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Ontogenetic Changes of 2-Propenyl and 3-Indolylmethyl Glucosinolates in *Brassica carinata* Leaves as Affected by Water Supply

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Concentrations of 2-propenyl and 3-indolylmethyl glucosinolates in two lines of *Brassica carinata* (Holeta-1 and 37-A) were assessed during the vegetative life cycle under optimal or drought-inducing water supply conditions. In the well-watered treatment, 2-propenyl and 3-indolylmethyl glucosinolate concentrations remained almost constant from the 6−8 to the 15−16 leaf stage, whereas a drought-induced water supply led to a distinct increase of these glucosinolates. Generally, the 2-propenyl concentration was higher in Holeta-1 at each leaf stage under drought stress as compared with 37-A, indicating a *B. carinata* line-specific drought response. The drought-induced glucosinolate accumulation seems to be integrated in the plant's process of osmotic adjustment. It seems that under drought, there is a shift from primary to secondary metabolism, thereby promoting glucosinolate synthesis. Thus, by keeping the relative soil−water content below 80%, glucosinolate concentrations could be increased up to the 15−16 leaf stage, resulting in better plant nutritional quality of *B. carinata*.

KEYWORDS: Glucosinolates; Ethiopian mustard; drought; leaf stage; secondary metabolites

INTRODUCTION

Glucosinolates are a group of secondary plant metabolites found almost exclusively in plants of the order Brassicales, including horticulturally important crop plants of the Brassicaceae family (*1*). Breakdown products of glucosinolates have generated considerable pharmacological interest due to their anticarcinogenic properties (*2*, *3*). Although about 120 glucosinolates are known, only hydrolysis products of certain individual glucosinolates have been found to induce health-promoting effects. Recently, aliphatic isothiocyanates derived from 2-propenyl glucosinolate have been proposed to bear strong anticarcinogenic properties (*4*−*6*). Moreover, the indole glucosinolate 3-indolylmethyl glucosinolate is reported to be the most effective messenger against cancer development (e.g., see refs *7* and *8*). In *Brassica* species, glucosinolate synthesis is known to be regulated both ontogenetically (e.g., see refs *9*−*11*) and environmentally (e.g., see refs *12*−*14*). Exploitation of these regulatory factors could have pronounced effects on anticarcinogenic glucosinolate concentrations, and in turn, this has strong implications for the production of health-promoting food for human nutrition.

Scenarios of global climate change predict increasing limitation of water resources, especially in arid and semiarid regions. In these areas, reduced water availability is aggravated by the great seasonal and annual irregularity of precipitation, leading to a further accentuation of the water shortage (*15*−*17*). Several studies on *Brassica* species have established a distinct drought-induced increase of glucosinolate concentration (*18*−*23*). However, the intensity of drought appears to be a decisive factor in glucosinolate accumulation, since under mild drought stress, glucosinolate concentrations were unchanged (*23*) or decreased (*24*). In addition to the plants' water status, these contradictious results lead to the assumption that the plant response to limited water supply is also modified by additional influencing factors such as the plant's climatic adaptation, the genotype, and the developmental stage.

Commonly known as Ethiopian mustard, *Brassica carinata* is one of the major traditional leafy vegetables in east Africa, particularly in Ethiopia, and is a well-established integral part of the local food system and diet (*25*) containing relatively high glucosinolate levels (*11*). Because *B. carinata* serves also as an oil seed, glucosinolate research has mainly focused on seeds (e.g., see ref *26*), whereas detailed investigations of ontogenetic effects on leaf glucosinolate concentration have not been performed. This is surprising, as *B. carinata* has relatively high concentrations of health-promoting 2-propenyl and 3-indolylmethyl glucosinolates in the leaves, which also serve as a key food source (*27*). To date, for aliphatic and indole glucosinolates, only one single stage with fully developed leaves was investigated during the lifecycle of *Brassica* species (e.g., see refs *9*, *11*, and *28*). Therefore, in contrast to previous studies and to advance our understanding of ontogenetic effects under drought stress to glucosinolate metabolism,

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glucosinolate concentrations during several leaf stages of *B. carinata* were investigated in this study.

Because the scarcity of water is rising globally, we investigated how dynamic and thus actual drought conditions during the entire vegetative phase of two *B. carinata* lines affected glucosinolate concentrations in the leaves, particularly potentially anticarcinogenic 2-propenyl and 3-indolylmethyl glucosinolates. In this study, we first investigated a *Brassica* species—*B. carinata*—which is characterized by its well adaptation to arid and semiarid climate conditions, since previous studies regarding limited water supply focus on *Brassica* species adapted to temperate climate conditions (*18*−*24*). We hypothesized that variations in the plants' water status at specific developmental stages may induce specific glucosinolate responses modified by the *Brassica* species' drought adaptation and by line-specific variations in drought sensitivity, thereby enabling possible identification of developmental stage and water supply optima in terms of 2-propenyl and 3-indolylmethyl glucosinolate concentrations.

MATERIALS AND METHODS

Experimental Setup. The experiment was carried out in a completely randomized block design comprising four replications with planting on April 26, 2006, and harvest from May 11 to 18, 2006. Corresponding to the climate conditions of the lower Ethiopian highlands, the experiment was conducted in a controlled greenhouse environment with day/night length of 12 h/12 h and with 18 °C/16 °C (day/night) heating temperature and 22 °C ventilation temperature set point at the Institute of Biological Production Systems, Leibniz Universität Hannover, Germany. Germination was carried out in peat trays. Three seedlings in the two leaf stage were transplanted in loess soil-filled 15 L containers. To prevent evaporation, the soil surface was covered with a 5 cm layer of quartz gravel.

Plant Material. Two *B. carinata* lines of Ethiopian source, Holeta-1 and 37-A, were chosen as experimental plant material on the basis of their differentiation in the predominant 2-propenyl glucosinolate per g dry weight (DW). Holeta-1 showed pronounced low leaf concentrations in 2-propenyl glucosinolate (2.20 mg g^{-1} DW), whereas 37-A was marked by high concentrations of the major aliphatic 2-propenyl glucosinolate (4.98 mg g^{-1} DW) in the leaves (*27*). These genotypic variations in 2-propenyl concentration resulted in distinct differences in total glucosinolate concentration (Holeta-1, 2.25 mg g^{-1} DW; and 37-A, 5.05 mg g^{-1} DW). The indole 3-indolylmethyl glucosinolate was the second main glucosinolate found in both lines and ranged between 0.04 and 0.06 mg g^{-1} DW (*27*).

Water Supply Treatments. Before planting, the water content in the containers was adjusted to 80% water holding capacity. The water supply was differentiated 1 week after transplanting. Both water supply treatments were implemented using two different irrigation schemes. In the treatment with optimal water supply (W1), plants were watered every other day to maintain 80% water holding capacity in the containers. For simulating dynamic and thus actual drought conditions, in the treatment with limited water supply (W2), drought stress was induced by withholding irrigation, and the soil−water content dropped to 40, 23, 17, and 15% in the 6−8, 11−12, 13−14, and 15−16 leaf stages, respectively.

Sample Preparation and Glucosinolate Analysis. For each *B. carinata* line, water supply treatment, and replication ($n = 4$), a mixed sample of leaves from 8, 6, 6, and 4 plants in the 6−8, 11−12, 13−14, and 15−16 leaf stage, respectively, was used. For glucosinolate determination, about 200 g of leaf fresh matter was immediately deep frozen (−40 °C), then freeze-dried, and finely ground (≤0.25 mm using an ultracentrifugal mill, Retsch GmbH, Haan, Germany).

The high-performance liquid chromatography (HPLC) method reported by Krumbein et al. (*29*) was used for the determination of desulfo-glucosinolates. Duplicates of freeze-dried sample material (0.5 g) were heated to and incubated at 75 °C for 1 min, extracted with 4 mL of a methanol/water mixture (v/v = 7:3, $T = 70$ °C), and then, after 1 mL of 0.4 M barium acetate was added, centrifuged at 4000 rpm for 10 min. The residue was extracted twice more with 3 mL of the methanol/water mixture (v/v = 7:3, $T = 70$ °C). The supernatants were pooled and made up to 10 mL with methanol/water mixture (v/v = 7:3, $T = 70$ °C). From this, 5 mL of the extract was applied to a 250 μL DEAE-Sephadex A-25 ion-exchanger (acetic acid-activated, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and rinsed with 10 mL of bidistilled water. Next, 250 μL of a purified solution of aryl sulfatase (Boehringer-Mannheim GmbH, Mannheim, Germany) was applied and left for 12 h before the desulfo compounds were flushed with 5 mL of bidistilled water. Desulfoglucosinolate analysis was conducted using a Merck-Hitachi HPLC system (Merck-Hitachi, Darmstadt, Germany) with a Spherisorb ODS2 column (Bischoff, Leonberg, Germany, 5 μm, 250 mm × 4 mm). A gradient of 0−20% acetonitrile in water was selected from 2 to 34 min, followed by 20% acetonitrile in water until 40 min, and then 100% acetonitrile for 10 until 50 min. Determination was conducted at a flow of 1.3 mL min^{-1} and a wavelength of 229 nm. Glucosinolate concentration was calculated using 2-propenyl glucosinolate as external standard (Sigma-Aldrich Chemie GmbH) and using the response factor of each compound relative to 2-propenyl glucosinolate (*30*). Determination of glucosinolates was performed in duplicate. The well-known desulfoglucosinolates were identified according to previous work (*31*) from the protonated molecular ions $[M + H]^+$ and the fragment ions corresponded to $[M + H - \text{glucose}]^+$ by HPLC-ESI-MS^2 using Agilent 1100 series (Agilent Technologies, Waldbronn, Germany) in the positive ionization mode.

Relative Water Content (RWC). To characterize the plant water status, the RWC was determined in the youngest fully expanded leaves. Leaf discs of 8 mm diameter were punched using a cork borer, weighed immediately after sampling to determine fresh weight (FW), and placed in distilled water for 24 h at 20 °C under dim illumination. Turgid weight (TW) was obtained after blotting. Thereafter, the leaf discs were dried at 70 °C to a constant weight for determining the DW. The RWC was calculated as RWC = (FW − DW)/(TW − DW).

Statistical Analysis. The concentrations of individual glucosinolates in leaves were analyzed using multifactorial analysis of variance (ANOVA), and least-significant differences were calculated with the Tukey's honest significant difference (HSD) test. All statistical tests were performed at a significance level of $P < 0.05$. Calculations were carried out using the software package Statistica for Windows (version 6.1, Statsoft Inc., Tulsa, OK).

Table 1. Glucosinolates in Two Lines of *B. carinata* under Two Water Supply Treatments at the End of the Drought Cycle[a]

		glucosinolates (mg g^{-1} DW)							
line	water supply	5-MSP	2P	3B	2H3B	3IM	4H3IM	4M3IM	1M3IM
Holeta-1	W1	0.01 a	6.10 b	0.02 a	0.03 a	0.05 b	0.01 a	0.02 a	0.01 a
	W2	0.01 a	11.02 a	0.02 a	0.02 a	0.11 a	0.02 a	0.02 a	0.01 a
37-A	W1	0.01 a	3.22 b	0.02 a	0.03 a	0.06 b	0.01 a	0.01 a	0.01 a
	W2	0.01 a	7.34 a	0.02 a	0.03 a	0.12 a	0.02 a	0.01 a	0.01 a

[a] Tukey's HSD, least HSD; each value represents the mean of four samples. Mean values are compared for each *B. carinata* line and each glucosinolate. Values followed by the same letter are not significantly different. 5-MSP, 5-methylsulfinylpentyl; 2P, 2-propenyl; 3B, 3-butenyl; 2H3B, 2-hydroxy-3-butenyl; 3IM, 3-indolylmethyl; 4H3IM, 4-hydroxy-3-indolylmethyl; 4M3IM, 4-methoxy-3-indolylmethyl; and 1M3IM, 1-methoxy-3-indolylmethyl.

RESULTS AND DISCUSSION

The aliphatic 2-propenyl glucosinolate and the indole 3-indolylmethyl glucosinolate were quantitatively determined at all leaf stages in both lines. Also, the aliphatic 5-methylsulfinylpentyl glucosinolate, 3-butenyl glucosinolate, and 2-hydroxy-3-butenyl glucosinolate were assessed in traces during the entire vegetative growth period (**Tables 1**−**3**). In addition to 3-indolylmethyl glucosinolate, the group of indole glucosinolates also comprised 4-hydroxy-3-indolylmethyl, 4-methoxy-3-indolylmethyl, and 1-methoxy-3-indolylmethyl glucosinolates but only in trace concentrations (**Tables 1** and **3**). The predominant glucosinolate in the leaves was 2-propenyl glucosinolate followed by 3-indolylmethyl glucosinolate.

In both lines under optimal water supply (W1), the 2-propenyl and 3-indolylmethyl glucosinolate concentrations remained

Table 2. 2-Propenyl Concentrations (mg g^{-1} DW) in Two Lines of *B. carinata* during Vegetative Development under Two Water Supply Treatments[a]

		leaf stage				
line	water supply	6−8	11−12	13−14	15−16	HSD
Holeta-1	W1	4.84 cd	2.86 d	4.74 cd	6.10 c	2.88
	W2	5.10 cd	8.02 b	7.48 b	11.02 a	
37-A	W1	3.95 ab	4.23 ab	4.35 ab	3.22 b	3.37
	W2	2.59 b	3.19 b	4.01 ab	7.34 a	
line (L)	*					
leaf stage (LS)	*					
water supply (W)	*					
L × LS	NS					
L × W	*					
LS × W	*					
L × LS × W	*					

[a] Tukey's HSD, least HSD; multifactorial ANOVA; NS, not significant; *$P < 0.05$. Each value represents the mean of four samples. Mean values are compared for each *B. carinata* line. Values followed by the same letter are not significantly different.

Table 3. 3-Indolylmethyl Concentrations (mg g^{-1} DW) in Two Lines of *B. carinata* during Vegetative Development under Two Water Supply Treatments[a]

		leaf stage				
line	water supply	6−8	11−12	13−14	15−16	HSD
Holeta-1	W1	0.04 b	0.02 b	0.04 b	0.05 b	0.04
	W2	0.04 b	0.05 b	0.03 b	0.11 a	
37-A	W1	0.04 b	0.06 b	0.05 b	0.06 b	0.05
	W2	0.03 b	0.04 b	0.04 b	0.12 a	
line (L)	*					
leaf stage (LS)	*					
water supply (W)	*					
L × LS	NS					
L × W	*					
LS × W	*					
L × LS × W	*					

[a] Tukey's HSD, least HSD; multifactorial ANOVA; NS, not significant; *$P < 0.05$. Each value represents the mean of four samples. Mean values are compared for each *B. carinata* line. Values followed by the same letter are not significantly different.

almost constant from the 6−8 to the 15−16 leaf stage (**Tables 2** and **3**). This is in line with investigations on *Brassica napus*, where the developmental stage was also found not to affect the concentrations of aliphatic 3-butenyl and 4-pentenyl glucosinolates as well as indole 3-indolylmethyl glucosinolate and aromatic phenylethyl glucosinolate (*32*). Moreover, in fully developed leaves of *Brassica juncea*, with the major glucosinolate being 2-propenyl glucosinolate, the total glucosinolate concentration remained unchanged (*33*). However, in *Nasturtium officinale* leaves, phenylethyl isothiocyanate, the hydrolysis product of phenylethyl glucosinolate, increased up to 40 days after emergence with no further increase in ongoing vegetative development (*10*). Taken together, these findings indicate that ontogenetic variation of individual glucosinolates in *Brassica* leaves is species-specific.

A limited water supply is known to increase the glucosinolate concentration in several Brassica species adapted to temperate climates, for example, *N. officinale* (*19*, *20*), *B. oleracea* var. *capitata* (*21*), *B. oleracea* var. *italica* (*22*), and *B. napus* (*23*). However, the intensity of drought appears to be a decisive factor in glucosinolate accumulation, since under mild drought stress, glucosinolate concentrations did not increase in *B. napus* plants (*23*) and decreased in *Brassica oleracea* var. *italica* (*24*).

Table 4. Vegetative Growth Parameters of Two Lines of *B. carinata* under Two Water Supply Treatments at the End of the Drought Cycle[a]

parameter	line	W1	W2	relative reduction (%)
leaf length (cm)	Holeta-1	16.02 a	5.10 b	68.16 a
	37-A	13.40 a	5.12 b	61.79 b
leaf area (cm^2)	Holeta-1	1110 a	305 b	72.52 a
	37-A	1200 a	302 b	74.83 a
plant height (cm)	Holeta-1	15.20 a	9.12 b	40.00 a
	37-A	12.00 a	9.07 b	24.42 b
plant dry matter (g)	Holeta-1	4.80 a	1.27 b	73.54 a
	37-A	5.15 a	1.85 b	64.08 b

[a] Each value represents the mean of four samples. Mean values of W1 and W2 are compared for each *B. carinata* line. Mean values of the relative reduction are compared for each vegetative growth parameter. Values followed by the same letter are not significantly different. W1, optimal water supply; W2, limited water supply; relative reduction (%): 100 × (W1 − W2)/W1.

Table 5. RWC (%) in Two Lines of *B. carinata* during Vegetative Development under Two Water Supply Treatments[a]

		leaf stage				
line	water supply	6−8	11−12	13−14	15−16	HSD
Holeta-1	W1	80.49 a	80.67 a	82.93 a	81.16 a	7.04
	W2	73.32 b	60.94 cd	61.13 c	50.28 d	
37-A	W1	80.02 a	79.00 ab	82.23 a	82.49 a	5.44
	W2	74.60 b	63.38 c	61.91 c	52.56 d	
line (L)	NS					
leaf stage (LS)	*					
water supply (W)	*					
L × LS	NS					
L × W	NS					
LS × W	*					
L × LS × W	NS					

[a] Tukey's HSD, least HSD; multifactorial ANOVA; NS, not significant; *$P < 0.05$. Each value represents the mean of four samples. Mean values are compared for each *B. carinata* line. Values followed by the same letter are not significantly different.

In comparison with the regularly watered treatment (W1), the drought stress applied in our experiment (W2) led to a distinct increase (80−120%) of 2-propenyl and 3-indolylmethyl glucosinolate concentration in the leaves of both lines at the final leaf stage (**Tables 2** and **3**). The response of the two lines to the reduced water supply (W2) varied considerably in 2-propenyl and 3-indolylmethyl concentrations. In Holeta-1, 2-propenyl concentration increased immediately between the 6−8 and the 11−12 leaf stage (from 5.10 to 8.02 mg g^{-1} DW) where the soil−water content was ≤40%, and the largest 2-propenyl glucosinolate concentration was obtained at the 15−16 leaf stage (11.02 mg g^{-1} DW), whereas 37-A was mainly marked by a late but considerable 2-propenyl glucosinolate increase between the 13−14 and the 15−16 leaf stage (from 4.01 to 7.34 mg g^{-1} DW) at a soil−water content ≤17% (**Table 2**), resulting in an approximately 2-fold enhancement of the 2-propenyl concentration in the final leaf stage (15−16). For the W2 treatment, no differences were found for indole 3-indolylmethyl glucosinolate between both lines (**Table 3**). However, a doubling of 3-indolylmethyl glucosinolate occurred in both lines at the end of the drought cycle between the 13−14 and the 15−16 leaf stage (Holeta-1, 0.11 mg g^{-1} DW; and 37-A, 0.12 mg g^{-1} DW) when the soil water content dropped to < 17%. Thus, the glucosinolate accumulation in *B. carinata* at limited water supply was dominated by its major glucosinolate 2-propenyl. In *B. rapa* ssp. *rapifera*, also the predominant phenylethyl glucosinolate was reported to show the strongest increase under drought stress (*34*). The increase of the alkenyl glucosinolate 2-propenyl in *B. carinata* adapted to

Table 6. Correlations between Individual Glucosinolates (GS) and the Relative Water Content (RWC) in the Leaves of Two Lines of *B. carinata*[a]

line	samples	equation	R^2	*F* value
Holeta-1	W1 + W2 (RWC values <80%)	[2-propenyl GS] = 17.80 − 0.16 × RWC	0.59*	41.64
	W1 + W2 (RWC values <80%)	[3-indolylmethyl GS] = 0.15 − 0.0015 × RWC	0.39*	18.71
37-A	W2 (RWC values <75%)	[2-propenyl GS] = 14.58 − 0.17 × RWC	0.48*	13.05
	W2 (RWC values <75%)	[3-indolylmethyl GS] = 0.23 − 0.0028 × RWC	0.44*	10.85

[a] *$P < 0.05$. Holeta-1: Sample size (including W1 and W2 samples, RWC values <80%), $n = 32$; degrees of freedom, $n = 29$; and critical *F* value = 4.18. 37-A: Sample size (including W2 samples; RWC values <80%), $n = 16$; degrees of freedom, $n = 13$; and critical *F* value = 4.67. W1, optimal water supply; and W2, limited water supply.

semiarid and arid conditions was as strong as the alkenyl glucosinolate rise (3-butenyl glucosinolate) in *B. rapa* ssp. *rapifera* (*34*) under reduced water conditions, which is adapted to temperate regions, suggesting a drought-induced glucosinolate accumulation independent of the climate adaptation of the *Brassica* species.

The enhanced concentrations of methylsulfinyl glucosinolates (3-methylsulfinylpropyl, 4-methylsulfinylbutyl glucosinolates) in *B. oleracea* ssp. (*21*, *22*) and in *B. rapa* ssp. *rapifera* (*34*) by limited water supply might indicate an increased oxidation of the methylthio group in alkyl glucosinolate side chains. In combination with increasing alkenyl glucosinolate concentrations (2-propenyl and 3-butenyl glucosinolates), as it was found in *B. carinata* (**Table 2**), *B. oleracea* var. *capitata* (*21*), and *B. rapa* ssp. *rapifera* (*34*) under drought stress, also an enhanced alkenylization in the side chain modification process of the corresponding alkyl glucosinolate in these *Brassica* species could be assumed. This shift within the aliphatic glucosinolate profile to an emphasis on methylsulfinyl and alkenyl glucosinolates could be attributed to drought stress. Because *B. carinata* as a hybrid amphidiploid species is succeeded by the parentals *B. oleracea* and *B. nigra* and genome evolution of *B. rapa* is based on wild *B. oleracea* (*35*). These genetic relationships between *B. oleracea* and *B. carinata* and *B. rapa*, respectively, might explain the similar aliphatic glucosinolate response to limited water supply.

As found in *B. carinata*, limited water supply also increased the indole glucosinolate concentration in leaves of *B. oleracea* (*21*), whereas in *B. oleracea* var. *italica* indole glucosinolates decreased (*24*) and in *B. rapa* ssp. *rapifera* indole glucosinolates were unaffected by reduced available soil water conditions (*34*), suggesting genotypically different responses to drought also with respect to indole glucosinolates.

In *B. carinata*, the drought-induced increase in glucosinolate concentration, especially 2-propenyl and 3-indolylmethyl glucosinolates, might be considered as an unspecific stress response. Similarly, other studies report that abiotic stress factors, for example, CO_2 enrichment in the atmosphere, lead to increased glucosinolate concentrations in *B. oleracea* varieties (*13*, *36*). Our results strongly suggest different drought stress sensitivities of the two *B. carinata* lines: Holeta-1 had already accumulated the major glucosinolate 2-propenyl glucosinolate at a soil water content of less than 40% from 8.02 mg g^{-1} DW up to 11.02 mg g^{-1} DW, whereas 37-A showed this response first at a soil water content less than 17% up to a distinctly lower concentration of 7.34 mg g^{-1} DW. This different sensitivity to limited water supply was also reflected by a mostly stronger relative reduction of leaf length, leaf area, plant height, and plant dry matter of Holeta-1 as compared with 37-A at the end of the drought cycle (**Table 4**). Because limited water supply caused a reduction in several vegetative growth parameters, although to different extents in both *B. carinata* lines, this restricted growth could cause a shift from growth-related primary metabolism to a preferred allocation of carbon and nitrogen into the secondary metabolism (*37*), thereby promoting the biosynthesis of carbon- and nitrogen-based secondary metabolites such as glucosinolates.

In both *B. carinata* lines, the increase in leaf glucosinolate concentrations was inversely correlated with the RWC of the leaves. This indicates that the water status of the plant as influenced by the water supply and hence by the soil water content is linked with the glucosinolate metabolism (**Tables 5** and **6**). In 37-A, 2-propenyl and 3-indolylmethyl glucosinolate concentrations showed a linear increase when the RWC was less than 75%. At RWCs above 75%, concentrations of these glucosinolates were nearly unchanged (data not shown). For every 10% drop in RWC of 37-A, the 2-propenyl concentration increased by 1.7 mg g^{-1} DW and 3-indolylmethyl concentration by 0.028 mg g^{-1} DW (**Table 6**). Similarly, in Holeta-1, when the RWC dropped below 80%, 2-propenyl and 3-indolylmethyl glucosinolate concentrations started to increase linearly. A 10% drop in RWC also led to an increase of 1.6 mg g^{-1} DW of 2-propenyl glucosinolate as well to an increase of 0.015 mg g^{-1} DW of 3-indolylmethyl glucosinolate (**Table 6**). Similarly, Jensen et al. (*23*) reported a correlation between total glucosinolate concentration in *B. napus* seeds and the plant's water status. There, total glucosinolates accumulated when the RWC was less than 82% comparable to *B. carinata*, especially for Holeta-1, in our study. Total glucosinolates in seeds of *B. napus* increased by about 7.0 mg g^{-1} DW per MPa decrease in the water potential corresponding to a drop in RWC of about 2% (*23*). The stronger response to drought stress of *B. napus* seeds might be due to the generally higher glucosinolate concentration in seeds as compared with vegetative plant parts (*9*, *31*, *38*). In *B. napus*, Stroeher et al. (*39*, *40*) have identified drought-induced genes with an up to 6-fold increase in their expression at a RWC of 63% in the leaves. This finding suggests that these drought-induced genes might be responsible for adaptation to osmotic stress and may thus also be responsible for the increase in glucosinolates as drought response. The involvement of glucosinolates in the process of osmotic adjustment is supported by Lopez-Berenguer et al. (*41*) who observed increasing glucosinolate concentrations in salt-treated *B. oleracea* var. *italica*, although the sulfur concentration was unchanged during the salt treatments.

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