ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICALS COMPOUNDS ON LEAF CABBAGE (*BRASSICA OLERACEA* L. VAR. *ACEPHALA*) AND CHINESE CABBAGE (*BRASSICA RAPA* L. VAR. *CHINENSIS*)

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Abstract

The aim of the study was the comparative analysis of some varieties of leaf cabbage in terms of their content in total phenolic compounds, flavonoids, foliar pigments, antioxidant activity and antioxidant enzyme activity (superoxide dismutase, catalase and soluble peroxidase). The biological material was represented by two cultivars of kale: Dwarf Green Curled and Nero Di Toscana and by the cultivar Pak Choi White, a variety of Chinese cabbage, cropped under the same conditions. The total phenolics, flavonoids, chlorophyll and carotene content were determined by colorimetric methods. The antioxidant activity was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and enzymatic antioxidant activities were determined colorimetric using appropriate substrate. The results show that Nero Di Toscana cultivar has the highest content of phenolic compounds (275.1 mg GAE/100 g fm), total soluble peroxidase (50.33 $\Delta A/min/g$ f.m) and low Superoxide dismutase activity (44.96 mM H₂O₂/g fm/min) and the highest antioxidant activity (1294 TE/100 g fm). The study recommends the introduction into the diet of the varieties studied due to the high content of phytochemicals with health-promoting benefits.

Key words: leaf cabbage, total phenolics, flavonoids, chlorophyll.

INTRODUCTION

Green leafy vegetables are sources of important nutritional value for human diet and health. Recent studies suggest that foods rich in carbohydrates and dietary fiber have the potential to reduce risks of diseases, including obesity, diabetes, cancer and heart disease.

Brassicaceae vegetables are an abundant source of health-promoting substances and which reduce the risk of diseases (Leja et al., 2010; Podsedek, 2007).

Brassica species are vegetables rich in phytochemicals that include polyphenols, phenolic acids, flavonoids, carotenoids (zeaxanthin, lutein, β -carotene), alkaloids, tannins, saponins, anthocyanins, chlorophyll phytosterols, glucosinolates, phytosteroids, E vitamin. (Nawaz et al., 2018). Flavonoid compounds produce biological activity with beneficial effects on human health: suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and up-regulate and protect antioxidant defenses (Agati et al., 2012).

The phenolic compounds are an important group of biologically active substances found in plants. They are secondary metabolites that are able to neutralize the free radicals and numerous studies indicate that phenols may play a significant role in protecting biological systems from the effects of oxidative stress.

This is especially important because under oxidative stress, excessive formation of reactive oxygen species (ROS) can damage biomolecules, such as DNA, proteins, lipids and carbohydrates and can lead to many diseases. Superoxide dismutase enzyme (SOD) catalyzes the elimination (O^{-2}) of H₂O₂, whereas peroxidase (POX) and catalase (CAT) are involved in the reduction of H₂O₂ in cells (Babeanu et al., 2017).

Kale (*Brassica oleracea* var. *Acephala*) is one of the oldest forms of the cabbage family. It has origins in the eastern Mediterranean (Acikgoz,

2011). Kale is commonly cultivated in Central and Northern Europe and North America (Korus, 2011).

Pak Choy plants (*Brassica rapa* var. *Chinensis*) belong to a group of plant crops derived from the Far East (Kalisz, 2011). Chinese cabbage is a very popular vegetable in China that is also widely known in the USA and in European countries there are attempts to introduce it into the culture (Kalisz, 2011). These species of *Brassica* are of major importance due to their nutritional but also medicinal value, being rich in proteins, minerals, cellulose and antioxidants.

Due to its antioxidant, anti-inflammatory and antibacterial properties, cabbage is widely used in traditional medicine to relieve symptoms associated with gastrointestinal disorders. As food plants can be used raw in the form of salads or juices, or other preparations much appreciated by consumers.

Kale (*Brassica oleracea* L. *var. Acephala*) and Chinese cabbage (*Brassica rapa var. Chinensis*) are little known in Romania as sources of vitamins and mineral salts. The regions most favorable for cabbage culture are represented by the river beds in the area of Transylvania, Moldova, but also in the Plain of the South and West of the country (Soare et al., 2016a; Balcău et al., 2013).

The crop of these species can have multiple advantages through the high income of the producers and the introduction in the diet of vegetables with high nutritional value. The aim of the study was the comparative analysis of the content of total phenols, flavonoids, antioxidant activity and enzymatic activity in varieties of leaf cabbage and Chinese cabbage, in order to promote them for cropping in southwest Romania.

MATERIALS AND METHODS

The biological material was represented by two cultivars of leaf cabbage (*Brassica oleracea* var. *Acephala*): Dwarf Green Curled and Nero Di Toscana and a variety of Chinese cabbage (*Brassica rapa* var. *Chinensis*), cultivar Pak choi White.

The crop was established at the Didactic Station of the University of Craiova, Romania (44°19'N and 23°48'E), in 2018-2019, a

favorable area for the cropping of the species of *Brassicaceae*, by seedlings. The sowing was done at the beginning of April, and the planting of seedlings in the field was carried out in the last decade of May. At planting, the seedlings were 50 days old. Planting distances were 80 cm between rows and 60 cm between plants in a row for leaf cabbage, and in Chinese cabbage 40 cm between rows and 25 cm between plants in a row. Within the experimental trials, the specific technological sequences were applied.

Characterization of genotypes

Dwarf Green Curled Kale genotype is characterized by large, densely curled, fleshy, petiolated, dark green leaves. It forms a vigorous plant, with rich foliage, spirally arranged. Also, the leaves are obliquely upward oriented and outward rolled.

Nero Di Toscana genotype has the appearance of a palm tree, with oblong, embossed bluegreen leaves, covered with a thick layer of rhyme, vertically oriented. These cultivars have a dual purpose both for food and ornamental, being winter resistant.

Pak Choi White genotype forms a rosette of green, smooth, leaves, with well-developed petiole, whose color is white. The leaves are sessile, they have a very well developed central string, which is white and a lush foliar limb, soft and with a fine texture (Soare et al., 2016a).

To carry out the study, fresh leaves were harvested from the cultivated varieties and the content of phenolic compounds, flavonoids, foliar pigments, antioxidant activity and antioxidant enzyme activity (superoxide dismutase, catalase and soluble peroxidase) were analyzed.

Chemical analysis methods

For the spectrophotometric determination of chlorophyll a, chlorophyll b and carotenoids, 1 g leaf samples were extracted in 100 mL methanol and absorbance of the extracts was measured at 470, 653 and 666 nm.

The results were calculated using the formulas of Lichtentaler and Wellburn (1983). Chlorophyll a (mg/mL) = 15.65*A666 - 7.340*A653; Chlorophyll b (mg/mL) = 27.05*A653 - 11.21*A666; Total carotenes (mg/mL) = (1000*A470 - 2.860*Chlorophyll a - 129.2*Chlorophyll b)/245

Taking into account the extraction rate (1 g: 100 mL), the amount of pigments in leaves was expressed as mg/g fm (fresh matter).

For antioxidant enzymes extraction, fresh tissue was homogenised with 0.1 M phosphate buffer (pH 7.5) containing 0.1 mM EDTA. The homogenates were centrifuged for 20 min at 6000 rpm and the supernatants were used for enzyme assays.

The Superoxide dismutase (EC 1.15.1.1) activity (SOD) was assayed by measuring ability of sample extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Soare et al., 2017). SOD activity was expressed as the amount of sample (mg) which caused 50% inhibition of photochemical reduction of NBT.

Total soluble peroxidase (guaiacol-type E.C.1.11.1.7) activity (POX) was assayed by measuring the increase in A470 due to guaiacol oxidation to tetraguaiacol on addition of H_2O_2 and their activity was expressed as $\Delta A/min/g$ fm (Matei et al., 2014).

Catalase activity (E.C.1.11.1.6) CAT was assayed through the spectrophotometric method at 240 nm (Soare et al., 2017) and expressed as as mM H_2O_2 /min/g fm at 25°C.

The extracts for the determination of total phenolic content, total flavonoids content and antioxidant activity were prepared into 80% aqueous methanol (1: 10 w/v) at 24°C for 16 h. The resulting slurries were centrifuged at 4000g for 5 min and the supernatants were collected.

Total phenolic content was determined colorimetrically with Folin - Ciocalteu reagent (Dinu et al., 2018a). To 1mL methanolic extract 5 mL reactive Folin - Ciocalteu (diluted 1: 10 with ultrapure water) were added. After two minutes, 4 mL 7.5% sodium carbonate was added and the samples were kept in the incubator at the room temperature for 90 minutes. The absorbance was measured at 765 nm and the total phenolic content was calculated using a standard curve with gallic acid and expressed as mg GAE/100 g fm.

The total flavonoids content was determined by colorimetric method with 10% Al(NO₃)₃ and 5% sodium nitrite (NaNO₂) in alkaline medium (Soare et al., 2015a). The absorbance was read at 500 nm and the results were calculated from

quercetin calibration curve and expressed as mg QE/100 g fm.

Antioxidant activity, DPPH (2,2-diphenyl-1picrylhydrazyl) radical scavenging assay: The capacity of extracts to scavenge the DPPH radical has been evaluated colorimetrically at 517 nm (Dinu et al., 2018b). The Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The results were expressed as μ M Trolox equivalents (TE)/100 g fm.

All the spectrophotometric measurements were carried out with Evolution 600 UV-Vis spectrophotometer, Thermo Scientific, England, with VISION PRO software.

Statistical method

All analyzes were performed in three repetitions, and mean values were processed by analysis of variance (ANOVA). The significance of the differences was estimated with the Duncan test at the $p \leq 0.05$ level The correlation coefficients between the analyzed characters were also calculated, their significance being determined by Pearson values.

RESULTS AND DISCUSSIONS

Brassicaceous species biosynthesize high levels of chlorophylls and carotenoids (Hanson et al., 2009). The pigment content confers certain sensory characteristics on the edible organs and is a feature with a role in the evaluation and acceptability of consumers of these species.

Carotenoids (α and β carotene, xanthophylls and oxygenated carotenoids) are compounds with antioxidant properties that play an important role in scavenging free radicals and maintaining the immune system. Of these, betacarotene (provitamin A), lutein and zeaxanthin, are involved in the process of vision. Carotene content has shown great interest recently because of its importance in human nutrition (Dinu and Soare, 2017).

Other components that contribute to the beneficial effects of consuming Brassicaceae are chlorophylls. Recent studies have investigated a wide range of medicinal and pharmacological properties of chlorophylls a including antimutagenic, and b. antiinflammatory and antioxidant properties and their use in cancer therapy (Gopi et al., 2014). Regarding the content of chlorophyll and

carotene in the present study, the values were higher in the varieties of leaf cabbage compared to the Chinese cabbage. The cultivar Nero Di Toscana recorded the highest values in chlorophyll a of 1.83 mg/g f.m., in chlorophyll b of 0.833 mg/g f.m. and in carotene, of 0.753 mg/g f.m. Our results are similar to those recorded by Jurkow, (2019) for two kale cultivars (blue green and red purple leaves) harvested before frost, a content of chlorophyll a by 1.24 mg/g f.m., chlorophyll b 0.632 mg/g fm and 0.142 mg/g fm carotenoids in 2015/2016 season and chlorophyll a by 1.531 mg/g f.m. chlorophvll b 0.49 mg/g f.m. and 0.324 mg/g fm carotenoids in 2016/2017 season, and Samec (2018), raported at Kale, chlorophyll a by 2.27 mg/g dw, Ch b de 1.19 mg/g dw and on caroten by 0.53 mg/g dw, and at Chinese cabbage, chlorophyll a by 1.88 mg/g dw, chlorophyll b 0.85mg/g dw, caroten 0.38 mg/g dw.

Also, Korus and Kmiecik (2007) report for 3 kale crop investigated in 2 successive years an average content of total chlorophylls of 118-145 mg/100g f.w.; carotenoids 23.1-26.0 mg/g fw and beta-carotene 3.8-4.53 mg/g fw. It can be stated that this variability in chlorophyll and carotene content can be greatly influenced by the species, cultivar, date harvest and leaf maturity.

Table 1. Content in chlorophyll and carotene in <i>Brassica</i>
oleracea var. Acephala and Brassica rapa
var Chinensis

var. Chinensis					
Indices Cultivar	chlorophyll a (mg/g fm)	chlorophyll b (mg/g fm)	Carotene content (mg/g fm)		
Nero Di Toscana	1.83a	0.833a	0.753a		
FC Dwarf Green Curled	1.40b	0.749b	0.533b		
Pak choi White	0.32c	0.114c	0.21c		
LSD $p \le 0.05$	0.11	0.13	0.13		

Values represent the mean in the same column followed by different superscript letters are significantly different at $p \le 0.05$

In a study by Kapusta-Duch and Leszczyńska (2013), the difference in carotene content was influenced according to the variation of climatic and agrotechnical conditions

(cultivated organic farms compared to vegetables grown in steel or retail available).

The content of total phenolic compounds varies between 217.9 mg GAE/100 g fm (Pak choi White) and 275.1 mg GAE/100 g fm (Nero Di Toscana) (Table 2). The investigated species have a high content in phenolic compounds, justifying the beneficial effects of the diet rich in *Brassicaceae*. The data obtained in this study are similar to those presented by Korus (2011) in kale, variety Winterbor, a content in phenolic compounds ranging from 273 to 381 mg chlorogenic acid/100 g fw., and Polish variety, medium high Green Curly between 256 and 395 mg chlorogenic acid/100 g fw at different stages of maturity, and Samec et al. (2018), report for kale 15.13 mg GAE/g dm (dry weight 11.53%) and for Chinese cabbage 13.2 mg GAE/g dm (dry weight 12.03%).

In a previous study showing the content of bioactive compounds in some varieties of Brassicaceae, our group (Soare et al., 2015b), reported the highest content of phenolic compounds in kale (206 mg GAE/100 g fw) followed by broccoli (101.66 mg GAE/100 g fw), red cabbage (100 mg GAE/100 g fw), white cabbage (73.6 mg GAE/100 g fw) and cauliflower (33 mg GAE/100 g fw) and Samek et al. (2018) found for total phenolic content a variation in the order of white cabbage > kale. > broccoli > Chinese cabbage. There is a significant variation in the content of phenolic compounds in the two varieties of Brassica influenced primarily by the species, from the moment of harvest and the crop conditions.

The results obtained for total flavonoids content are shown in the Table 2.

The content of total flavonoid compounds was higher in Chinese cabbage, 124.2 mg QE/100 g fm cultivar Pak choi White, and leaf cabbage 109.86 mg QE/100 g fm Dwarf Green Curled cultivar 117.12 mg QE/100 g fm cultivar Nero Di Toscana.

The literature shows that in the composition of the studied varieties, a wide range of flavonoids has been identified and quantified, the majority of which are: quercetin, kaempferol and rutin (Cartea et al., 2011).

Table 2. Content in total phenols, total flavonoids and antioxidant activity in *Brassica oleracea var. Acephala* and *Brassica rapa* var. *Chinensis*

Indices Cultivar	Total Phenols (mg GAE/100g) fm	Total Flavono ids (mg QE/100 g)	Antioxidant activity (μmol TE/100g fm DPPH)
Nero Di Toscana	275.10ª	117.12ª	982.04 ^b
Dwarf Green Curled	267.43ª	109.86 ^b	830.92°
Pak choi White'	217.09 ^b	124.30 ^a	1294ª
LSD 0.05	25.24	8.74	67.48

Values represent the mean in the same column followed by different superscript letters are significantly different at $p \le 0.05$

Some authors have evaluated the content of flavonoid compounds in different species of Brassicaceae. Samec et al. (2018) analyzing comparative phytochemicals content in five Brassicaceae species reports a TFC of 3.67 mg CE/g dw for kale and 2.57 mg CE/g dw for Chinese cabbage, Hagen et al. (2009) finds in kale variety Reflex, total flavonol content 661 mg/100g dm, Agarwal et al. (2017) determine for kale 13.98 mgO/g dw (dw = 9.08%) while Bahorun et al. (2004) show for Chinese cabbage TF of 944 µg QE/g fw. It can be stated that there is a great variability of the flavonoid content influenced by the species. variety, genetic potential, the extra time for the plants remaining in the field after the coming of the frost and different harvesting times.

Numerous studies indicated that the beneficial effects of eating fruits and vegetables are partly explained by the content in compounds with antioxidant activity that scavenge free radicals and contribute to the defense against oxidative stress (Agati et al., 2012; Gopi et al., 2014; Podsedek, 2007).

The antioxidant activity of the studied *Brassica* vegetables was determined by the ability of extracts to reduce the DPPH radical. The results obtained are shown in table 2.

The antioxidant activity determined by DPPH recorded 1294 μ M TE/100 g fm in Chinese cabbage, Pak choi and Kale cultivars, from 830.92 μ M TE/100g fm (Dwarf Green Curled) to 982.04 μ M TE/100 g fm (Nero Di Toscana). In some reports, DPPH values determined for radical scavenging activity ranged from 56.3 μ mol TE/100 g fw (cauliflora) and 965.76 μ mol TE/100 g fw (kale), high values were

also recorded for red cabbage of 566.08 μ mol TE/100g fw and also of 388.56 μ mol TE/100 g fw for broccoli (Soare et al., 2015b). The variation of the antioxidant potential depends on the species, the maturity stage of the plant or the variety.

Korus (2011) reports for kale, the antioxidant activity measured by DPPH (1,1-diphenyl-2picrylhydrazyl) radical scavenging method values ranging from 14.7 μ M Trolox/g to 19.6 μ M Trolox/g at different stages of maturity. (Samec et al., 2018; Korus, 2011) show for kale and Chinese cabbage 34 μ M Trolox/g dw (dw = 11.53%, respectively 12.03%). Within the *Brassica* species, there is a great deal of variability, and depending on the cultivated genotype (Soare et al., 2016b; Korus and Kmiecik, 2007).

To evaluate the enzymatic antioxidant capacity of the investigated *Brassica* species, the activity of the enzymes was determined: superoxide dismutase-SOD, catalase - CAT and soluble peroxidase - POX.

SOD is an enzyme that catalyzes the dismutation of the superoxide anion into oxygen and hydrogen peroxide, peroxidase (POX) catalyzes oxidation of various electron donor substrates concomitant with the decomposition of H_2O_2 . Peroxidase is an enzyme involved in the control of various essential physiological processes of plant growth and development, lignification, biotic and abiotic stress responses and the catabolism of growth regulators (Caverzan et al., 2012). Catalase catalyses the reduction of H_2O_2 to H_2O and molecular oxygen.

The obtained results show that the activity of antioxidant enzymes varies with the investigated variety (Table 3). The enzymatic activity of CAT depending on the species and genotype, varies in the order: Dwarf Green Curled (26.8 mM H₂O₂/g fm/min) <Nero Di Toscana (36.18 mM H₂O₂/g fm/min) <Pak choi White (44.96 mM H₂O₂/g fm/min). In previous research, our group determined for the enzymatic activity of CAT at kale, 33.6 mM H₂O₂/min/g fm, and this enzymatic activity determined for some varieties of Brassicaceae, varied in the order: broccoli> kale> cabbage> white cabbage> red cauliflower (Soare et al., 2017). The results obtained by us are very close to those reported

by Samec et al., (2018) for kale and Chinese cabbage.

Indices Cultivar	CAT (mM H ₂ O ₂ /g fm/min)	POX (ΔA/min/g fm)	SOD (IC 50% (mg)
Nero Di Toscana	38.6 ^b	50.33ª	30.06 ^a
Dwarf Green Curled	26.8°	19.6°	12°
Pak choi White	44.96 ^a	34.85 ^b	21.6 ^b
LSD 0.05	3.86	6.95	4.17

 Table 3. Enzyme activity in Brassica oleracea var.

 Acephala and Brassica rapa var. Chinensis

Values represent the mean in the same column followed by different superscript letters are significantly different at $p \le 0.05$

In the present study, the enzymatic activity of POX ranges from 19.6 $\Delta A/min/g$ fm (Dwarf Green Curled) to 50.33 Δ A/min/g fm (Nero Di Toscana). Of the two kale cultivars, Nero Di Toscana has the highest enzyme activity of CAT and POX, 35%, respectively 156.78% higher than the value determined in the Dwarf Green Curled cultivar. In a previous study investigating antioxidant enzyme activity in five species of Brassica, our group showed a 54.6 $\Delta A/min/g$ fm enzyme activity of POX, the highest value for peroxidase activity was determined at red cabbage, followed by broccoli, white cabbage, kale and cauliflower (Soare et al., 2017). In other studies, for 2 varieties of kale, values between 3.89 and 16.06 Δ A/min/g fm are reported, depending on the degree of maturity (Korus, 2011). Peroxidase activity among different vegetables varies significantly. In a comparative study evaluating the enzymatic activity of peroxidase in selected vegetables the authors report the greatest activity in the case of cabbage while lowest peroxidase activity was observed in the case of green chilli and spinach (Pradeep et al., 2017).

The enzymatic activity of SOD varies with genotype and species. A low IC 50% expressed as the amount of sample (mg) which caused 50% inhibition of photochemical reduction of nitrobluetetrazolium corresponds with high superoxide dismutase enzymatic activity. The results obtained for superoxide dismutase activity, expressed as IC 50% (mg), varied between 12.00 mg (Dwarf Green Curled) and 30.06 mg (Kale Nero Di Toscana) (Table 3).

Of the cultivars investigated, Dwarf Green Curled with the highest enzyme activity of SOD 2.5 times higher than the value determined at Nero Di Toscana and 1.8 times higher than the value determined at Pak choi White. Our previous results indicate values for the kale species of 32.4 IC 50% mg (Soare et al., 2017). Although a high level of enzyme activity investigated increases the value of antioxidant capacity, bioavailability and food quality, high enzymatic activity can cause unwanted changes in foods that occur as a result of oxidation of different substrates leading to loss of quality attributes such as aroma and appearance.

The enzymatic activities and the antioxidant activity vary depending on the analyzed species and cultivar (Korus, 2011; Babeanu et al., 2017; Soare et al., 2017). In a study of different species of *Brassica*, superoxide dismutase activity recorded high levels in kale, peroxidase recorded high values in red cabbage, highest value for catalase activity was determined in broccoli, and highest value for antioxidant activity in kale (Soare et al., 2017).

Regarding the correlation coefficient (r) between the analyzed phytochemicals, both positive and negative correlations were registered (Table 4).

Significant positive correlations were recorded between chlorophyll a and chlorophyll b (r = 0.974), between carotene and chlorophyll a and b, (r = 0.940; r = 0.890); between total phenols and chlorophyll b and between CAT and total flavonoids (0.862), between CAT and antioxidant activity (r = 0.913), as well as between SOD and POX (r = 0.934).

Chemical compound	Chlorophyll a	Chlorophyll b	Carotenes	Total phenols	Total flavonoids	Antioxidant activity	POX	CAT
Chlorophyll b	0.974^{**}							
Carotenes	0.940**	0.890**						
Total phenols	0.935**	0.965**	0.84^{**}					
Total flavonoids	-0.514	-0.580	-0.607	-0.540				
Antioxidant activity	-0.802**	-0.896**	-0.717	-0.870**	0.784*			
POX	0.279	0.098	0.348	0.065	0.434	0.326		
CAT	-0.509	-0.655	-0.476	-0.642	0.862**	0.913**	0.634	
SOD	0.244	0.063	0.363	0.092	0.359	0.352	0.934**	0.615

Table 4. Correlations between the analyzed compounds in the investigated *Brassica* species

p 5% = 0.67; p 1% = 80

CONCLUSIONS

The results of this study show that the species studied of cabbage for leaves, less known and marketed in Romania, are a rich source of compounds with antioxidant activity. Among the cultivars studied, Nero Di Toscana kale cultivar has the highest content of phenolic compounds, flavonoides and the highest enzyme activity of CAT and POX, while chinese cabbage, Pak choi White cultivar recorded the highest catalase activity and antioxidant activity.

Regarding the analysis of the compounds investigated, a positive correlation is observed between chlorophyll a and chlorophyll b, between carotene and chlorophyll a and b, between total phenols and chlorophyll b and between determined antioxidant activity and content in flavonoids and in the enzymatic activity of catalase. The results of the study indicate that Chinese cabbage and kale are sources of antioxidants. The emergence of new species of *Brassica* on the market, such as kale and Chinese cabbage, can have multiple benefits for humans, such as sources of antioxidants in food, opportunities to diversify the vegetable assortment and as ornamental plants.

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