Original research paper

EVALUATION OF ELICITATION EFFECT IN UNDERSTUDIED CRUCIFEROUS VEGETABLES

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ABSTRACT: Alteration of the secondary metabolism is one the responses of plants to environmental changes. As part of this secondary metabolism, *Brassicaceae* is distinguished by the presence of numerous bioactive compounds with health-promoting properties such as glucosinolates, phenolics and anthocyanins. These bioactive compounds are enhanced after elicitors' application in sprouts and young plants. The most effective pathway involved in such enhancement has been reported to be related to jasmonic acid (JA). JA and its volatile methyl ester methyl jasmonate (MeJa) act through transduction pathway resulting in a significant accumulation of glucosinolates and flavonoids. However, optimization of secondary metabolites production depended on crop, concentration of MeJa, number of applications, and developmental stage of the plant. As the effects of MeJa on phytochemicals on Bimi® (a new commercial broccoli coming from a hybrid between conventional broccoli and Chinese cabbage) and red cabbage, have not been studied in adult plants, in this work, we were focused on the effects on production of secondary metabolites and mineral concentrations in commercial plants. Also, the evaluation of the quality and stability of extracts was carried out. The increases of bioactive compounds obtained were promising for obtaining high quality extracts.

Key words: *brassicaceae, glucosinolates, phenolic compounds, elicitors*

INTRODUCTION

Cruciferous vegetables, specifically from the Brassicaceae family, are well known due to their wide variety of nutrients and bioactive compounds with health-promoting benefits, such as phenolics, antocians, flavonoids and glucosinolates (Hallmann et al., 2017; Scalzo et al., 2008). In particular, interest has been focused on these last mentioned biomolecules, which are nitrogen and sulfur-containing secondary metabolites found in Brassica species. Glucosinolates (GLSs) are the precursors of isothiocyanates (ITCs), which are released by the enzyme myrosinases after mechanical disruption, like chewing (Dinkova-Kostova and Kostov, 2012). In addition, ITCs have an antiinflamatory and chemoprotective activity, as well as an antiproliferative effect in cancer cells (Surh and Na, 2008)

It has been reported that some substances, known as elicitors, can induce changes in the phytochemical composition of plants by altering the different pathways in the secondary

metabolism (Angelova et al., 2006). Thus, improvement of the production of these secondary metabolites depends on elicitor specificity, concentration and duration of the treatment, and developmental stage of the plant (Jawahar et al., 2014). In this way, elicitors such as Jasmonic Acid (JA) and its volatile methyl ester (MeJA) act through their signaling transduction pathway, resulting in a significant accumulation of glucosinolates (Gu et al., 2015). Diverse studies performed in sprouts of different Brassica species have demonstrated a higher amount of GLSs when MeJA was applicated. For example, it has been observed a 2.4-fold increasing of indole GLSs after in red cabbage sprouts (*Brassica oleracea* var. *capitata*) (Hassini et al., 2017). However, few are known about elicitation with MeJA in adult plants of red cabbage and Bimi®. Both species have developed a growing interest in recent years. On the one hand, Bimi®, a hybrid between kalian (*Brassica oleracea* var. *alboglabra*) and broccoli (*Brassica oleracea* var. *italica*), has become popular due to its milder flavour (Martínez-Hernández et al., 2013). On the other hand, red cabbage presents a large variety of bioactive compounds with benefits for human health, such as anthocyanins (Arapitsas and Turner, 2008).

For all this, the aim of this work was to study the effects of MeJA elicitation to enhance the glucosinolate composition of Bimi® (*Brassica oleracea* var. *alboglabra* × *Brassica oleracea* var. *italica*) and red cabbage (*Brassica oleracea* var. *capitata*) and to evaluate the response of the plants in terms of growth and mineral content to such biofortification treatments.

MATERIAL AND METHODS

Plant material and treatment with elicitors

Seeds of Bimi® (*Brassica oleracea* var. *alboglabra* × *Brassica oleracea* var. *italica*) and red cabbage (*Brassica oleracea* var. *capitata*) from Sakata Seed Iberica S.L.U. (Valencia, Spain), were cultivated during spring period in soil of experimental farm, under a semi-arid Mediterranean climate. They were planted in March and collected in May 2018. Daily average temperatures and relative humidity were recorded every 10 min using dataloggers (AFORA S.A., Braloworld Scientific, Murcia, Spain). In the case of Bimi®, the elicitation with 100 µM MeJA in 0.2 % ethanol solution (Patent Application ES 201830674) was performed at the appearance of the central bud and five days after. On the contrary, for the red cabbage, the same MeJA solution was applied at flower bottom stage and during the development of the inflorescence, 5 and 10 days after. All plants were drip irrigated with a diluted (1:4) Hoagland nutrient solution. The plants were harvested 90 days after transplant. Then, the leaves and inflorescences from control and treated plants were sampled and fresh weighed in four technical replicates, and transported to the lab for further analysis (sampling time and transport always in less than 3 hours using coolers).

Extraction and determination of glucosinolates

Samples were freeze-dried and glucosinolates were extracted and analyzed from 100 mg of powder samples using the same procedure of Baenas et al. (2016). First, the separated intact glucosinolates were identified from extracted samples following their MS2 [M-H] fragmentation patterns in HPLC-DAD-ESI-MS^n (Agilent Technologies HPLC 1200,

Waldbronn, Germany; coupled to an Ion Trap mass detector Bruker in series, model Ultra- HCT, Bremen, Germany).

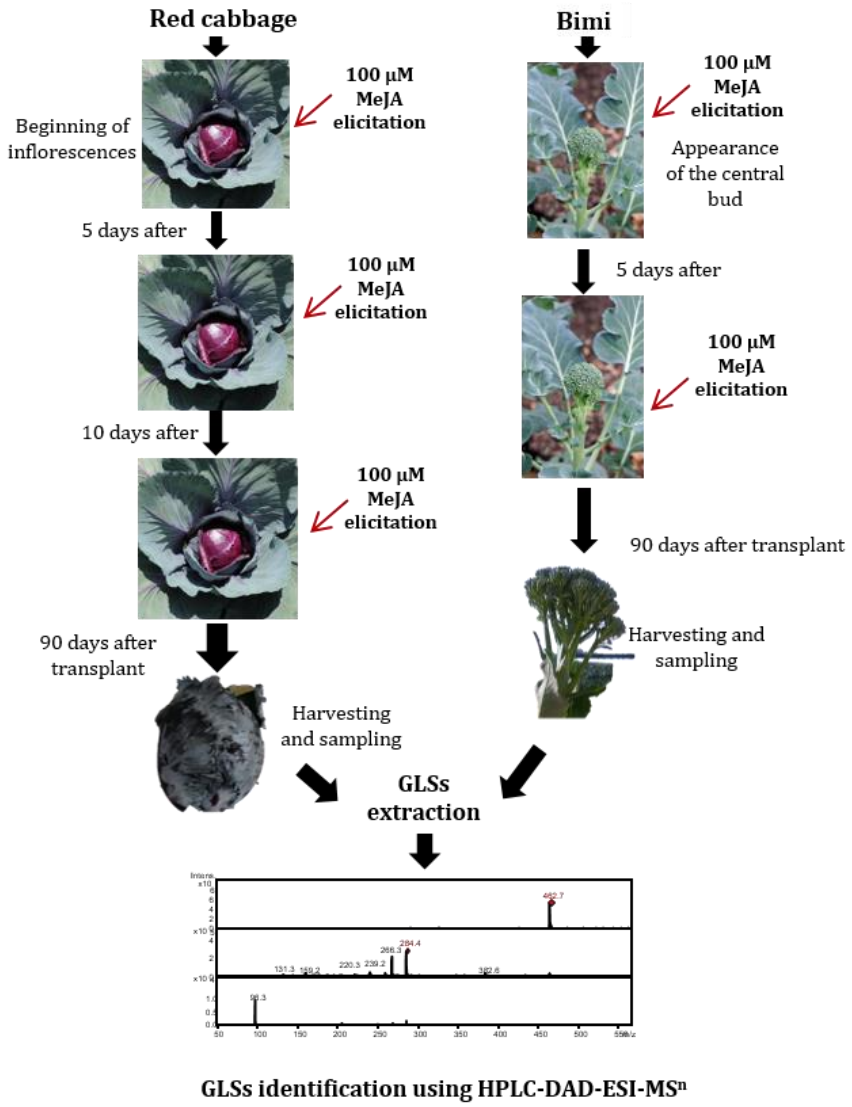

Figure 1. Outline of the methodology followed for field and HPLC-DAD-ESI-MSⁿ experiments

RESULTS AND DISCUSSION

In Table 1, dry biomass analysis is shown for both, Bimi® and red cabbage. The statistical analysis in leaves and inflorescences of Bimi® indicated that there were no statistically significant differences between the control and the treatment with MeJA. However, there was a slightly increase in biomass in leaves and inflorescence of red cabbage with MeJA treatment.

Table 1. Dry biomass in leaves and inflorescences from controls and elicited samples in Bimi® and red cabbage.

	Bimi®		Red Cabbage	
	Control (g)	MeJA (g)	Control (g)	MeJA (g)
Leaves	3.00 ±0.82[a]	3.50 ±0.58[a]	14.75 ±1.26[b]	17.50 ±1.73[a]
Inflorescences	4.75 ±0.96[a]	5.50 ±0.57[a]	36.75 ±7.63[b]	38.00 ±6.73[a]

Data are shown as mean ± SD (n=4). Same latter indicates that statistically significant differences were not found (P>0.05 in Tukey's test)

The analysis of the profile of GLSs is indicated in Table 2. In the case of Bimi® (Figure 2A), the MS2 analysis revealed that glucoraphanin, gluconapin, glucobrasscin, 4-methoxyglucobrassicin (MGB) and neoglucobrassicion (NGB) were present in both organs; leaves and inflorescences, and in control and MeJA samples. However, glucoerucin, glucoalyssin, progoitrin and gluconasturtiin were identified in Bimi® leaves treated with 100 µM MeJA. In addition, the majority of aliphatic GLSs detected by MS2 were under the limit of quantification, so other methods will be needed to obtain a reliable measurement. Surprisingly, 4-hydroxyglucobrassicin (HGB) was not found in leaves after treatments with the elicitor.

Figure 2. (A) Photography of a Bimi® sample. (B) Photography of a sample of red cabbage.

On the other hand, in red cabbage (Figure 2B), glucoiberverin, glucoraphanin, progoitrin, glucobrassicin, HGB, glucoiberin and sinigrin were found in both leaves and inflorescences with and without treatment. Nevertheless, it was interesting that the last two GLSs mentioned before were only detected in red cabbage but not in Bimi® samples. Furthermore, six GLSs were only identified in red cabbage inflorescences compared with the leaves: glucoerucin, gluconapin, glucotropaeolin, gluconastrutiin, MGB and NGB. The further study on contents and accumulation in the plant is ongoing to help in the clarification of the effects of the treatments.

In both brassicas, the presence of progoitrin as trace compound is beneficial since it has been reported an anti-nutritional effect. In this way, the anti-thyroid activity may be reduced compared with other cruciferous such as rapeseed (Mawson et al., 1994).

Table 3. GLSs profile from MS2[H-M] detection in leaves and inflorescence with or without MeJA elicitation in Bimi® and red cabbage

		Bimi®				Red Cabbage			
		Leaves		Inflorescences		Leaves		Inflorescences	
GLS common name	*[M-H]-*	*Control*	*MeJA*	*Control*	*MeJA*	*Control*	*MeJA*	*Control*	*MeJA*
glucoiberverin	406					*	*	*	*
glucoerucin	420		<LOQ	<LOQ	<LOQ			*	*
glucoiberin	422					<LOQ	<LOQ	<LOQ	<LOQ
glucoraphanin	436	<LOQ	<LOQ	<LOQ	<LOQ	*	*	*	*
glucoalyssin	450		<LOQ	<LOQ	<LOQ				
sinigrin	358					*	*	*	*
gluconapin	372	<LOQ	<LOQ	<LOQ	<LOQ			*	*
progoitrin	388		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
glucotropaeolin	408							*	*
gluconasturtiin	422		*	*	*			*	*
glucobrassicin	447	*	*	*	*	*	*	*	*
4-hydroxyglucobrassicin	463	*		*	*	*	*	*	*
4-methoxyglucobrassicin	477	*	*	*	*			*	*
neoglucobrassicin	477	*	*	*	*			*	*

*GLS was identified in a quantifiable amount; LOQ: Limit of Quantification

CONCLUSIONS

In conclusion, this preliminary study shows differences in the presence of GLSs between adult Bimi® and red cabbage leaves and inflorescences. Also, differential GLSs profiles were identified between control and MeJA elicited Bimi® leaves. In this way, further research is guaranteed to get a better insight and understanding on the response of these understudied cruciferous foods to obtain ingredients enriched in glucosinolates with environmentally-friendly practices of food production.

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