZ 5052. These data are in agreement with a study on 3 green broccoli varieties which found a rising trend in phenolic content with floret maturity. [32] Year had a significant main effect on both phenolic and flavonoid content with levels of both lower in the year 2 trial (2009-20 10), which was cooler, drier and brighter than the preceding year (Table 1). Calculation of correlation coefficients with climate variables indicated that phenolic and flavonoid content was affected by differences in temperature (T, Tm and TM) and rainfall (PP and RA) but not affected by humidity (IT). Solar radiation showed a relatively weak negative correlation with flavonoid but not phenolic content (Table 4).

Glucosinolate profile of selected sprouting broccoli varieties

Sprouting broccoli varieties TZ 5052, TZ 6002 and Red Admiral were selected for further analysis since they showed high levels of total flavonoids combined with good yield and quality attributes. Levels of total glucosinolates based on the sum of individual compounds were determined and compared to the levels in a commercial white sprouting variety TZ 4039 and a commercial green broccoli variety Ironman. Total glucosinolate concentration in the five cultivars tested ranged from 11.3 µmol g^{-1} DM in Ironman to 33.8 µmol g^{-1} DM in the white spouting variety TZ 4039 (Table 5). Purple sprouting cultivars (TZ 5052, TZ 6002, Red Admiral) had a significantly higher content of total glucosinolates than green broccoli variety Ironman. Previous studies have indicated a strong genotype effect on glucosinolate content in broccoli, with

glucoraphanin or glucobrassicin the predominant glucosinolate in green broccoli (reviewed in [13]) . In this study glucoraphanin was only a quantitatively dominant glucosinolate in the green broccoli variety Ironman. Glucobrassicin was found in all studied cultivars and represented more than 22-32% of the total glucosinolate content in purple varieties TZ 6002, TZ 5052 and Red Admiral. Glucoiberin and sinigrin were the predominant glucosinolates in sprouting broccoli varieties TZ 6002, TZ 5052 and TZ 4039. Red Admiral also had a high content of glucoiberin, whilst sinigrin was not found in this sprouting broccoli variety. In terms of bioactivity sulforaphane (the breakdown product of glucoraphanin) has been most extensively studied. More recently a breakdown product of glucobrassicin (indole-3-carbinol and its condensation product 3,3' diindolylmethane (DIM)), have been shown to exhibit potent antitumor activity with low levels of toxicity in a wide range of human cancer cell lines. [33] The isothiocyanate breakdown products of the aliphatic glucosinolate glucoiberin and sinigrin are iberin and allyl isothiocyanate respectively. Both have shown potent anticarcinogenic activity. [34, 35]

CONCLUSIONS

Levels of total phenolics, total flavonoids and total glucosinolates were higher in purple sprouting broccoli than in green broccoli varieties, with levels of glucobrassicin notably higher in purple sprouting as compared to green broccoli or white sprouting types. Phenolic and flavonoid levels in sprouting broccoli compared well to reported levels in a range of fruits and vegetables [20] and increased sprouting broccoli consumption would provide a rich source of bioactive compounds in the diet. Crop wastes contain high levels of both phenolic and flavonoid compounds and could be explored as a source of functional ingredients. Current spacing recommendations for sprouting broccoli are

acceptable and did not significantly affect flavonoid content, although phenolic content was affected in one year only. Climatic factors influenced levels of both phenolic and flavonoid accumulation in sprouting broccoli with higher levels measured in warmer wetter years.

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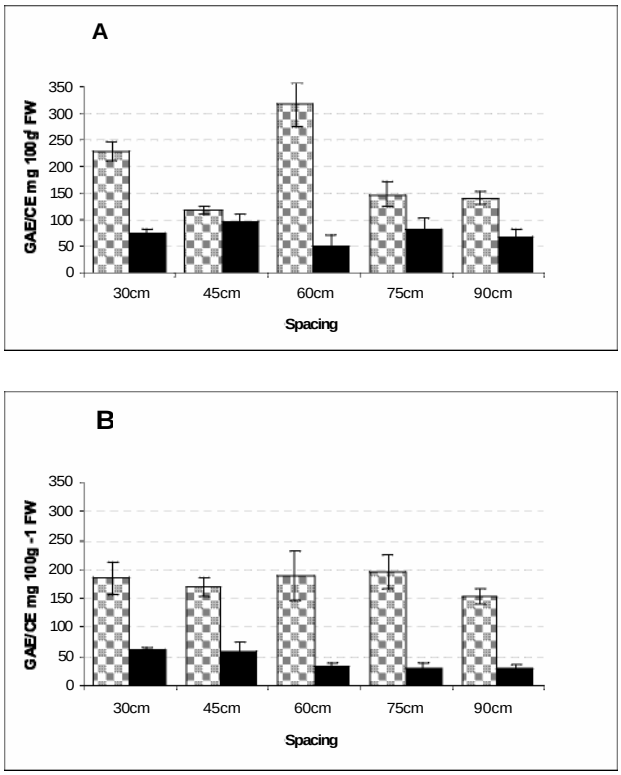

igure 1. Total phenolics (hatched columns) and total flavonoids (shaded columns) in condary florets of cultivar TZ 6002 grown in 2008 (panel A) and 2010 (panel B). Bar harts show mean ± standard error (n=4).

Table 1. Climatic conditions during the growing season.

	T	TM	Tm	H	PP	RA	SR
2008 August to:							
Feb. 2009	8.3	11.4	5.0	86.5	84.0	23.0	1133
March2009	8.1	11.3	4.7	86.0	76.6	22.8	1424
April2009	8.2	11.4	4.6	85.8	75.9	22.7	1790
2009 August to:							
March2010	7.6	11.0	3.7	86.9	66.1	22.5	1534
April2010	7.6	11.2	3.6	85.9	61.7	21.6	1922
May2010	7.9	11.6	3.7	85.2	58.4	21.4	2478
2010 August to:							
Feb. 2011	7.7	11.1	3.9	86.8	63.4	22.9	1368
March2011	7.5	11.1	3.5	86.5	57.8	22.3	1687
April2011	7.9	11.6	3.7	85.7	54.4	21.9	2044

T = Mean temperature (°C), TM = Average maximum temperature (°C), Tm = Average minimum temperature (°C), H = Mean humidity (%), PP = Precipitation amount (monthly total) (mm), RA = Indicator for occurrence of rain or drizzle (days), SR = Cumulative solar radiation since transplanting (MJ m^{-2}).

Table 2. Total phenolics, total flavonoids and agronomic evaluation of 24 sprouting broccoli varieties.

Variety	Yield (kg per plot)	White blister (% florets affected)	Wet rot (% florets affected)	Total phenolic content (GAE mg 100g $^{-1}$ FW)	Total flavonoid content (CE mg 100g $^{-1}$ FW)	Harvest dates year 1 trial (2009-2010)	Harvest dates year 2 trial (201 0-2011)
Nine Star Perennial*	4.57±0.61 ^{a}	0	0	189.51±26.99 ^{ab}	73.61±8.66 ^{abc}	23rd April -21st May	14th April - 29th April
EarlyBroccoli*	4.28±0.44 $^{a b}$	0	0	117.30±18.62^{b}	41.69±11.24^{c}	6thMay -13thMay	14thApril–29thApril
White Star	3.89±0.42 $^{a bc}$	0	0	193.67±22.85 ^{ab}	71.10±13.49 ^{abc}	23rd April – 21st May	14th April – 29th April
SproutingEarly White *	3.78±0.41^{abcd}	0	3	183.22±23.94 ^{ab}	77.91±7.35 ^{abc}	18thMarch–7thMay	13thDecember–20thApril
Cardinal	3.46±0.34 ^{abcde}	0	0	212.28±17.06 ^{ab}	79.63±7.68 ^{abc}	23rd April – 21st May	3rd March – 29th April
TZ6002	3.36±0.37^{abcdef}	0	0	184.18±8.48^{ab}	73.61±6.50 ^{abc}	24thMarch–7thMay	21st March–5thApril
White Eye	3.34±0.34 ^{abcdef}	0	0	177.52±11.91 ^{ab}	66.08±4.17 ^{abc}	1st April – 13th May	13th December– 14th April
TZ 5052	3.22±0.37^{abcdef}	0	0	243.26±32.21 ^{a}	104.58±4.89 ^{a}	24th March -7th May	13th December – 20th April
Improved White Sprouting	2.96±0.41 ^{abcdefg}	0	0	214.15±30.25 ^{ab}	85.08±16.90 ^{abc}	23rd April – 13th May	5th April – 20th April
Purple Cape	2.99±0.34 ^{bcdefg}	0	12	169.77±23.33^{ab}	51.38±8.51^{bc}	24thMarch- 7thMay	21st March–5th April
Late Purple Sprouting	2.98±0.19 ^{abcdefg}	0	0	188.36±24.57 ^{ab}	75.3±11.77 ^{abc}	23rd April -13th May	14th April - 29th April
Redhead	3.07±0.08 ^{abcdefg}	0	0	228.37±17.42 ^{a}	78.98±7.17 ^{abc}	23rd April -21stMay	5thApril–20thApril
Red Spear	2.59±0.50 ^{cdefg}	0	0	215.76±12.35 ^{ab}	80.42±14.26 ^{abc}	24th March – 7th May	21st March – 5th April
TZ5055	2.73±0.34 ^{bcdefg}	0	0	254.42±12.78 ^{a}	91.53±4.60^{ab}	18thMarch–7thMay	21st March–5thApril
Purple Sprouting	2.91±0.14 ^{bcdefg}	0	0	190.09±14.95 ^{ab}	71.88±8.45 ^{abc}	23rd April – 13th May	5th April – 20th April
Rudolf	2.57±0.42 ^{cdefg}	0	0	219.96±23.98 ^{a}	83.50±10.77 ^{abc}	3rd March –31st May	13th December – 5th April
Sicilian Purple Early Autumn	2.27±0.7 1 ^{cdefgh}	0	26	nd.	nd.	1 8thDecember–24th March	20th November -3rd March
RedArrow	2.37±0.40 ^{cdefgh}	0	0	200.21±20.08^{ab}	77.37±6.88 ^{abc}	1stApril–7thMay	21st March–5thApril
TZ7035	2.15±0.58 ^{defgh}	1	7	192.83±21.89^{ab}	64.64±11.51 ^{abc}	3rd March–15thApril	3rd March–5thApril
TZ 7033	2.53±0.19 ^{cdefg}	0	0	186.16±14.08 ^{ab}	84.36±7.04 ^{abc}	1 8th December–13th May	21st March – 20th April
HRI 6223 *	2.07±0.22 ^{efgh}	0	0	200.32±15.96 ^{ab}	80. 78±10.98 ^{abc}	23rd April – 13th May	5th April – 20th April
RedAdmiral	1.77±0.32 ^{fgh}	0	3	240.73±28.08 ^{a}	93.40±11.77^{ab}	18thMarch–23rd April	3rd March–21stMarch
HRI 8721 *	1.52±0.23 ^{gh}	0	0	177.70±18.54^{ab}	60.70±12.61 ^{abc}	7thMay–21stMay	14thApril–29thApril
Sicilian Purple Lake	0.8±0.25 ^{h}	0	26	nd.	nd.	3rd March – 24th March	20th November -3rd March
ANOVA p values							
variety	**<0.0001**	0.5603	**0.0132**	**0.0004**	**0.0065**		
year	0.1341	0.5045	0.3404	**0.0107**	0.0569		
block	0.4585	0.2966	0.2448	0.8175	**0.0046**		
variety * year	**<0.0001**	0.7107	0.1460	**0.0017**	0.0805		

* Heritage seed accessions obtained from HRI. Purple sprouting varieties are shown in bold. 'Purple Cape', 'Sicilian Purple Early Autumn' and 'Sicilian Purple Lake' were morphologically similar to green heading broccoli or cauliflower (*B.oleracea* var. *botrytis*) with a large central primary floret. Data shown are the mean of two year trial data ± standard error. Numbers with different letters within the same column were significantly different ($p<0.05$). ANOVA p values significant at $p < 0.05$ are shown in bold.

Table 3. Total phenolic and flavonoid content in different tissues of 3 purple spouting broccoli varieties.

Variety	Tissue type	Total phenolic content (GAE mg 100g $^{-1}$ FW)	Total flavonoid content (CE mg 100g $^{-1}$ FW)	Harvest date year 1 trial (2008-2009)	Harvest date year 2 trial (2009-2010)
TZ6002	Secondary floret	184.25 ±23.08 ^{a}	89.88 ± 20.31 $_{a}$	23rd February–5thMarch	15thApril
	Mature primary floret	104.43 ±10.41 ^{bc}35.20	± 5.61 $_{de}$	23rd February	8th April
	Immatureprimary floret	87.95 ±9.19 ^{c}	34.96 ± 8.30 $_{cde}$	23rd February	18thMarch
	Leaf	184.24 ± 23.12 ^{a}	78.23 ± 17.12 $_{abc}$	23rd February	18th March
	Flower	169.93 ± 6.59 ^{ab}	46.33 ± 7.60 $_{bcde}$	21st April	15th May
TZ 5055	Secondary floret	185.00 ± 14.98 ^{a}	46.98 ± 8.33 $_{bcde}$	23rd February– 5th March	8th -15th April
	Mature primary floret	149.47 ± 15.69 $_{abc}$	28.00 ± 4.89 $_{e}$	23rd February– 5th March	23rd March – 8th April
	Immatureprimary floret	107.40 ± 14.37 ^{bc}38.13	±6.86 $_{cde}$	23rd February	18th–23rdMarch
	Leaf	192.12 ± 16.65 ^{a}	72.75 ± 12.51 $_{abcd}$	23rd February	18th March
	Flower	182.16 ± 17.12 ^{a}	81.82 ± 15.03 $_{ab}$	21st April	15th May
TZ5052	Secondary floret	141.46 ±4.01 $_{abc}$	54.16 ± 9.01 $_{abcde}$	5$_{th}$ March	8th April
	Matureprimary floret	150.88 ±12.38 $_{abc}$	42.16 ± 6.11 $_{bcde}$23rd	February	23rd March
	Immatureprimary floret	141.11 ±16.75 $_{abc}$	46.04 ±10.90 $_{bcde}$23rd	February	18th March
	Leaf	206.73 ± 23.83 ^{a}	73.86 ± 12.66 $_{abcd}$	23rd February	18th March
	Flower	163.00 ± 9.27 ^{ab}	72.11 ± 15.80 $_{abcd}$	21st April	15th May
ANOVA p values					
year		**0.0096**	**<.0001**		
variety		0.2169	0.7714		
tissue type		**<.0001**	**<.0001**		
block		0.2277	0.2086		
variety * tissue		**0.0239**	**0.0005**		
year * variety		0.7745	0.1534		
year * tissue		0.0732	**<.0001**		

Total phenolic and flavonoid values are the mean of two year trial data ± standard error. Numbers with different letters within the same column were significantly different ($p<0.05$). ANOVA p values significant at $p < 0.05$ are shown in bold.

Table 4. Correlations between phenolic content and climatic conditions

	CE	T	TM	Tm	H	PP	RA	SR
GAE	0.554 ***	0.336 ***	0.288 ***	0.303 ***	ns	0.260 **	0.169 *	ns
CE		0.590 ***	0.351 ***	0.593 ***	ns	0.540 ***	0.454 ***	-0.308 ***

Note. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns not significant. CE = flavonoid content (catechin equivalents), GAE = phenolic content (gallic acid equivalents, T = Mean temperature (°C), TM = Average maximum temperature (°C), Tm = Average minimum temperature (°C), H = Mean humidity (%), PP = Precipitation amount (monthly total) (mm), RA = Indicator for occurrence of rain or drizzle (days), SR = Cumulative solar radiation since transplanting (MJ m^{-2}).

Table 5. Average content (µmol g $^{-1}$ DM) ± standard error of major glucosinolates in five broccoli varieties. ND indicates compound was not detected.

	Aliphatic					Indolyl				Aromatic	Total
	Glucoiberin	**Glucoraphanin**	**Progoitrin**	**Singrin**	**Gluconapin**	**4-OH-Glucobrassicin**	**Glucobrassicin**	**4-MeO-Glucobrassicin**	**Neo-glucobrassicin**	**Gluconaturtiin**	
TZ 4039	11.98 ± 1.26 ^{a}	1.71 ± 0.12 ^{b}	2.92 ± 0.23 ^{a}	15.49 ± 0.96 ^{a}	0.20 ± 0.01 ^{a}	0.03 ± 0.00 ^{b}	2.10 ± 0.09 ^{c}	0.04 ± 0.00 ^{b}	0.09 ± 0.01 ^{c}	0.26 ± 0.02 ^{a}	33.80 ± 2.58 ^{a}
TZ 5052	5.21 ± 0.23 ^{b}	ND	1.86 ± 0.08 ^{b}	8.91 ± 0.42 ^{b}	ND	0.20 ± 0.03 ^{a}	8.51 ± 0.42 ^{a}	0.62 ± 0.03 ^{a}	1.01 ± 0.06 ^{b}	ND	26.30 ± 1.22 ^{bc}
TZ 6002	12.16 ± 0.45 ^{a}	0.38 ± 0.03 ^{b}	1.50 ± 0.08 ^{b}	10.68 ± 0.43 ^{b}	ND	ND	7.14 ± 0.29 ^{ab}	0.13 ± 0.01 ^{b}	0.25 ± 0.01 ^{c}	ND	32.03 ± 1.32 ^{ab}
Red Admiral	11.94 ± 1.14 ^{a}	ND	0.11 ± 0.07 ^{c}	ND	ND	0.14 ± 0.02 ^{a}	6.70 ± 0.88 ^{b}	0.75 ± 0.11 ^{a}	0.82 ± 0.19 ^{b}	ND	20.46 ± 1.03 ^{c}
Ironman	0.67 ± 0.05 ^{c}	4.25 ± 0.31 ^{a}	ND	ND	ND	0.18 ± 0.01 ^{a}	3.50 ± 0.26 ^{c}	0.77 ± 0.06 ^{a}	1.89 ± 0.15 ^{a}	ND	11.27 ± 0.83 ^{d}

Numbers with different letters within the same column were significantly different ($p<0.05$).

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