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Environmental and Genetic Effects on Yield and Secondary Metabolite Production in *Brassica rapa* Crops

Marta Francisco,* María Elena Cartea, Ana María Butrón, Tamara Sotelo, and Pablo Velasco

Misión Biológica de Galicia (CSIC), P.O. Box 28, E-36080, Pontevedra, Spain

ABSTRACT: Twelve *Brassica rapa* varieties grown, such as turnip green and turnip top, were evaluated in seven environments to determine the environmental and genotypic variables that have an influence on crop production and on the content of glucosinolates and phenolic compounds. Factorial regression analysis showed that, in general, crop production was favored by high temperatures all along the crop cycle. However, the lack of a period of intense cold could be a limiting factor. The metabolite content seems to be regulated by extreme temperatures (daily maximum and minimum temperatures) rather than by average daily temperatures. With regard to genotypic covariables, turnip top production was significantly affected by traits related to the vegetative development and time to flowering. Meanwhile, turnip green production was largely affected by a sinapoyl derivative compound, which is a precursor of cell wall components. Cross-talk between glucosinolate biosynthesis and phenylpropanoid signaling pathways is suggested.

KEYWORDS: *Brassica rapa, environmental covariates, factorial regression, genotypic covariates, secondary metabolites, yield*

INTRODUCTION

Brassica rapa is an important species of the genus *Brassica*, cultivated worldwide, which includes a variety of vegetable crops such as Chinese cabbage, Pak-choi, turnip, and turnip green as well as oilseed crops such as turnip rape and sarson.[1] Like all *Brassica* crops, this species contains secondary metabolites, mainly glucosinolates (which are found almost exclusively in the Brassicaceae family) and phenolic compounds, including flavonoids and hydroxycinnamic acids.[1−4] The presence of these compounds in the diet has increased for the past years because of their beneficial health properties.[5−7]

In Galicia (northwestern Spain) and in the coldest regions of Portugal, *B. rapa* ssp. *rapa* L. includes turnip green and turnip top as vegetable products for culinary profit as well as turnips for fodder.[8] Turnips are the thickened roots, turnip greens are the young leaves harvested in the vegetative period, while turnip tops are the floral shoots and surrounding leaves. In these regions, they constitute a unique supply of vegetables during winter[2] together with other *Brassica* crops.

Both yield and quality-related characters, such as health-beneficial compounds content, have important implications on *Brassica* crop cultivation. All of these traits are genetically controlled, although genetic control varies widely across species, or even families, and depends on the plant stage of development.[6,9,10] On the other hand, there are many environmental factors that play a role in regulating the pathways involved in the synthesis of glucosinolate and phenolic compounds.[11−13] Moreover, unfavorable external conditions may produce a stress on plants, resulting in lower yields.[14] However, there have been few attempts to analyze the effect of the interaction between genetic and environmental factors on yield and on the variation of metabolite content in *Brassica* crops. In our recent studies,[15,16] 12 varieties of a collection of *B. rapa* ssp. *rapa* from northwestern Spain, currently kept at the Misión Biológica de Galicia (CSIC, Spain), were agronomically and nutritionally evaluated in different environments. The stability of the genotypes and the effect of the environment (E), genotype (G), and genotype × environment interaction (GE) on crop production and metabolite content were determined by using the sites regression method (SREG). These studies showed a significant GE interaction in the traits studied, which means differential responses of genotypes in different environments. The SREG method's disadvantage is that it is unable to incorporate additional information about genotypes and environments and provides no explanation of the GE interaction.[17] Therefore, little is known about the most relevant environmental or genotypic variables that determine *B. rapa* crop quality.

When information on environmental and/or genotypic variables is available, such as meteorological data, earliness, or time to flowering, other statistical models, including factorial regression models,[18] can be used to determine which environmental and genotypic variables influence G, E, and GE interaction of the trait studied. Factorial regression models are usually linear models accounting for GE interaction by differential cultivar sensitivity, which can be explained in part by differences in genotypic characteristics, to explicit environmental covariables. The influence of these variables on GE interaction can be tested statistically. Environmental and genotypic characteristics are regressed on main additive effects and/or on interaction terms.[19,20]

Crop production and metabolite synthesis are complex traits. Their contribution to variation is usually unexplained. For the first time, this actual work has been aimed to determine which genotypic and environmental covariates explain the G, E, and GE effects on turnip green and on turnip top production and

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the metabolite content on 12 local varieties tested in seven environments.

MATERIALS AND METHODS

Plant Material. Twelve local varieties of *B. rapa* were evaluated in this study. Ten varieties were chosen based on the study carried out by Padilla et al.[8] because of their high fresh production of turnip top and/or turnip green (MBG-BRS0082, MBG-BRS0143, MBG-BRS0173, MBG-BRS0184, MBG-BRS0401, MBG-BRS0433, MBG-BRS0451, MBG-BRS0461, MBG-BRS0472, and MBG-BRS0550). In addition, two varieties were obtained after three cycles of mass selection for fresh yield [MBG-BRS0163(S)C3 and MBG-BRS0197(S)C3]. Varieties were transplanted in 3 years (2006, 2007, and 2008) at three locations that represent standard *B. rapa* production areas in northwestern Spain: Oroso (A Coruña) (43°1′N, 8°26′W, 280 m.a.s.l.), Guitiriz (Lugo) (43°12′N, 7°53′W, 516 m.a.s.l), and Salcedo (Pontevedra) (42°24′N, 8° 38′W, 20 m. a s.l). Two trials were lost in Salcedo due to unfavorable climatic conditions in 2006 and to plant damage caused by *Delia radicum* L. immediately after transplanting in 2007. Varieties were transplanted in a randomized complete block design. Morphological and agronomical traits were recorded along the maturity cycle as it has been described in our previous works.[15,16]

Extraction and Determination of Glucosinolates and Phenolic Compounds. The secondary metabolite analysis was carried out by following the multipurpose chromatographic method that separates glucosinolates and phenolics simultaneously.[4] A portion of 150 mg from each sample was extracted in 4 mL of 70% MeOH at 70 °C for 30 min with vortex mixing every 5 min to facilitate the extraction. Samples were centrifuged (13000*g*, 15 min), 1 mL of supernatants was collected, and methanol was completely removed by using a sample concentrator (DB-3D, Techne, United Kingdom) at 70 °C. The dry material obtained was redissolved in 1 mL of ultrapure water and filtered through a 0.20 μm syringe filter (Acrodisc Syringe Filters, Pall Life Sciences). Chromatographic analyses were carried out on a Luna C18 column (250 mm × 4.6 mm, 5 μm particle size; Phenomenex, Macclesfield, United Kingdom). The mobile phase was a mixture of (A) ultrapure water/trifluoroacetic acid (TFA) (99.9:0.1) and (B) methanol/TFA (99.9:0.1). The flow rate was 1 mL min^{-1} in a linear gradient starting with 0% B at 0−5 min, reaching 17% B at 15−17 min, 25% B at 22 min, 35% B at 30 min, 50% B at 35 min, 99% B at 50 min, and 0% B at 55−65 min. The injection volume was 20 μL, and chromatograms were recorded at 330 nm for phenolic derivatives and at 227 nm for glucosinolates in a model 600 high-performance liquid chromatography (HPLC) instrument (Waters), equipped with a model 486 UV tunable absorbance detector (Waters). Glucosinolates were quantified by using sinigrin (sinigrin monohydrate from Phytoplan, Diehm and Neuberger GmbH, Heidelberg, Germany) as the standard. Caffeoylquinic and *p*-coumaroylquinic acid derivatives were quantified as chlorogenic acid (5-caffeoylquinic acid, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), flavonoids were quantified as kaempferol 3-rutinoside (Extrasynthese, Genay, France), and sinapic acid and derivatives were quantified as sinapic acid (Sigma, St. Louis, MO).

Dependent Variables. The present study focuses on explaining variability of fresh crop production and the secondary metabolite content (glucosinolates and phenolic compounds) in turnip green and in turnip top. Among the secondary metabolites present in these crops, we will focus on those that showed a higher concentration and variability. Thus, traits used as dependent variables were turnip green fresh production (average fresh weight of a leaf, expressed in g), turnip top fresh production (turnip top fresh matter × number of secondary stems, expressed in kg), and the contents of gluconapin, sinapic acid, 1-sinapoyl-2-feruloylgentiobioside, kaempferol-3,7-di-*O*-glucoside, and isorhamnetin-3,7-di-*O*-glucoside.

Genotypic Covariates. As stated before, genotypic covariates included agronomical and nutritional traits. The agronomical traits were turnip green fresh production, turnip top fresh production, time to turnip top production (days from transplanting until 50% of plants have the first turnip top), turnip top production period (difference between the time to flowering and the time to turnip top production), time to flowering (days from transplanting until 50% of plants have the first flower), early vigor (visual rating recorded 1 month after transplanting), and late vigor (visual rating recorded 3 months after transplanting). Glucosinolates studied as nutritional factors were progoitrin, glucoraphanin, gluconapin, 4-hydroxyglucobrassicin, glucobrassicanapin, glucobrassicin, neoglucobrassicin and gluconasturtiin. Flavonoids were kaempferol-3-*O* (methoxycaffeoyl) sophoroside-7-*O*-glucoside, kaempferol-3-*O* (caffeoyl) sophoroside-7-*O*-glucoside, kaempferol-3,7-di-*O*-glucoside, and isorhamnetin-3,7-di-*O*-glucoside. Hydroxycinnamic acids were sinapic acid, 1,2-disinapoylgentiobioside, and 1-sinapoyl-2-feruloylgentiobioside.

Environmental Covariates. Climatic data were obtained from meteorological stations located at the experimental fields; data were recorded every 10 min. On the basis of these data, averages of maximum, minimum, and mean temperatures and degree days throughout the crop cycle were calculated. Thus, the environmental covariates were mean of daily maximum temperature (maximum temperature), mean of daily minimum temperature (minimum temperature), average of daily mean temperature (mean temperature), number of days with a maximum temperature over 30 °C, number of days with a maximum temperature over 20 °C, number of days with a mean temperature over 20 °C, number of days with a mean temperature below 10 °C, number of days with a minimum temperature below 10 °C, and number of days with a minimum temperature below 0 °C. In addition, the same climatic variables were computed each month during the growing period of the plant.

Factorial Regression. Each environment was considered as the combination of a location and a year for this analysis. The general form for a factorial regression model with K genotypic and H environmental covariates is[18−21]

$$Y_{ij} = \mu + \left[\sum \rho_k \cdot G_{ik} + \alpha_i\right] + \left[\sum \delta_h \cdot E_{jh} + \beta_j\right] + \left[\sum G_{ik} \cdot \theta_{kh} \cdot E_{jh} + \sum \alpha_{ih}' \cdot E_{jh} + \sum \beta_{jk}' \cdot G_{ik} + \varepsilon_{ij}\right]$$

where ρ_k and δ_h are the regression coefficients of genotypic (G_{ik}) and environmental covariates (E_{jk}), respectively; α_i and β_j are the residuals of genotype and environmental main effects, respectively; θ_{kh} is the regression coefficient of the cross-product of covariates G_{ik} and E_{jh}; and α_{ih}' and β_{jk}' are the genotype i and environment j specific regression coefficients of E_{jh} and G_{ik}, respectively. The term ε_{ij} is the residual interaction effect. All sources of variation were considered fixed. Stepwise regressions of each dependent variable averaged across environments or genotypes were performed to determine which genotype and environmental covariates, respectively, should be used in the factorial regression model.[18−22] After the standardization of covariates, factorial regression analyses were performed by using the computer package INTERA.[23] All terms were tested with the residual experimental error.

RESULTS AND DISCUSSION

Fresh Production. Results showed that E seems to be decisive for variation in turnip green production (Figure 1A), whereas variation in E, G, and GE had similar effects on variation in turnip top production (Figure 1B). Factorial regression analyses showed that the number of days with a maximum temperature over 20 °C, degree days of maximum temperatures, and the maximum temperature in November significantly affected the E component of fresh turnip green production variability. They also explained 45, 28, and 20% of E variation, respectively (Figure 1A); the residual effect was not significant. The number of days with a maximum temperature over 20 °C and degree days of maximum temperatures had significant and positive effects on fresh production with regression coefficients of δ = 7.12 and δ = 7.59, respectively. On the other hand, the maximum temperature in November had a negative effect on this trait (δ = −9.21). Many research

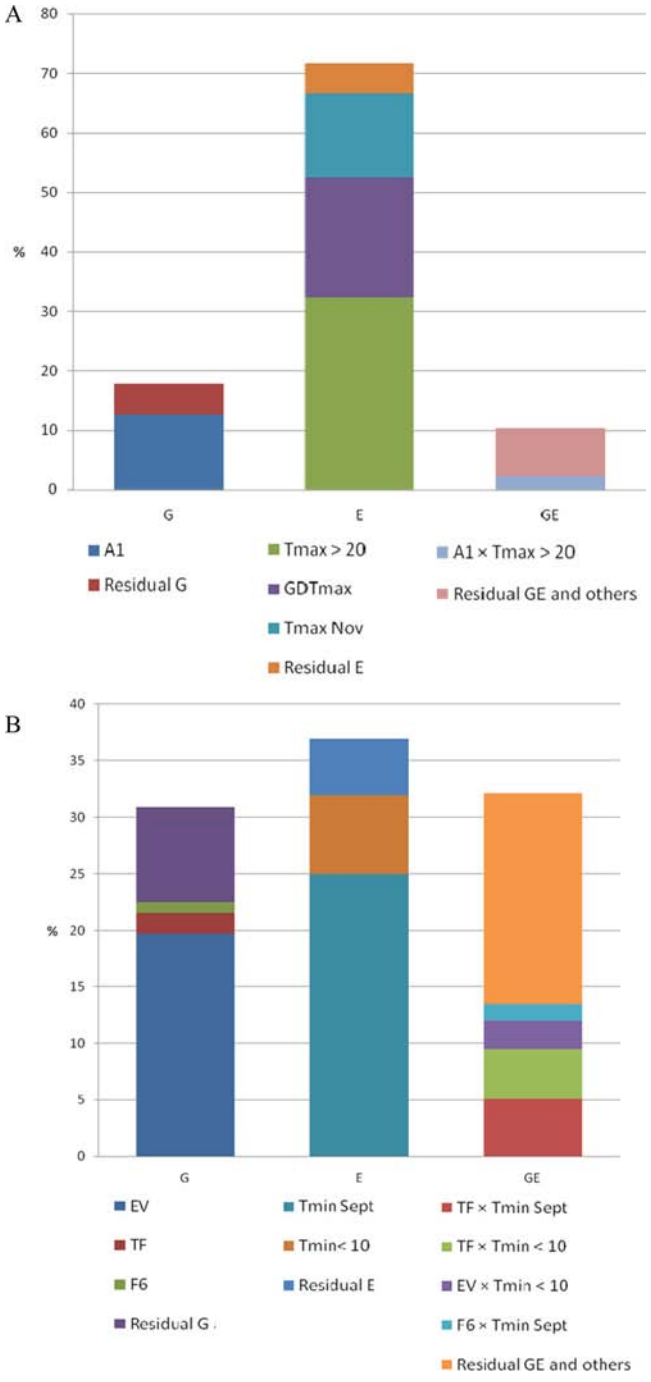

Figure 1. Estimated proportion of variation genotype (G), environment (E), and genotype × environment (GE) interactions explained by each covariate for turnip green (A) and turnip top (B) fresh production of 12 turnip green and turnip top varieties evaluated in seven environments. Environmental and genotypic covariates used in the factorial regression analysis were previously detected with the stepwise method. A1, 1,2-disinapoylgentiobioside; Tmax > 20, number of days with maximum temperature over 20 °C; GDTmax, degree days of maximum temperatures; TmaxNov, average of daily mean temperature of November; EV, early vigor; TF, time to flowering; F6, isorhamnetin-3,7-di-*O*-glucoside; TminSept, mean of daily minimum temperature of September; and Tmin < 10, number of days with a mean temperature below 10 °C.

studies have shown that *B. rapa* crop yields vary from year to year due to weather conditions.[24−26] In this work, we found that, in general, like it happens to other *Brassica* crops,[27] turnip green production increases with moderate to high maximum temperatures all along the crop cycle. Nevertheless, during November, just before harvest, turnip green production was favored by a low temperature. It is possible that, at this point of the growing plant cycle, higher temperatures could induce early flowering, thus stopping the vegetative growth and therefore decreasing production.

Among the genotypic covariates studied, only one had a significant effect on G variation in turnip green fresh production; the residual was not significant (Figure 1A). The 1,2-disinapoylgentiobioside content explained more than 70% of the variation in G and had a positive regression coefficient on fresh production (ρ = 4.35), thus indicating that turnip green varieties with a higher content of 1,2-disinapoylgentiobioside are more productive. Although a great deal is still unknown regarding the roles of phenolic acids in plants, they have been related to diverse functions, including nutrient uptake, protein synthesis, enzyme activity, photosynthesis, and allelopathy,[28,29] which may have an influence on crop production.

For turnip top, environmental covariates detected as significant by the stepwise method were the minimum temperature in September and the number of days with a minimum temperature below 10 °C, whereas early vigor, days to flowering and isorhamnetin-3,7-di-*O*-glucoside content were detected as significant genotypic covariates (Figure 1B).

The minimum temperature in September together with the number of days with a minimum temperature below 10 °C explained 85% of the E variability (Figure 1B) approximately and had significant and positive effects on turnip top fresh production (δ = 684.99 and δ = 274.81, respectively). These results indicate that a period of low temperatures is decisive to increase turnip top production. The effect of low temperature on bolting of other *B. rapa* crops, such as Chinese cabbage, has been confirmed.[26,30,31] It is generally accepted that mean daily temperatures higher than 15−18 °C during plant growth significantly reduce bolting as compared with lower temperature.[32]

Early vigor, days to flowering, and isorhamnetin-3,7-di-*O*-glucoside content explained approximately 64, 6, and 3% of variation in G, respectively. The residual was significant and accounted for 27% of G variation (Figure 1B). The coefficient of regression of early vigor was negative (ρ = −12.87), whereas those for time to flowering and isorhamnetin-3,7-di-*O*-glucoside content were positive (ρ = 308.15 and ρ = 173.74, respectively). Nonvigorous genotypes at early stage had better turnip top production, thus indicating that the excess of vegetative development may limit the available nutrients to the reproductive stage. The earliest genotypes with rapid leaf area development yield less than the latest genotypes. The coefficient of regression of turnip top production on isorhamnetin-3,7-di-*O*-glucoside content was also positive; flavonoids exhibit a diverse spectrum of biological functions and play an important role in the plant−environment interaction, which may affect crop yield.

The factorial regression model explained more than 85% of variation due to the GE interaction in turnip top fresh production, although the residual GE was significant. Six GE covariate cross-products explained a large portion of variation (45%), and four of them were significant (Figure 1B).

The regression coefficients of turnip top production on the covariate cross-products early vigor × number of days with minimum temperatures below 10 °C, days to flowering × minimum temperature in September, days to flowering ×

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number of days with minimum temperatures below 10 °C, and isorhamnetin-3,7-di-*O*-glucoside content × minimum temperature in September were positive (θ = 89.59, θ = 731.84, θ = 117.24, and θ = 202.93, respectively). In general, climatic conditions that favored turnip top production were especially beneficial for genotypes with higher vegetative development. It is well-known that long cycle varieties require longer cool periods for effective vernalization, while long cool periods could be stressful for short cycle varieties.

Among the remaining covariate cross-products, variability in GE was largely explained by the interaction of environmental covariates with the residual genotype variation. There were significant genotype-specific responses to the minimum temperature in September, which could not be explained by differences in any of the genotypic covariates used.

Secondary Metabolites. *Glucosinolates.* Genotypic and environmental variation in eight different glucosinolates belonging to the three chemical classes (aliphatic, indolic, and aromatic) was studied in turnip green and turnip top varieties. Factorial regression analyses only detected significant covariates in gluconapin content. This was expected since gluconapin was the main glucosinolate, representing more than 60% of the total glucosinolate content (mean value of 10.21 μmol g^{-1} dw for turnip green and 17.39 μmol g^{-1} dw for turnip top).

E and G main effects were of great importance for gluconapin content variation in turnip green (Figure 2A). The factorial regression analysis showed that the number of days with a minimum temperature below 0 °C was the only significant environmental covariate, whereas time to flowering and the glucobrassicanapin content were detected as significant genotypic covariates.

The number of days with a minimum temperature below 0 °C explained 63% of the E variation and had negative effects on gluconapin content (δ = −2.95); the residual effect was significant. As it has already been reported, temperatures clearly affect the glucosinolate content in many *Brassicaceae* species. Several studies indicate that winter or autumn seasons seem to lead to lower glucosinolate content due to short days, cool temperatures, and less radiation.[12,33−35]

Time to flowering explained 23% of G variation in gluconapin content in turnip greens, and glucobrassicanapin content explained 15% of G variation (Figure 2A). As most G variation (62%) has not been explained by the model, other variables should have been included to achieve a better understanding of the variation among genotypes. Thus far, no studies have been reported on how plant earliness affects glucosinolate content. The negative coefficient of regression of time to flowering (ρ = −2.04) suggested that earliness favors gluconapin synthesis. The regression coefficient of glucobrassicanapin content was positive (ρ = 1.46). It was expected since both glucosinolates are aliphatic, and their syntheses are controlled by the same genetic system, except for one elongation cycle.[6,7]

The factorial regression model explained 49% of variation due to the GE interaction for total gluconapin content in turnip green, and the residual GE was not significant. Variability in GE was largely explained by the interaction of the number of days with a minimum temperature below 0 °C with the residual genotype variation (26%) (Figure 2A). The interaction of time to flowering with residual environment was significant and explained more than 7% of GE (Figure 2A). Therefore, there were significant environment and genotype-specific responses,

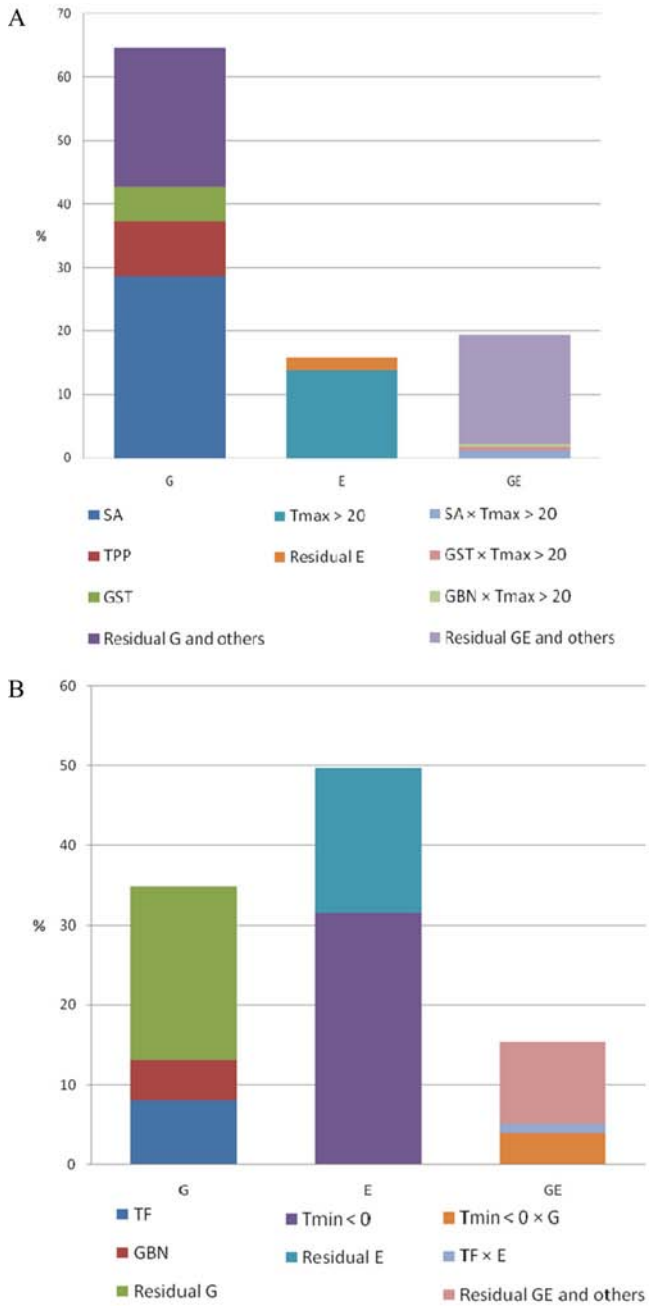

Figure 2. Estimated proportion of variation genotype (G), environment (E), and genotype × environment (GE) interactions explained by each covariate for turnip green (A) and turnip top (B) for gluconapin content of 12 turnip green and turnip top varieties evaluated in seven environments. Environmental and genotypic covariates used in the factorial regression analysis were previously detected with the stepwise method. TF, time to flowering; GBN, glucobrassicanapin; Tmin < 0, number of days with minimum temperature below 0 °C; SA, sinapic acid; TPP, time to turnip top production; GST, gluconasturtiin; and Tmax > 20, number of days with a maximum temperature over 20 °C.

which could not be explained by differences in any of the environmental and genotypic covariates tested.

The factorial regression analysis showed that the number of days with maximum temperatures over 20 °C, which explained 87% of E variation (Figure 2B), had a positive effect on gluconapin content in turnip tops (δ = 1.84). Unlike what

happened to turnip green, for which minimum temperatures seem to be a limiting factor for gluconapin synthesis, for turnip top, variation of the number of days with moderate or high temperatures explains the most variability in gluconapin content, suggesting that extreme temperatures seem to be more important than mean temperatures in the regulation of the synthesis of this major glucosinolate. These results are in agreement with those reported by other authors who found that *Brassica* crops grown under high temperatures significantly increased glucosinolate concentration.[12,35,36]

The G main effects were of great importance for gluconapin content in turnip tops. More than 65% of G variation was explained by variations in sinapic acid and gluconasturtiin contents and in the turnip top production period (Figure 2B), although other covariates, such as the content of 1,2-disinapoylgentiobioside, 1-sinapoyl-2-feruloylgentiobioside, and glucobrassicanapin, were also significant. The concentration of gluconasturtiin, sinapic acid, and 1,2-disinapoylgentiobioside had positive regression coefficients on gluconapin content (ρ = 5.81, ρ = 1.55, and ρ = 0.75, respectively), while turnip top production period and the concentration of 1-sinapoyl-2-feruloylgentiobioside, as well as glucobrassicanapin content, had negative regression coefficients on gluconapin content (ρ = −4.05, ρ = −1.55, and ρ = −0.23, respectively). Glucosinolates and phenolic compounds are secondary metabolites in plants involved in pathogen−plant interactions. Although there are many substrates and products in plant secondary metabolism, there are only a few types of reactions. Cross-talk between different signaling pathways is very common in the plant defense response. Hemm et al.[36] found that the *Arabidopsis ref2* mutant that showed reduced levels of different phenolic acids derived from phenylpropanoid pathway had also reduced the levels of all aliphatic glucosinolates and increased the levels of indolic glucosinolates in their leaves. Therefore, cross-talk among glucosinolate and phenolic signaling pathways may be the cause of the significant correlations between compounds belonging to both groups.

The factorial regression model explained 68% of the GE interaction sums of squares for gluconapin content. Three covariate cross-products were significant (Figure 2B). The regression coefficients of the cross-products between sinapic acid and glucobrassicanapin contents and the number of days with maximum temperatures over 20 °C were negative (θ = −0.36 and θ = −0.82, respectively), while the regression coefficient for the interaction of gluconasturtiin content with the number of days with maximum temperatures over 20 °C was positive (θ = 0.39). This indicated that varieties with a lower content of sinapic acid and glucobrassicanapin and a higher content of gluconasturtiin were especially favored by a larger number of days with maximum temperatures over 20 °C to increase gluconapin content.

Phenolic Compounds. Hydroxycinnamic Acids. The major phenolic compound in both plant organs was the sinapic acid, which reaches 46 and 18% of total hydroxycinnamic acids in turnip green and turnip top, respectively (mean values of 20.25 μmol g^{-1} dw for turnip green and 6.63 μmol g^{-1} dw for turnip top). Besides, the sinapic acid derivative 1-sinapoyl-2-feruloylgentiobioside in turnip top is also a relevant compound, representing 12% of the total phenolic content in this plant organ and varying significantly across genotypes and environments.[16]

Factorial regression analyses showed significant effects on these two major hydroxycinnamic acids. Both compounds were affected by the minimum temperature in November (Figure 3A,B), and the regression coefficients of this covariate were negative (δ = −4.87 and δ= −0.63 for turnip green and top, respectively). On the other hand, the mean temperature and maximum temperature in November had a positive effect on sinapic acid content (δ = 3.19 and δ = 1.62, respectively). These results suggest that warm temperatures during the growth cycle favor hydroxycinnamic acid production, but the drop of minimum temperatures in November seems to be decisive for the synthesis of these compounds. Accumulation of phenolic acids in winter oilseed rape leaves, grapevine, apple trees, and sugar cane subjected to low temperature treatments was also reported.[37,38] The authors suggested that changes in phenolic content and composition could increase the adhesion of the membrane to the cell wall and thus reduce membrane collapse during freeze-induced dehydratation.

Among genotypic variables, only the concentration of total indolic glucosinolates significantly affected the G component of sinapic acid content variability (Figure 3A). This variable explained 44% of the G variation and the regression coefficient was negative (ρ = −1.41). According to the results found by Hemm et al.,[36] our results may suggest again that there is a metabolic link between glucosinolate biosynthesis and phenylpropanoid metabolism.

The genotypic covariates that had significant effects on G variation in 1-sinapoyl-2-feruloylgentiobioside content were the content of another sinapoyl derivative, 1,2-disinapoylgentiobioside, and total flavonoids, explaining 45 and 30% of the variation in G, respectively (Figure 3B); the residual was also significant. The regression coefficients of these two genotypic covariates on 1-sinapoyl-2-feruloylgentiobioside content were positive (ρ = 0.47 and ρ = 0.37, respectively). It was expected since these compounds are synthesized in the same metabolic pathway. The same substrate is common for the enzyme chalcone synthase, which catalyzes the formation of the flavonoid skeleton, and for hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase, which leads to the biosynthesis of sinapyl glucosides, among others.[39]

The factorial regression model explained 60% of the GE interaction sums of squares for sinapic acid content in turnip green and 38% of the GE interaction sums of squares for 1-sinapoyl-2-feruloylgentiobioside content in turnip top. The residual GE was significant for 1-sinapoyl-2-feruloylgentiobioside. No cross-products between the genotypic covariates and the environmental covariates were significant. However, there were environment and genotype-specific responses that could not be explained by differences in any of the environmental and genotypic covariates tested (Figure 3A,B).

Flavonoids. The most abundant class of flavonoids was kaempferol derivatives, which varied between 64 and 75% of total flavonoid content in turnip green and turnip top, respectively. Among them, kaempferol-3-*O* (methoxycaffeoyl) sophoroside-7-*O*-glucoside, kaempferol-3-*O* (caffeoyl) sophoroside-7-*O*-glucoside, and kaempferol-3,7-di-*O*-glucoside were the major kaempferol derivatives in both plant organs (mean values from 1.90 to 3.42 μmol g^{-1} dw). In addition, these *B. rapa* varieties showed high isorhamnetin-3,7-di-*O*-glucoside content, which is a compound derived from isorhamnetin, which was the major flavonoid in turnip top (mean value of 2.96 μmol g^{-1} dw).[16]

Factorial regression analyses showed significant effects of genotypic and environmental covariates on two of the major flavonoids in turnip top (kaempferol-3,7-di-*O*-glucoside and

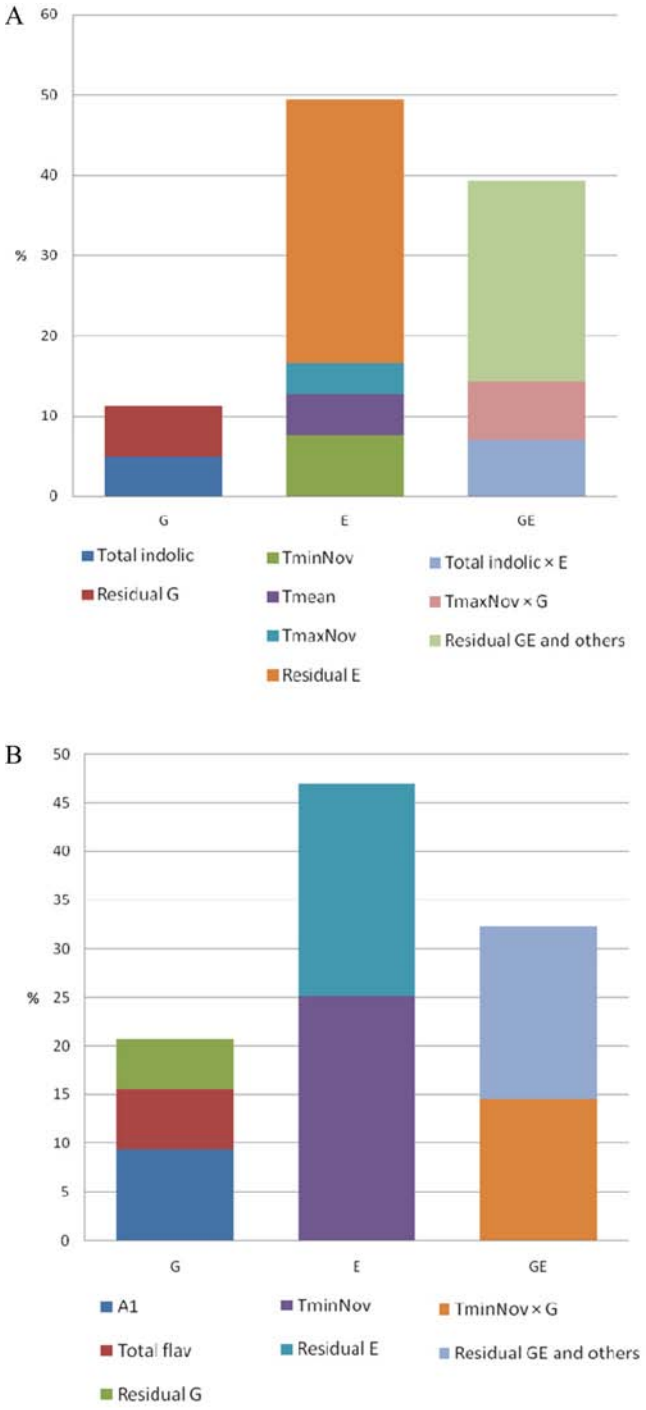

Figure 3. Estimated proportion of variation genotype (G), environment (E), and genotype × environment (GE) interactions explained by each covariate for turnip green (A) and turnip top (B) for sinapic acid and 1-sinapoyl-2-feruloylgentiobioside content of 12 turnip green and turnip top varieties evaluated in seven environments. Environmental and genotypic covariates used in the factorial regression analysis were previously detected with the stepwise method. Total indolic, total indolic glucosinolate content; TminNov, mean of daily minimum temperature in November; Tmean, average of daily mean temperature; TmaxNov, mean of daily maximum temperature in November; A1, 1,2-disinapoylgentiobioside; and Total flav, total flavonoid content.

isorhamnetin-3,7-di-*O*-glucoside). For these two flavonoids, the E main effect accounted for almost 50% of total variation (Figure 4A,B). The mean of the minimum temperature and the number of days with maximum temperatures over 20 °C explained almost 75% of E variation in both flavonoids. The minimum temperature had a negative effect on kaempferol-3,7-di-*O*-glucoside and isorhamnetin-3,7-di-*O*-glucoside content (δ = −0.48 and δ = −0.49, respectively), whereas the number of days with maximum temperatures over 20 °C had a positive effect on these flavonoids (δ = 0.10 and δ = 0.19, respectively). This suggests that contrasting temperatures between day and night promote flavonoid synthesis. Other authors have described an increment of flavonoids at both higher and cooler temperatures related with the induction of freezing resistance[40−42] and with antioxidant activity against harmful radiation,[42] respectively.

Genotypic covariates that showed significant effects on G variation of kaempferol-3,7-di-*O*-glucoside were the content of 1, 2-disinapoylgentiobioside, kaempferol-3-*O* (methoxycaffeoyl) sophoroside-7-*O*-glucoside, and 1-sinapoyl-2-feruloylgentiobioside. These covariates explained 20, 11, and 7% of the G variation, respectively (Figure 4A). The regression coefficient of 1-sinapoyl-2-feruloylgentiobioside was negative (ρ = −0.29), whereas the regression coefficients of 1,2-disinapoylgentiobioside and kaempferol-3-*O* (methoxycaffeoyl) sophoroside-7-*O*-glucoside were positive on kaempferol-3,7-di-*O*-glucoside content (ρ = 0.10 and ρ = 0.59, respectively). For the other flavonoid studied, isorhamnetin-3,7-di-*O*-glucoside, the factorial regression analysis showed that kaempferol-3,7-di-*O*-glucoside and 1-sinapoyl-2-feruloylgentiobioside explained 40 and 22% of the G variation and the effect was positive (ρ = 0.21 and ρ = 0.05, respectively), whereas gluconapin content explained 12% of the G variation and the effect was negative (ρ = −0.01) (Figure 4B). In general, genotypic covariates that influenced the final content of these two kaempferol and isorhamnetin derivative flavonoids are other major flavonoid and sinapate derivatives. Although the expression of flavonoid biosynthesis and sinaptate pathway genes is differentially coordinated to produce pathway-specific metabolites, these results suggested that the flavonoid and sinaptate pathways could be organized as enzyme complexes and are able to compete for common intermediates.

The factorial regression model explained 49 and 57% of the GE interaction sums of squares for kaempferol-3,7-di-*O*-glucoside and isorhamnetin-3,7-di-*O*-glucoside content, respectively. The residual GE was significant only for kaempferol-3,7-di-*O*-glucoside, and no cross-products between genotypic and environmental covariates were significant in this compound. One covariate cross-product, gluconapin content × number of days with maximum temperatures over 20 °C, was significant in isorhamnetin-3,7-di-*O*-glucoside (Figure 4B). The regression coefficient of this cross-product was negative (θ = −0.23), suggesting that varieties with lower gluconapin contents were more favored by the number of days with maximum temperatures over 20 °C to increase isorhamnetin-3,7-di-*O*-glucoside content than those with higher gluconapin content.

Moreover, variability in GE was largely explained by the interaction of environmental covariates with the residual genotype variation (approximately 30%) of GE (Figure 4A,B). Therefore, there were significant environment-specific responses that could not be explained by differences in any of the genotypic covariates tested.

In conclusion, it is necessary to consider the relative roles that genetics and environment play on relevant crop traits before breeders can plan strategies for their improvement. In

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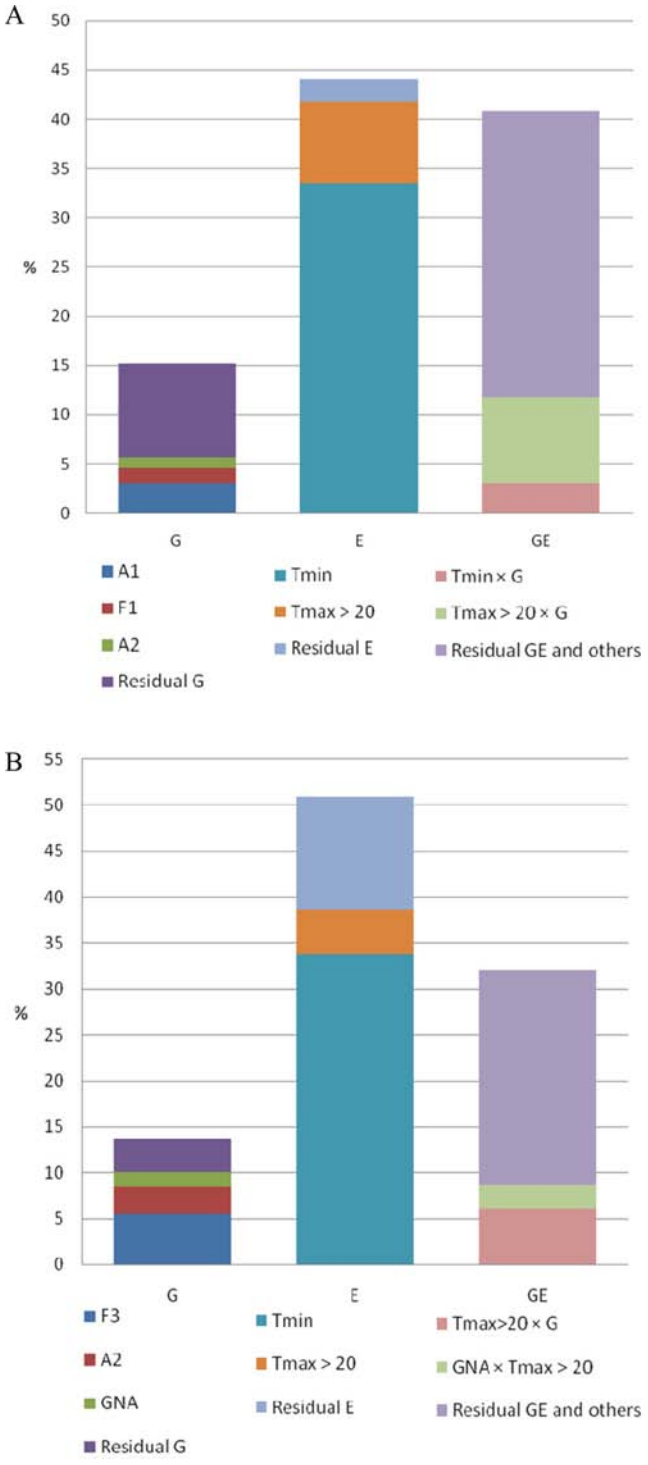

Figure 4. Estimated proportion of variation genotype (G), environment (E), and genotype × environment (GE) interactions explained by each covariate for kaempferol-3,7-di-*O*-glucoside in turnip green (A) and isorhamnetin-3,7-di-*O*-glucoside in turnip top (B) of 12 turnip green and turnip top varieties evaluated in seven environments. Environmental and genotypic covariates used in the factorial regression analysis were previously detected with the stepwise method. A1, 1,2-disinapoylgentiobioside; F1, kaempferol-3-*O* (methoxycaffeoyl) sophoroside-7-*O*-glucoside; A2, 1-sinapoyl-2-feruloylgentiobioside; Tmin, mean of daily minimum temperature; Tmax > 20, number of days with a maximum temperature over 20 °C; F3, kaempferol-3,7-di-*O*-glucoside; and GNA, gluconapin.

this study, we have reported the influence of each genotypic and environmental factor for the first time, as well as their interaction on turnip green and turnip top fresh production and also on the major glucosinolate and phenolic compounds present on these crops, indicating that, in general, the daily maximum and minimum temperatures were the main limiting factors for most of the traits studied. However, for turnip top production and turnip top glucosinolate content, genetic variation was equally or more important than environmental variation. Early vigor, time to flowering, and the content of certain phenols, such as sinapic acid and their derivatives, were the genotypic covariates with the largest influence on fresh production, gluconapin and flavonoids content, thus showing a clear relationship between production and secondary metabolism and also cross-talks among glucosinolate and phenol signaling pathways.

AUTHOR INFORMATION

Corresponding Author

*Tel: 0034-986854800. Fax: 0034-986841362. E-mail: mfrancisco@mbg.cesga.es.

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