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Metabolic profiling of cauliflower under traditional and reduced tillage systems

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Abstract

In this study we determined the nutritional value of cauliflowers under traditional or reduced tillage cultivation system. The content of carbohydrates, proteins, free amino acids, glucosinolates, ascorbate and glutathione were determined separately in immature flowers and corymb stems of cauliflowers. A heat map analysis was applied to all the obtained results for the different tissues and different tillage treatments. The most surprising result was the 1.9 fold higher average concentration of starch and the 2.2 and 1.6 fold lower average concentration of glutamine and tyrosine, respectively, in flowers compared to corymbs. The tillage treatment, on the contrary, did not affect significantly the metabolites profile of the plants which were substantially similar, from a nutritional point of view, except for the free amino acids and glucosinolates. In particular the essential free amino acids average content was 1.4 fold higher under reduced tillage than under traditional tillage and this was mainly due to the 3.5 fold increase of tryptophan. On the contrary the glucosinolates content was about 1.3 fold higher under traditional tillage than under reduced tillage. However a cluster analysis confirmed that the different distributions of metabolites between flowers and corymbs from the same plants were much higher than the difference in metabolites determined by farming methods, suggesting that nutritional characteristics of cauliflower were not significantly affected by reduced tillage.

Keywords: Cauliflower, Reduced tillage, Glucosinolates, Ascorbate, Glutathione, Essential amino acids, Glutamine.
Abbreviations: RT – Reduced tillage; TT – Traditional tillage; FW – Fresh weight; DW – Dry weight.

Introduction

Dietary factors have been recognized as primary risk for the development of cancer and chronic and degenerative diseases (Key et al., 2004). However, the epidemiological evidence that fruit and vegetable consumption may reduce the risk of such diseases has directed much research attention toward the chemo-protective role of certain plant compounds known as "phytochemicals" (Steinmetz and Potter, 1991). Cruciferous plants contain high levels of dietary fiber, folate, water, vitamins and phytochemicals: many studies have provided convincing evidence of an inverse association between their consumption and cancer risk (Verhoeven et al., 1996). Some of the Brassica vegetables, such as broccoli and cauliflower, contain vitamin C, in amounts similar to citrus fruits. Recent epidemiological evidence has revealed that vitamin C, which mainly occurs as ascorbic acid and its oxidation product dehydroascorbic acid, can reduce levels of C-reactive protein (CRP), a marker of inflammation and possibly a predictor of heart disease (Block et al., 2004). Nevertheless, cruciferous vegetables are unique in that they are rich sources of glucosinolates, a group of glycosides stored within cell vacuoles, that are responsible for their pungent aromas and spicy (some say bitter) taste (Steinmetz and Potter, 1991; van Poppel and Verhoeven, 1999). These metabolites are enzymatically transformed by myrosinase to isothiocyanates compounds when the plant tissues are damaged, as in harvest, chow or digestion by intestinal microflora (Fimognari and Hrelia, 2007). Gluroraphanin, the major glucosinolate in young sprouts of broccoli and cauliflower (55% of total glucosinolates) is the direct precursor of sulforaphane (Higdon et al., 2007). Recently, Clarke and coworkers (2011) showed that sulforaphane selectively targets benign hyperplasia cells and cancerous prostate cells while leaving the normal prostate cells unaffected. Cauliflower (*Brassica oleracea* L. var. botrytis L.) is one of the most important crops among vegetables grown in open field; it is very appreciated for its nutritional and nutraceutical properties. Its cultivation in Italy began at the end of 19th century, and in 1883 Italian cauliflower was already exported to Germany. With a broccoli and cauliflower harvest of 444,600 tonnes, Italy was the largest producer of broccoli and cauliflower in Europe in 2009, accounting for 19% of total European production, and the third largest producer in the world according to data from the Statistics Division of the Food and Agricultural Organization of the UN (Faostat, 2010). Cauliflower is grown in a surface of about 25,000 hectares. The cultivation is mostly concentrated (90% of total) in the regions of the Centre and South of Italy (Candido et al., 2003). Cauliflower is, however, a high impact crop. In fact, it requires not only high inputs of water, fertilizers and pesticides but above all repeated tillage that can cause soil erosion and runoff and dramatically decreases water infiltration, soil organic matter content and soil biological activity, thus reducing the sustainability of agriculture (Annunziata et al., 2012). Research has given, until now, an

inadequate attention to the sustainable horticultural cropping systems, but in the last years, the demand for high quality foods and government policies focused on environmentally sustainable agricultural systems have stimulated a rapid expansion of new farming methods. Studies comparing the productivity of reduced tillage (RT) or no-till practices to traditional tillage (TT) provide an excellent example of the wide range of benefits that may result from a conversion to sustainable agricultural methods. The studies, carried out primarily in the U.S. and Australia, showed that the conservation tillage system increases the benefits of the plant itself and reduces the production costs while fully respecting the environment and soil fertility (De Vita et al., 2007; Grooms, 2002; Holland, 2004). Notwithstanding, there can be a considerable risk that RT could impact the nutritional content of the resulting food products (poor food quality) (Chevalier and Ciha, 1986; Nakamoto et al., 2006; Neelam et al., 2008; Westermann and Sojka, 1996). Therefore, a clear understanding, from a nutritional point of view, of the advantages of using a RT farming system could be critical for the expansion of this practice (Gadermaier et al., 2012). In this view, we determined the effect of RT or TT cultivation system on the nutritional values of cauliflowers (cv Atalaya) by using biochemical or HPLC methods.

Results

Cauliflower yield under RT and TT farming

In RT system there was a significant ($p < 0.05$) increase of both the fresh marketable yield of 16.7 % (10.8 and 9.0 T/ha in RT and TT, respectively) and the number of leaves per plant of 9.9 % (17.1 and 15.4 leaves plant^{-1} in RT and TT, respectively) compared to TT farming (not shown). The dry matters of immature flowers (flower) and corymb stems or heads (corymb) in both treatments were quite similar accounting for about 6.7 % in average of the fresh weight (Table 2).

Metabolites profile under RT and TT farming

All the results from metabolic profiling analysis are shown in Table 2 and 3. The starch content was about 2 fold higher in flowers than in corymbs independently of tilling treatment ($p < 0.05$). The opposite was observed for the glucose content, which was significantly higher in corymb than in flower ($p < 0.05$). The average protein content was 1.4 % of the fresh weight. There was no significant difference between the proteins produced in cauliflowers from RT and TT, nor was there any difference between the different plant parts. Regarding the free amino acid content, it significantly differed ($p < 0.05$) depending on treatment and tissue (Table 2 and 3). Very high content of glutamine stood among the highest relative to the other free amino acids. It was about 1.1 % of the fresh weight in the corymbs, more than twice the concentration present in the flowers regardless of the cultivation system, accounting for 42 % and 33 % of the total free amino acids in TT and RT, respectively. The second most concentrated free amino acid was glutamate which showed no variation between tissues, but was significantly ($p <0.05$) higher in the RT condition. A similar pattern, although at lower concentrations, was shown by serine. Tyrosine, even though at lower concentrations, showed a pattern similar to that of glutamine, being more concentrated in corymbs than in flowers, while alanine, aspartate and proline were not significantly different ($p <0.05$), neither in dependence on cultivation nor on tissue.

Among essential free amino acids (Table 3), valine showed the highest content, in particular its average content was higher in RT treatment but the difference was not statistically significant because of the high standard deviation. There was highly significant difference ($p<0,01$) between the two treatments in relation to the tryptophan content, which was 3.5 fold higher in RT than in TT system, independently of the plant tissue considered. For the other essential free amino acids, the highest value, except for threonine, was always present in the RT treatment than in the TT one, but there was no significant difference in the distribution of the free amino acids between the flower and corymb. Ascorbate content was higher in corymbs than in flowers, but this difference was significant ($p< 0.05$) only in RT system (Table 2). While there was no significant difference ($p <0.05$) in dependence on cultivation system. The glutathione (Table 2) was present essentially in the reduced form and showed no significant changes ($p <0.05$) neither for the cultivation system nor for the plant part. Glucosinolates concentration significantly differed ($p < 0.05$) depending on tissue and treatment (Table 2). In fact, it was about 1.7 fold higher in flowers than in corymbs, while its average content decreased of 1.25 fold under RT in both tissues. Glucosinolates content varied from 65.5 mg/100g FW of RT corymb to 146.5 mg/100g FW of TT flower, corresponding to a range of about 21-46 µmol/g DW.

Cluster analysis of the growth and metabolic profiling data

The cluster analysis was applied to all the obtained results for the different tissues and different tillage treatments. All the data were cross-analyzed vs. alternative cultivation systems. The clusters of responses provided some useful indication to highlight the main differences between RT and TT treatments and between the different plant parts. The results of the cluster analysis are shown by a dendrogram (Fig 1), which lists the samples on the horizontal axis and indicates on the vertical axis the value of similarity among these samples. The most significant result was represented by the identification of two separate main clusters of samples from flower and corymb samples, which points to plant tissues as the most critical variables. The level of similarity between these two clusters was about 56 %, while the level of similarity of RT and TT flowers and RT and TT corymbs were about 66 % and 71 %, respectively.

Discussion

Although, literature data report unequivocal findings concerning the effect of both growing management practices and genotypes on nutritional value and elemental composition of Brassica vegetables (Cartea et al., 2010; Kopsell et al., 2005; Wang et al., 2012); in this study Atalaya was the only cauliflower cultivar tested. The reason is that, unlike other cultivars, it has a good leaf covering which ensures that the vigorous and compact heads are well protected resulting in a better resistance to fungal and bacterial diseases and ideal for autumn-winter harvesting (Veneto Agricoltura, 2010). In this way we could evaluate the direct influence of the two tillage systems on productivity and nutritional profile of cauliflowers regardless of genotype-related cold season yield depressing effects. As also found previously (Azooz and Arshad, 1998; De Vita et al., 2007; Gooms, 2002; Holland, 2004), cauliflower production level was better in the RT system (+21 %) as a result of the greater number of leaves, the highest weight of the corymb and the most significant root mass. To this end, it's important to

Table 1. Mean maximum and minimum temperature and monthly rainfall for the growing season 2007-2008 compared to long-term data recorded at Foggia (1980-2010), Italy.

	2007-2008			Long-term data		
	T_{max} (°C)	T_{min} (°C)	Rainfall (mm)	T_{max} (°C)	T_{min} (°C)	Rainfall (mm)
August	34.0	18.9	-	32.6	19.0	40.1
September	26.6	13.4	23.6	27.6	15.5	39.5
October	20.6	11.5	47.0	22.7	12.0	46.1
November	14.8	6.2	50.4	16.6	7.7	49.6
December	10.9	3.7	48.8	12.7	4.7	67.8
January	15.3	4.2	14.6	11.8	3.3	46.3
Sum			184			289

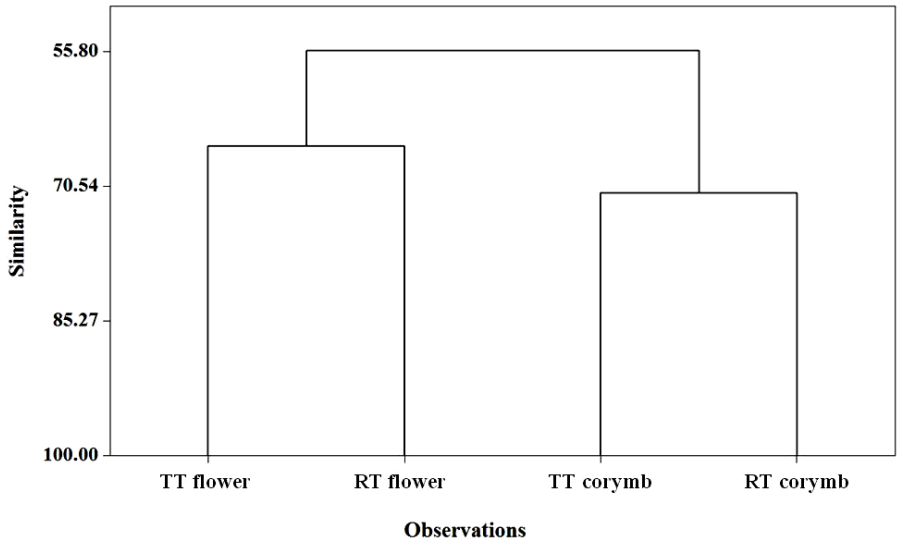

Fig 1. Dendrogram showing the cluster aggregation analysis of all results obtained from the different tissues (flower and corymb) under traditional tillage (TT) and reduced tillage (RT). All the data were cross-analyzed vs. alternative cultivation systems. The dendrogram indicates the samples on the horizontal axis and the value of similarity among the samples on the vertical axis.

highlight that in experimental studies on barley (Azooz and Arshad, 1998) and durum wheat (De Vita et al., 2007) no-tillage increased yield only during dry-seasons, a weather condition similar to which our plants were exposed. This result could be ascribed to the higher residue retention present in conservative tillage systems that increases organic carbon in the upper layer of the soil, which in turn leads to higher root density allowing the ready absorption of water and minimizing water loss by evaporation (De Vita et al., 2007). However, the novelty of our paper has consisted in considering the effects of RT on nutritional value of cauliflower crop. The analysis of nutrients, and in particular of certain classes of metabolites, was found to be a suitable tool to describe and trace the growing conditions. In addition, the separation of the sample into the immature flowers and the stems of the corymb allowed appreciate the finest alterations related to the plant tissues that could not have been detected by analyzing all the plant parts together. The heat map representing the changes in metabolite levels provided an integrated view of the differences between the plants tissues, under both RT and TT treatment (Table 2). The most surprising result was the higher concentration of starch and the lower concentration of glutamine and tyrosine in flowers compared to corymbs, regardless of the farming treatment. One possible reason why flowers had more starch and less glucose compounds is that in developing tissues soluble sugars are used to lay down storage reserves, such as starch, which will be mobilized in the following developmental stage (Lunn, 2008). Many genetic engineering studies have been done recently in order to enrich crop plants with essential amino acids, given the inability of animals, including humans, to synthesize them and that must be obtained from the diet (Hounsome et al., 2008; Ufaz and Galili, 2008). Therefore it is absolutely noteworthy the presence of larger amounts of essential free amino acids and in particular of tryptophan that confers a higher nutritional value to cauliflowers under RT system. Finally, it is interesting to note that cauliflowers were substantially similar, independently of farming system, except for the lower content of glucosinolates and the higher content of essential free amino acids in cauliflowers coming from RT system. An higher essential free amino acids content was also found in potatoes under organic farming (Carillo et al., 2012). In fact, the cluster analysis confirmed that the different distribution of metabolites between the different parts of the same plant was much higher than the difference in metabolites determined by farming methods, suggesting that nutritional characteristics of cauliflower were not affected by RT.

Materials and methods

Plant material and growth conditions

Cauliflower plants (*Brassica oleracea subsp. botrytis* cv Atalaya) were grown in the CRA-CER Cereal Research Centre of Foggia (90 m a.s.l., 41° 27' N, 15° 36' E, Southern Italy) on a silty-clay vertisol soil of alluvial origin (1.20 m

Table 2. Composition of cauliflowers under traditional tillage (TT) and reduced tillage (RT) treatments. Values are mean ± SD (n = 5). On the right a heat map summarizes the differences between TT and RT samples. Logarithm base 2 (Log_2) ratios were calculated for each value by dividing RT by TT values to estimate the effect of farming system or flower by corymb values to estimate the relevance of the different tissues. Log_2 ratios are visualized according to the scale bar given in the figure below the table.

Farming system

	TT				RT				RT/TT		Flower/Corymb	
	Flower		Corymb		Flower		Corymb					
g/100g FW									Flower	Corymb	TT	RT
DW	7.4 ± 0.8 a		6.2 ± 0.8 ab		7.3 ± 1.1 ab		5.8 ± 0.6 b					
Water content	92.6 ± 0.8 a		93.8 ± 0.8 ab		92.7 ± 1.1 ab		94.2 ± 0.6 b					
Glucose	8.1 ± 1.6 a		13.6 ± 3.3 b		10.2 ± 1.5 a		15.0 ± 1.0 b					
Fructose	7.0 ± 1.1 a		8.9 ± 1.5 ab		8.0 ± 0.5 a		10.1 ± 0.4 b					
Sucrose	2.5 ± 0.6 a		2.8 ± 0.4 a		3.1 ± 0.4 a		2.7 ± 0.5 a					
Starch	2.1 ± 0.7 a		1.1 ± 0.3 b		2.0 ± 0.3 a		1.1 ± 0.1 b					
Total proteins	1.6 ± 0.2 a		1.3 ± 0.1 a		1.6 ± 0.3 a		1.2 ± 0.3 a					
Total free amino acids	2.2 ± 0.3 a		2.8 ± 0.2 b		2.6 ± 0.2 ab		3.3 ± 0.1 c					
Essential free amino acids	0.27 ± 0.03 a		0.28 ± 0.05 ab		0.37 ± 0.06 b		0.38 ± 0.06 b					
mg/100g FW												
Glucosinolates	147 ± 12.2 a		86.9 ± 8.0 b		120 ± 9.9 c		65.5 ± 5.3 d					
Ascorbate	40.1 ± 5.8 a		66.5 ± 21.9 ab		41.2 ± 8.7 a		75.5 ± 11.3 b					
Glutathione	10.2 ± 1.5 a		8.1 ± 2.3 a		9.6 ± 1.2 a		7.3 ± 1.6 a					

log_2 ratio -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5

Table 3. Free amino acids content of cauliflowers under traditional tillage (TT) and reduced tillage (RT) treatments. Values are mean ± SD (n = 5). The description of the heat map, on the right, as in Table 2.

Farming system

	TT				RT				RT/TT		Flower/Corymb	
	Flower		Corymb		Flower		Corymb					
mg/100g FW												
Non Essentials amino acids									Flower	Corymb	TT	RT
Alanine	201 ± 34.5 a		203 ± 32.8 a		242 ± 30.0 a		228 ± 26.5 a					
Arginine	51.6 ± 18.1 a		44.9 ± 13.4 a		65.7 ± 11.9 a		98.8 ± 15.1 b					
Asparagine	209 ± 67.4 ab		270 ± 41.2 a		168 ± 30.6 b		199 ± 18.7 ab					
Aspartate	203 ± 37.3 a		210 ± 50.0 a		174 ± 48.2 a		226 ± 17.7 a					
Glutamine	477 ± 74.0 a		1103 ± 157 b		492 ± 34.4 a		1112 ± 185 b					
Glutamate	502 ± 75.9 a		420 ± 105 a		670 ± 89.9 b		630 ± 110 ab					
Glycine	22.9 ± 7.6 ab		18.0 ± 3.9 a		31.4 ± 6.7 b		23.4 ± 9.9 ab					
Histidine	23.6 ± 7.7 ab		23.5 ± 5.8 a		34.8 ± 2.6 b		30.8 ± 5.6 ab					
Proline	26.3 ± 7.6 a		30.2 ± 5.0 a		24.1 ± 9.8 a		25.7 ± 6.8 a					
Serine	142 ± 37.2 a		129 ± 40.3 a		226 ± 36.6 b		213 ± 22.4 b					
Tyrosine	22.0 ± 5.1 a		35.2 ± 7.0 b		28.1 ± 10.8 ab		45.8 ± 15.5 b					
Essential amino acids												
Isoleucine	39.0 ± 11.9 a		34.9 ± 9.3 a		48.1 ± 6.6 a		44.2 ± 9.7 a					
Leucine	22.1 ± 3.6 a		26.6 ± 7.4 a		31.1 ± 7.1 a		29.1 ± 5.1 a					
Lysine	24.7 ± 6.6 a		29.8 ± 6.7 ab		36.5 ± 4.9 b		36.0 ± 4.0 b					
Methionine	11.0 ± 2.4 a		14.3 ± 3.3 ab		15.3 ± 4.6 ab		20.7 ± 6.6 b					
Phenylalanine	16.1 ± 4.3 a		15.5 ± 3.4 a		21.1 ± 3.7 a		17.8 ± 3.6 a					
Threonine	56.7 ± 17.8 a		57.6 ± 23.9 a		47.9 ± 12.6 a		47.1 ± 18.0 a					
Tryptophan	37.1 ± 17.2 a		33.2 ± 14.3 a		126 ± 29.9 b		117 ± 30.3 b					
Valine	98.4 ± 19.6 a		112 ± 29.0 a		128 ± 37.4 a		144 ± 43.4 a					

log_2 ratio -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5

depth) (Typic Chromoxerert, fine, thermic, according to the Soil Taxonomy-USDA), with the following characteristics: sand, 38%; loam, 33%; clay, 29%; organic matter, 2.1%; total N (Kjehldahl) 0.128%; mineral N-NH_4, 4.74 ppm; mineral N-NO_3, 1.96 ppm; NH_4O Ac-extractable K_2O, 1098 ppm; Olsen P, 31 ppm; pH (2:1 water extraction) 8.3 (Elia and Conversa, 2012). The climate, classified as "accentuated thermo-Mediterranean" (FAO - Unesco classification), is characterised by scanty rains, mainly concentrated in the winter months, summer temperatures often higher than 40 °C and winter ones lower than 0 °C, by late frost (in April) and strong windiness (NW in winter, SW in summer) (Maiorana et al., 2004). Foggia was equipped with a weather station (Alba et al., 2010). Historical meteorological data were retrieved from a free database (www.ilmeteo.it) covering 30-year period. The trend of monthly average minimum and maximum temperatures and the distribution of monthly average rainfall for the 1980-2010 periods in comparison with the data of the experimental period are shown in Table 1. Cauliflowers growth occurred from September to December. As is typical of the Mediterranean climate, quantity and distribution of rainfall were highly variable. There was no rain in August; then rainfall increased from the end of September to October and remained constant until December, while it strongly decreased in January. In comparison with long-term data, the season 2007-2008 was less rainy/drier than average (-36 %), but temperatures slightly moved away from the mean maximum and minimum data. Cauliflower seedlings were transplanted on a field previously cultured with durum wheat. The crop residues were left on soil surface to decrease evaporation and erosion and increase the amount of organic matter (nutrients) (Campiglia et al., 2010; Troccoli et al., 2008). The experimental field 8.5 m wide x 11.8 m long was divided into two main blocks according to the two treatments: RT and TT. Each block was 8.5 m wide x 5.9 m long. A spacing of 170 cm was used between the rows and then the plants were 40 cm apart in the row. The two treatments consisted of a TT system, with mouldboard ploughing to 30 cm depth followed by secondary tillage with a soil grubber and harrow for plant-bed preparation and a conservative technique of RT in which there was no weeding, with crop residues cut by the combine, chopped and spread evenly with a combine-attached chopper on the surface (De Vita et al., 2007). The day before the transplanting, the experimental field was watered for 10 h, irrigating directly the area where the cauliflower seedlings had to be placed by a drip irrigation system. Cauliflower seedlings were transplanted at a density of 3 plants m^{-2} on September, 12, 2007. The farming was managed both for RT and TT plants according to the standard local cultivation protocols (Troccoli et al., 2008). Immediately after transplantation, the two plant systems were irrigated providing in fertigation 100 kg/ha of P_2O_5 and a preventive treatment of bio-insecticide for click-beetles, Ethoprophos 19%, (Bayer, CBG, Germany). Until the end of the crop cycle other 4 irrigations were performed (total water volume 1,280 m^3), distributing also 90 kg/ha of nitrogen by fertigation divided equally at post-transplant, beginning of differentiation and full inflorescence, and two pyrethroid-based insecticide treatments (deltametrine 2.8% and lambdacialotrine 2.5%, BASF, Italy). Both systems also had a graminicide treatment (quizalofop-ethyl D-isomer 5.27%, Bayer, CBG, Germany). Cauliflowers were manually harvested three months after transplanting when the curds were still compact and surrounded by leaves. Usually before cooking, the outer leaves and thick stalks are removed, and only the cauliflower florets and part of the main stem are cooked. For this reason at harvest we discarded several layers of green leaves attached to the stem and took for analysis only the edible parts of the crops, which are the corymb heads. These were further divided in immature flowers and corymb stems, parts of which were immediately used to determine fresh and dry weights or cut into slices, frozen in liquid nitrogen and stored at -80 °C.

Standards, reagents and other materials

All the reagents and standards (included the A9906 Fluka amino acid standard solutions) were purchased from Sigma-Aldrich (Milan, Italy), except for the Bio-Rad protein assay dye reagent which was purchased from Bio-Rad Laboratories Srl (Milan, Italy), the phosphoglucose isomerase from Roche Diagnostic (Milan, Italy) and ethanol, methanol and tetrahydrofuran (HPLC grade) which were purchased from Delchimica Scientific Glassware srl (Naples, Italy). Polypropylene and UV microplates were purchased from Corning (Wiesbaden, Germany), while centrifuge microtubes from Eppendorf (Hamburg, Germany).

Measurements of fresh and dry weight

The cauliflowers were divided in flowers and corymbs and immediately weighed to give the fresh weight. They were then oven-dried at 70 °C to steady state weight (48 h) and the dry weight was measured. The average values were determined from five different cauliflowers for each treatment.

Fresh sample preparation for chemical analysis

The frozen plant material was homogenized by a swing mill (MM 200, Retch, Haan, Germany) at 30 Hz for at least 1 min in order to obtain a fine powder. The metal balls (1 cm) and containers (stainless steel of 20 ml volume) were pre-cooled in liquid nitrogen before homogenization. Then, the fine powder was transferred to several pre-cooled 1.5 or 2 ml tubes (Eppendorf) and stored at –80 °C. Aliquots were used to determine the content of protein, carbohydrates, ascorbate and glutathione, free amino acids and glucosinolates.

Metabolites analysis

Total protein content was determined according to (Carillo et al., 2012). Amino acids were extracted and assayed according to (Carillo et al., 2009). Proline was determined according to (Carillo et al., 2011a). Soluble sugars and starch were extracted and determined according to (Carillo et al., 2005). Starch was expressed as glucose equivalents. Ascorbate and glutathione were extracted as described by (Annunziata et al., 2012) and evaluated according to (Queval and Noctor, 2007). Total glucosinolates were extracted and assayed according to Conaway et al. (2000).

Statistical analysis

The measures were performed on samples of flowers and corymbs from five individual cauliflower plants under TT or RT treatments (twenty different biological samples). The analysis of variance (ANOVA) of the results was carried out using SigmaStat 3.1 software (Systat Software Inc., Richmond, CA, USA). The means, when significant, were compared by the Least Significant Difference (LSD) test (at

0.05 confidence level). A heat map, graphical representation of data where the values taken by a variable in a two-dimensional table are represented as colours, was used to summarise the differences between RT and TT cauliflowers and between flowers and corymbs. Logarithm base 2 (Log_2) ratios were calculated for each value by dividing RT by TT values to estimate the effect of farming system or flower by corymb to estimate the difference between tissues according to (Carillo et al., 2011b). Log_2 ratios were visualized according to a scale bar in which positive values were represented by blue squares and negative values by red squares. No differences were visualized by white squares. Correlations among all analyzed samples were obtained by cluster aggregation analysis using the statistical software MINITAB (Minitab Inc., State College PA, USA). The result of the cluster analysis was shown by a dendrogram which listed on the horizontal axis the analyzed treatments and tissues and indicated on the vertical axis at what level of similarity the clusters were joined.

Conclusions

Reduced tillage system produced higher cauliflower yield than traditional tillage, with a greater number of leaves, a highest weight of the corymb and a most significant root mass. However, in our opinion, the major finding from the present study was the absence of large alterations in cauliflower nutritional value under the two different tillage systems considered. We showed, in fact, that the variation in metabolites content as result of reduced tillage farming was within the metabolites range present in the different tissues of cauliflower under traditional tillage, with the exception of glucosinolates and tryptophan. The results obtained, even if preliminary in that a thorough study requires the analysis of at least three cultivation cycles (6 years), suggest that this horticultural species can advantageously be cultivated in a conservative system of management of the soil both for productivity and nutritional value.

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