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Comparison of the phenolic contents and epigenetic and genetic variability of wild and cultivated watercress (*Rorippa nasturtium* var. *aquaticum* L.)

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ABSTRACT

Background: Epigenetic modifications are key factors modulating the expression of genes involved in the synthesis of phytochemicals. The knowledge of plant epigenetic and genetic variations can contribute to enhance the production of bioactive compounds. These issues have been little explored thus far in *Rorippa nasturtium* var. *aquaticum* L. (watercress), an edible and medicinal plant. The aim of the current study was to determine and compare the phenolic composition and epigenetic and genetic variations between wild and cultivated watercress.

Results: Significant differences were found in the quantitative phenolic composition between wild and cultivated watercress. The eight primer combinations used in the methylation-sensitive amplification polymorphism (MSAP) method revealed different epigenetic status for each watercress type, the cultivated one being the most epigenetically variable. The genetic variability revealed by the *EcoRI*/*MspI* amplification profile and also by eight inter-simple sequence repeat (ISSR) primers was different between the two types of watercress. The results of the Mantel test showed that the correlation between genetic and epigenetic variations has diminished in the cultivated type. Cluster analyses showed that the epigenetic and genetic characterizations clearly discriminated between wild and cultivated watercress.

Conclusions: Relevant chemical, epigenetic, and genetic differences have emerged between wild and cultivated watercress. These differences can contribute to fingerprint and develop quality control tools for the integral and safety use and the commercialization of watercress. The richness of epialleles could support the development of tools to manipulate the watercress epigenome to develop high bioproduct–producing cultivars.
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1. Introduction

Epigenetic modifications play a key role in plant growth, development [1], and stress adaptation [2]. DNA methylation is an important epigenetic mechanism involved in the regulation of gene expression [3]. Several studies showed that abiotic and biotic stress cause heritable alterations in cytosine methylation patterns, which can produce sustained gene expression and new phenotypes, thereby providing plants the ability of rapid adaptation through expression of genes involved in the synthesis of bioproducts [4,5].

Counting with strategies for manipulating plant epigenome would enhance the production of plant bioproducts. However, for achieving this purpose, it is necessary to have knowledge of both the basal epigenetic and genetic variability of plants of interest. Some strategies of the epigenetic manipulation for the novo or enhanced production of bioactive compounds have been developed for some fungi species [6]. Gallusci et al. [5] proposed that the complete characterization of epigenetic variations enables the construction of predictive models of the transmission and stability of this variation, which has application in breeding. Additionally, determining the differential contribution of both genetic and epigenetic variability to the rapid adaptation of

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plants to environmental changes is important to reveal the epigenetic potential for plant breeding application. Studies on this topic have been carried out for *Spartina alterniflora* and *Borrichia frutescens* [4].

Watercress (*Rorippa nasturtium* var. *aquaticum* L., syn.: *Nasturtium officinale* W. T. Ayton) is an aquatic perennial plant of the family Brassicaceae native to Europe [7]. This plant is rich in secondary metabolites [8,9]. These bioproducts, having a wide spectrum of biological activities, convert this edible species into a medicinal plant with potent anticarcinogenic properties [10], among others. All these properties have aided research to improve the accumulation of bioactive phytochemicals of watercress. In this context, Voutsina et al. [11] described the first transcriptome of this plant, and Payne et al. [12] evaluated the gene expression and morphologic variation of commercial watercress, among other studies. However, a lack of knowledge still exists about the genetic and epigenetic variability of natural and cultivated watercress, as well as about the potential of epigenetic manipulation to improve the accumulation of its bioactive compounds.

In Mexico, watercress naturally occurs in springs of high valleys [7], where people consume it as a vegetable. This plant is also cultivated in hydroponic systems in some central regions of the country. The aim of the current study was to determine and compare the phenolic composition and the epigenetic and genetic variations between wild and cultivated watercress.

2. Materials and methods

2.1. Reagents

Ethanol, Folin–Ciocalteu reagent, gallic acid, aluminum chloride, polyvinylpyrrolidone (PVP40), vanillin, HPLC standards, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). *EcoRI*, *MspI*, *HpaII*, T4DNA ligase, T4DNA ligase buffer, Taq Polymerase, PCR buffer, dNTPs, and MgCl2 were purchased from Promega (Madison, WI, USA).

2.2. Plant material

Leaves of 20 accessions of wild watercress (named 1 to 20) were collected in springs located in Berros and La Constancia, Durango, Mexico (sampling area between 23° 93′ 19” N, 104° 27′ 23” W and 23° 91’ N, 104° 26’ W; altitude between 1760 and 1800 m), in July 2016. The voucher specimen was deposited at Herbarium CIIDIR (curatorial number 16895). The average annual temperature of the locations was 16°C, the minimum was 4°C, and the maximum was 23°C. Photoperiod was 13 h, and the maximum relative humidity was 87%.

Leaves of 20 individuals of cultivated watercress (named 21 to 40) were obtained from an equal number of different lots, in a local market in July 2016. These samples were hydroponically grown under greenhouse conditions in Queretaro, Mexico (20° 51′ 51” N, 99° 55′ 43” W; 1990 m altitude), where the average annual temperature was 17.4°C, the minimum was 12.5°C, and the maximum was 26.5°C. Photoperiod was 12.5 h, and the maximum relative humidity was 94%.

2.3. Preparation of extracts

Each type of sample was independently prepared and analyzed. Samples were dried (at 40°C) and ground. Three subsamples of each watercress type were formed and separately analyzed. Samples (1 g) were extracted with 10 mL of 80% ethanol (*v*/v) for 12 h. After centrifugation (8000 rpm, 10 min), the supernatant was recovered and the pellet was re-extracted under the same conditions. The two supernatants of the same sample were combined and concentrated to dryness. The dried extract was solved in 80% ethanol (*v*/*v*), at a concentration of 2 mg/mL. Aliquots were used in the determination of phenolic composition.

2.4. Phenolic composition

Total phenolic contents were determined according to Skotti et al. [13]. Phenolic contents were calculated by generating a calibration curve of gallic acid (slope = 0.0913, axis crossing point = −0.0144, r = 0.9963) and expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g DE).

Flavonoid contents were determined following Barriada-Bernal et al. [14]. The flavonoid contents were calculated by generating a calibration curve of quercetin (slope = 0.3261, axis crossing point = 0.0277, r = 0.9957) and expressed as milligrams of quercetin equivalents per gram of dry extract (mg GAE/g DE).

Condensed tannins were determined following Julkunen-Tiitto [15]. The contents were estimated by generating a calibration curve of epicatechin (slope = 4.8739, axis crossing point = 0.2050, r = 0.9983) and expressed as milligrams of epicatechin equivalents per gram of dry extract (mg EE/g DE).

The phenolic profile was determined by HPLC-DAD, in a PerkinElmer Series 200 HPLC system (Shelton, Connecticut, USA), using a PerkinElmer Brownlee Analytical C18 column (4.6 × 250 mm, 5 μm) and diode array detection (DAD) (PerkinElmer Series 200) by the gradient method [16]. Structural information of compounds was obtained by considering the number and λ_{max} of bands and shoulders, as well as the whole shape of the UV spectra according to the UV theory developed for flavonoids and phenolic acids [16] and also by comparing the retention time (RT) and UV spectra with those of the following reference compounds: chlorogenic acid (RT: 29.76 min; λ_{max}: 243sh, 296sh, 326), caffeic acid (RT: 53.13 min; λ_{max}: 239sh, 295sh, 318), *p*-coumaric acid (RT: 37.2 min; λ_{max}: 294sh, 308), quercetin (RT: 45.95 min; λ_{max}: 260, 268sh, 299sh, 370), rutin (quercetin-3-*O*-[rhamnosyl(1–6)glucoside]; RT: 33.74 min; λ_{max}: 255, 264sh, 294sh, 355), and apigenin (RT: 59.60 min, 267, 290sh, 335). The relative abundance of each compound was determined by area measurements, using a standard curve of rutin (slope = 8×10^6, axis crossing point = 42.373, r = 0.9987) for flavonols, and a standard curve of chlorogenic acid (slope = 8×10^6, axis crossing point = 9892.900, r = 0.9985) for phenolic acids. Concentrations were reported as milligrams per gram of dry extract (mg/g DE).

2.5. Epigenetic and genetic analysis

Total DNA of each individual was independently obtained and analyzed. DNA extraction was carried out by grinding the samples in liquid nitrogen and using 2% polyvinylpyrrolidone (PVP 40), according to Bhau et al. [17].

Table 1
MSAP (Methylation-Sensitive Amplification Polymorphism) primers used to assess the epigenetic variability of wild and cultivated watercress (*Rorippa nasturtium* var. *aquaticum*).

MSAP primers	*EcoRI* Sequence	*HpaII*/*MspI* Sequence
Pre-amplification primers	5′GACTGCGTACCAATTC-3′	5′-ATCATGAGTCCTGCTC GG-3′
Selective amplification primers	5′GACTGCGTACCAATTCAC-3′	5′-ATCATGAGTCCTGCTC GGTCAA-3′
	5′GACTGCGTACCAATTCAG-3′	5′-ATCATGAGTCCTGCTC GGAAT-3′
	5′GACTGCGTACCAATTCAAC-3′	
	5′GACTGCGTACCAATTCAT-3′	
Adapter pair	5′-CTCGTAGACTGCGT ACC-3′/3′-CATCTGACGCA TGGTTAA-5′	5′-GATCATGAGTCCTG CT-3′/3′-AGTACTCAGGA CGAGC-5′

The underlying sequences mean the 2 to 4 selective nucleotides added at the 3′ end of the selective amplification primers.