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Full Length Research Paper

Effects of cultivar and developmental stage on glucosinolates in garden cress (*Lepidium sativum* L.)

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Glucosinolates associated with health promoting properties were investigated in two commercial garden cress cultivars (Dadaş and Bahar) at optimum harvest time (1) and at the onset of flowering period (2). Results of glucosinolate analysis revealed that the predominant glucosinolate synthesized was glucotropaeolin of the benzyl group. The glucotropaeolin content varied between 1.79 to 4.57 µmolg^{-1} DW according to the cultivar and developmental stage of the plants. The highest content was found in cv. Dadas at the optimum harvest time. Mean glucotropaeolin content was slightly lower in samples collected at the onset of the flowering period compared to the optimum harvest time in both cultivars (P>0.05). The current study revealed for the first time the glucosinolate profile and content of two garden cress cultivars of Turkey, distinct from each other with their morphological features, at two different developmental stages.

Key words: Garden cress, glucosinolates, Brassicaceae.

INTRODUCTION

Garden cress (*Lepidium sativum* L.) is a herbaceous plant of the crucifers consumed mostly in salads. Two types of garden cress are cultivated in Turkey. These are “plain garden cress” and “curled garden cress” well distinguished with its curly and partial leaves. While the common cress is widespread throughout the country, the cultivation of curled cress is mostly localized in Eastern part of Turkey and has recently been registered as a new cultivar (Dadaş).

Garden cress is an exceptionally rich source of antioxidants provided by a research evaluating 26 vegetable species (Souri et al., 2004). In addition, it contains glucosinolates that are non-nutritive constituents present in crucifers associated with substantial health benefits when break down to specific compounds with the action of an enzyme (β-thioglucosidases) as a result of tissue damage (Li et al., 2010). They are the precursors of isothiocyanates (ITCs) that are thought to inhibit the development of cancer cells and reduce the risk of several forms of cancers by a number of mechanisms by inhibiting carcinogen activating enzymes, enhancing carcinogen detoxifying enzymes and inducing cell cycle arrest and apoptosis (Hamsa et al., 2011; Juge et al., 2007).

Garden cress contains glucotropaeolin, the benzyl glucosinolate, which is the parent compound of benzyl isothiocyanate (BITC) (Radwan et al., 2007). Of the different types of isothiocyanates, the anticarcinogenic activity of benzyl isothiocyanate (BITC) in garden cress has been studied extensively as a chemopreventive agent towards different classes of carcinogens (Kassie et al., 2002). Recent studies demonstrated the suppresion of pancreatic tumor growth (Boreddy et al., 2011) and inhibition of human breast cancer cells (Sehrawat and Singh, 2011) by BITC. In addition to its anticarcinogenic properties, benzyl glucosinolates from *Lepidium virginicum* root extracts was reported to reveal antiprotozoal activity as a therapeutic agent against diarrhoea and dysentery (Calzada et al., 2003). BITC has also been shown to exhibit high antibacterial activity (Jang et al., 2010) and toxicicity to several soil pathogens including nematod and fungi and thus can possibly be used as biofumigants (Jensen et al., 2010).

The glucosinolate profile is usually dependent on the genetic background of the individuals as well as on the environmental conditions during the growing period and conditions postharvest until reach the consumers as reported in several crucifers (Cartea et al., 2008; Jin et al., 2009; Song and Thornalley, 2007). In addition, the

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glucosinolate content can vary depending on the developmental stages of the plant as reported in *Arabidopsis thaliana* the model plant of the Brassicaceae family (Brown et al., 2003) and in kale (Sarıkamıs et al., 2008), the glucosinolate content can vary depending on the developmental stages of the plant. However, in garden cress, information regarding the factors influencing glucosinolate content is scarce except a recent study demonstrating an increase in the total glucosinolate content when seeds were germinated on different selenium solutions (Frias et al., 2010).

In order to explore factors influencing glucosinolate production in garden cress, the current research was designed to determine the effects of cultivar and developmental stage on glucosinolates. At the same time, determination of glucosinolates of garden cress cultivars growing in Turkey will be performed for the first time.

MATERIALS AND METHODS

Plant material

In this study two commercial garden cress cultivars Dadas and Bahar, (curled and plain garden cress) cultivated in Turkey were used as the plant material. Dadas is a new registered cultivar which has curly leaves, toothed partially at the leaf edge, commonly grown in the Eastern part of Turkey, while Bahar is a plain garden cress cultivar commonly grown in the country. The seeds for both cultivars were sown in an unheated greenhouse located at Ankara University Faculty of Agriculture, Department of Horticulture, at the end of September 2009. Leaves were harvested at two different developmental stages, at optimum harvest time (1) three weeks after sowing the seeds and at the onset of the flowering period (2), when first flower stalks appeared at the end of February 2010.

Analysis of glucosinolates

Leaf samples were collected and immediately taken to the laboratory and placed in -80°C deep freezer until analysis. Prior to the analysis, leaf samples were freeze-dried and ground to a fine powder. Extraction of glucosinolates, conversion to desulfo-glucosinolates and analysis by HPLC was as described previously (Sarikamis et al., 2006). Samples were analysed and separated by HPLC-UV (Shimadzu®) detection in the HPLC laboratory at Ankara University, Department of Horticulture. A volume of 50 µl from the extract was injected onto a Waters Spherisorb 5 µM ODS 2, 4.6 × 250 mm analytical cartridge. Analysis was carried out on a gradient of 99% water and 1% acetonitrile, at a flow rate of 1 ml/min for 24 min. The detection was carried out at a wavelength of 229 nm. Confirmation of the identity of the peak was performed by using comercially available glucotropaeolin (Applichem) as the standard, analysed at the same sequence with garden cress extracts. Sinigrin (16 mM) was used as the internal standard for the quantification of the peaks. The quantification of glucosinolates was carried out according to Sarikamis et al. (2006) and expressed as $\mu molg^{-1}$ dry weight (DW). Correction factors for glucotropaeolin was taken as 0.8 during quantification as reported by Brown et al. (2003).

Statistical analysis

Multifactorial variance analysis (ANOVA) was performed to evaluate tha data obtained, using MINITAB® version 14. Cultivars and plant developmental stages were taken into consideration as variables, while comparing the variation among cultivars and plant developmental stages. Significant differences were evaluated at $P<0.05$ error level. Data were presented as mean values ± standard error (SE) of the mean.

RESULTS AND DISCUSSION

The glucosinolates determined in two garden cress cultivars (Dadas and Bahar) at two different developmental stages revealed that glucotropaeolin (benzyl glucosinolate) was synthesized predominantly with a similar profile in both despite their significant morphological differences in between and discrete geographical locations they are adapted (Figure 1).
hese findings are also consistent with Radwan et al. (2007) reporting that glucotropaeolin is the major compound in garden cress. Therefore it can be deduced that genetic factors are the major determinants of the glucotropaeolin synthesis during the biosynthetic process and most probably these cultivars originate from a common genetic background distributed all over the world and conserved over the years.

In terms of the amount of glucotropaeolin found in two garden cress cultivars, the results revealed that, the glucotrapeolin content of Dadas ranged between 2.51 to 4.57 $\mu molg^{-1}$ DW with a mean of 3.63±0.601 $\mu molg^{-1}$ DW whereas, varied between 2.88 to 3.30 $\mu molg^{-1}$ DW with a mean of 3.13±0.0167 $\mu molg^{-1}$ DW in Bahar when the plants were at optimum harvest period (1) as demonstrated in Table 1. Subsequently, at the onset of flowering period (2), the glucotropaeolin content varied between 1.79 to 4.11 $\mu molg^{-1}$ DW with a mean value of 2.9±0.672 in Dadas and between 1.92 to 2.02 $\mu molg^{-1}$ DW with a mean value of 2.02±0.056 $\mu molg^{-1}$ DW in Bahar (Table 1 and Figure 2). Considering the mean glucotropaeolin content of the two cultivars, although a slight variation was observed, analysis of variance (ANOVA) reports suggested that the slight difference was not statistically important ($P>0.05$). Hence, the effect of cultivar on glucosinolate content was not evident in the current study.

Comparing the glucotropaeolin content at two different growth stages revealed that mean glucotropaeolin content slightly decreased in samples collected at the onset of the flowering period compared to the others taken at the optimum harvest period as presented in Table 1 and demonstrated in Figure 2, however, this variation was not significantly important at both cultivars ($P>0.05$). A few published studies have addressed the variation of glucosinolates among different organs and developmental stages in Arabidopsis during their life cycle, revealing variations in seeds, leaves, roots, inflorescences, siliques suggesting that the seeds had the highest concentration followed by inflorescences, siliques, leaves and roots (Brown et al., 2003). In broccoli, comparison of the glucosinolate content in

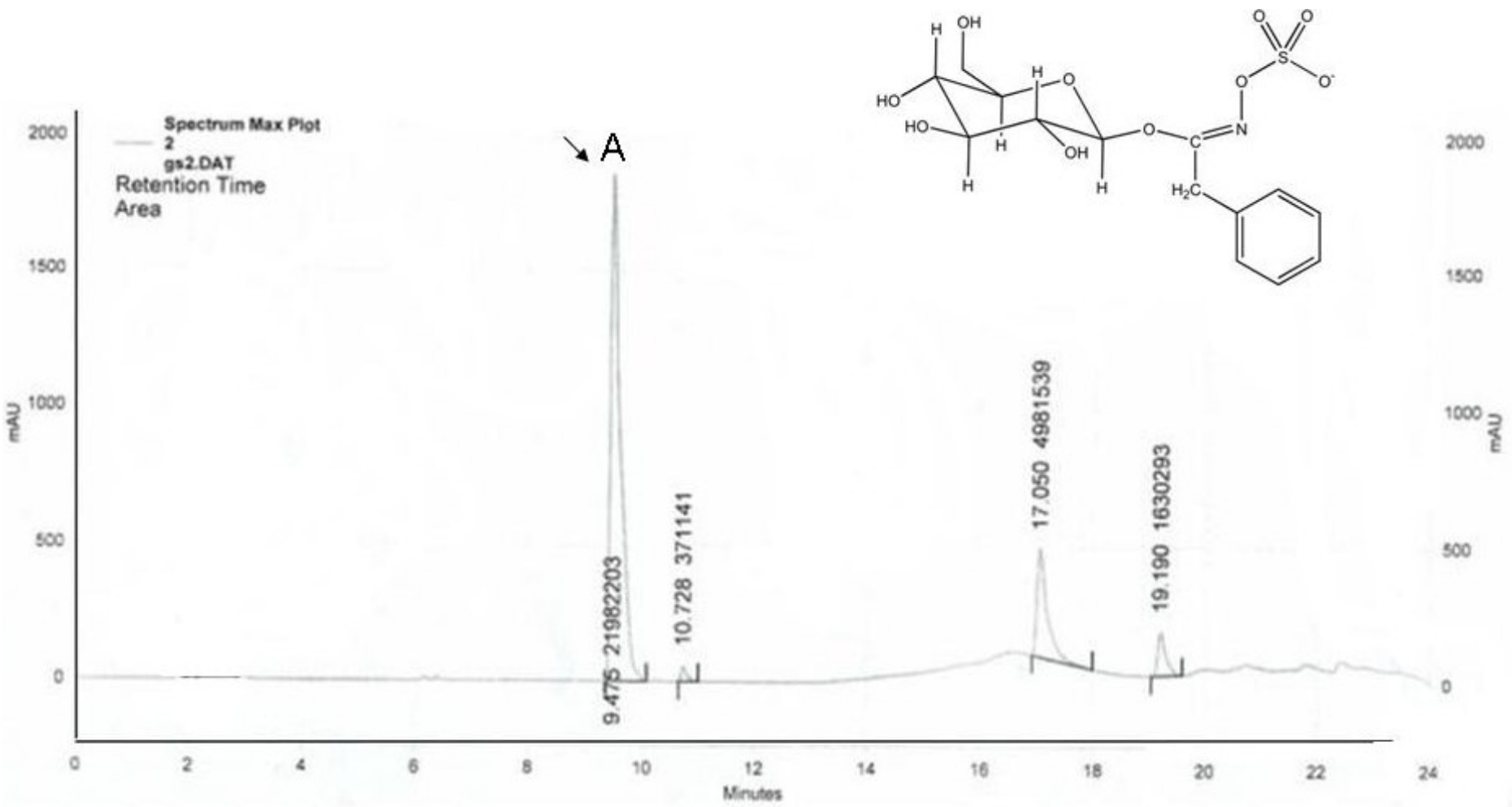

Figure 1. Glucosinolate profile of garden cress (Dadaş and Bahar). (A) Glucotropaeolin.

seeds and seedlings revealed that the seeds contained higher aliphatic and indole glucosinolates compared to the seedlings (Sarıkamış et al., 2010). Bennett et al. (2006), studied a group of plant secondary metabolites including glucosinolates in different rocket varieties such as salad rocket, wall rocket, wild rocket and Turkish rocket at different ontogenic stages. Their findings demonstrated the variation both in terms of different varieties and different plant organs in each cultivar. In the present study, we have analysed the Glucotropaeolin content at two different developmental stages in seedlings in order to determine the amount of glucosinolates at the optimum harvest time to see how much

Table 1. Glucotropaeolin content (in µmol g^{-1} dry weight) of two garden cress cultivars (Dadas and Bahar) at two different developmental stages at the optimum harvest time (1) and onset of flowering period (2).

Cultivars	Developmental stages	
	Optimum harvest time (1) Mean ± SE	Onset of flowering period (2) Mean ± SE
Dadaş	3.63±0.601	2.9±0.672
Bahar	3.13±0.0167	2.02±0.06

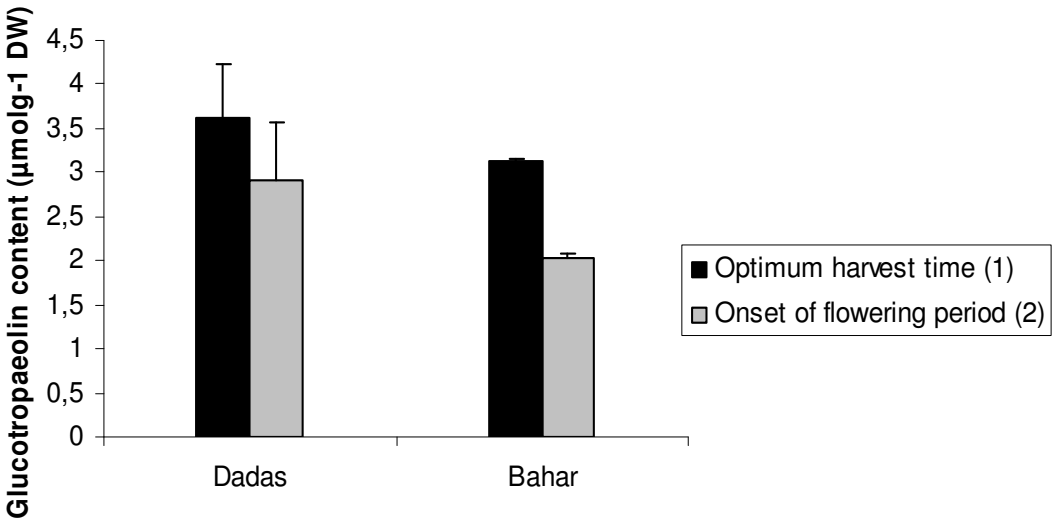

Figure 2. Glucotropaeolin content (µmolg^{-1} DW) of Dadas and Bahar at optimum harvest time (1) and at the onset of flowering period (2).

consumers can benefit and to figure out changes when plants get mature. However, in order to detect changes during the plants' life cycle, it would be much informative to consider an ontogenic profiling.

The present study demonstrated the glucosinolate content of two garden cress cultivars with distinct morphological features cultivated in Turkey for the first time and identified glucotropaeolin which is the parent compound from which benzyl isothiocyanate (BITC) is formed regarded as a promising chemopreventive agent towards different classes of carcinogens. Similar to other studies on different cruciferous models, the available concentrations are usually low in commercial cultivars and under the effect of several factors. Therefore precise knowledge of the changes in glucosinolate content during the growth cycle of plants is crucial in gaining the maximum benefits upon consumption.

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