Effect of environmental conditions and water status on the bioactive compounds of broccoli

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Abstract: Four experiments were carried out in 2010 and 2011 to determine how cultivation period (spring or autumn), harvest season (summer or autumn), and plant water status (irrigated or rainfed) influenced content and composition of broccoli cultivar Parthenon F1 with respect to sulforaphane and phenolics under field conditions in Gödöllő, Hungary. Sulforaphane content was significantly higher in the autumn harvests, regardless of irrigation treatments. Harvest season also influenced total phenolics content, with the highest values occurring in the spring season. Harvest season also affected trolox equivalent antioxidant capacity (TEAC) and this capacity was also the greatest in spring. Caffeic acid glucoside was a major phenolics component in both spring and autumn season harvests. The season and irrigation related changes in other phenolic component contents were also characterised in this study.

Keywords: Brassica oleracea convar. botrytis var. italica • Phenolics • Sulforaphane • TEAC • Irrigation

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1. Introduction

Glucosinolates are the main bioactive compounds found in the Brassicaceae family. Interest has grown over the past few decades in these phytochemicals of broccoli (Brassica oleracea convar. botrytis var. italica L.) and the role they may play in plant protection with regards to the influences of abiotic and biotic factors, but also in potential effects on human health [1-4]. This rising interest is primarily due to experimental evidence suggesting a therapeutic and preventative effect of the dietary constituents of broccoli on cancer progression [5]. Sulforaphane is classified under the isothiocyanates, which are formed from the parent compound glucoraphanin. Only glucoraphanin, an aliphatic glucosinolate, can form sulforaphane when hydrolyzed by myrosinase [6]. Glucosinolates are located within the vacuole of plant cells of Brassica crops, as opposed to the location of the enzyme myrosinase, which is localized to the interior of myrosin grains [7], so tissue rupture is necessary to bring them into contact. Recent scientific studies reported two controlling mechanisms affecting glucosinolate metabolism in broccoli. The first mechanism is the glucosinolate-myrosinase system, which is aimed at protection from herbivores [8,9], and the second mechanism appears to be associated with protection from environmental effects [6,10-12].

Sulforaphane is formed from glucoraphanin, by the action of myrosinase, when broccoli tissue is crushed or chewed [6]. Sulforaphane has been reported to have important anti-carcinogenic potential [13] in the case of several cancer types including skin carcinogenesis [14,15]. The effective indirect antioxidant role of sulforaphane has recently

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been reviewed. Sulforaphane is able to induce many cytoprotective proteins, including antioxidant enzymes that work to reduce oxidative stress in brain and neuronal injury, renal-, liver- hyperglycemia and β-cell-, and heart and cardiac cell-damage [16,17]. Broccoli flower head extract also possesses an antimutagenic property [18].

In addition, the effect of broccoli genotype on glucosinolate and phenolic compound content and profile has also received attention. A ten-fold difference in total aliphatic glucosinolate and glucoraphanin levels of broccoli florets has been observed among the genotypes examined [19]. The synthesis of aliphatic glucosinolates such as glucoraphanin was foremost regulated by genotype (60%) with environment x genotype (10%) and environmental (5%) factors exerting less effect. In contrast, indolyl glucosinolates such as like glucobrassicin were more closely associated with environment factors (33%) and environment x genotype (21%) than by genotype (12%) [19]. Significant differences have also been reported in vitamin C levels, as well as total glucosinolate and phenolic compounds content in B. oleracea genotypes examined [19]. The synthesis of aliphatic glucosinolate was foremost regulated by genotype (60%) with environment x genotype (10%) and environmental (5%) factors exerting less effect. In contrast, indolyl glucosinolates such as like glucobrassicin were more closely associated with environment factors (33%) and environment x genotype (21%) than by genotype (12%) [19]. Significant differences have also been reported in vitamin C levels, as well as total glucosinolate and phenolic compounds content in 8 genotypes of broccoli [20]. In the case of seeds and sprouts of broccoli cultivars, compared to indolic glucosinolates, aliphatic glucosinolates are primarily affected by genetic factors [19,21].

The glucosinolate-myrosinase system is the characteristic chemical defense mechanism against herbivores in plants of Brassicaceae [22]. The effect of spring or fall growing period on the glucosinolate-myrosinase system under field conditions has also been examined [10,11]. Total glucosinolate concentrations at harvest had a negative linear relationship with temperature and day length during the two weeks prior to harvest and a positive linear relationship with photon flux over the same two week period. Indole glucosinolate concentrations varied in a similar fashion with mean temperature, day length and photon flux during the four weeks preceding harvest. Glucoraphanin concentrations at harvest not only decreased linearly with mean photon flux from transplanting to harvest but also had a negative linear relationship with daylength from transplanting to harvest [10]. Myrosinase activity on a fresh weight basis (U g⁻¹) had a negative linear relationship with temperature, and a positive linear relationship with photon flux. Myrosinase activity on protein basis (U mg⁻¹) had a positive linear relationship with both temperature and photon flux [11].

Phenolic compounds of broccoli florets also possess anticarcinogenic and antioxidant properties [23,24], if prepared by steaming, and not by other cooking processes [25,26]. The effect of solar radiation on the flavonol content in broccoli florets grown in spring and fall cultivation periods under field conditions has also been experimentally described. Quercetin and kaempferol contents were highly positively correlated with total solar radiation from planting to harvest [27].

Under greenhouse conditions in Brazil, it has been reported that the low soil water content during growth combined with post-harvest cold storage gave the best preservation of antioxidant activity and L-ascorbic acid content in broccoli florets, while phenolic compound content was independent of cultivation or post-harvest storage conditions [28]. There are few literatures dealing with the effect of optimum or low soil water content on the bioactive components of broccoli under field conditions. In an experiment in Quebec, Canada the absence of irrigation significantly increased polyphenolics content in field grown broccoli [29]. Drought stress led to increased aliphatic glucosinolate [30] and flavonoid levels in broccoli [28,31] and *Arabidopsis* [22] also.

The aim of the present study was to investigate under field conditions, the effect of irrigation and spring/ fall cultivation period on the phytochemicals present in broccoli florets from identical genotypes.

2. Experimental Procedures

2.1 Plant material and experimental design

Four experiments were carried out in 2010 and 2011. Experiment I and III focused on spring cultivation between April-June and experiment II and IV examined the summer/fall cultivation periods between July and November. In all experiments, Parthenon F₁, cultivar was used [32, http://www.sakata-eu.com/vegetables/product_veg.asp?CategoryId=7&SpeciesId=2&VarietyId=189], and plants were planted in rows with a plant density of 6.25 plants/m². In experiment I and III seeds were sown on the 10 March 2010 and 9 March 2011, and seedlings were planted in the field on the 17 April 2010 and 14 April 2011. Harvesting dates were 24 June 2010 and 28 June 2011. In experiment II and IV seeds were sown on the 20 June 2010 and 2011 and seedlings were planted in the field on the 21 July 2010 and 24 July 2011, with harvesting dates of 30 September, 7, 14, 17, and 28 October 2010 and 12 October and 16 November 2011. In experiments II and IV side-florets were harvested during the last harvest date (28 October 2010 and 16 November 2011). The experimental design was a randomized block with four replications with four florets in each replication.

In addition to season, florets were subjected to either regular or no irrigation (rainfed control) treatments. The daily potential evapotranspiration of vegetable crops (tomato) is well estimated by expected daily average temperature divided by five expressed in millimeters [33]. The amount of irrigation needed is based on