



Research article

Accessing to the *Nicotiana tabacum* leaf antimicrobial activity: *In-silico* and *in-vitro* investigations

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ABSTRACT

In this research, in-silico and in-vitro approaches were adopted with the aim to investigate the relationship between the tobacco leaf structures (trichomes) and the production of secondary metabolites with antimicrobial activity. Machine learning techniques were used to know the correlation between phenotypic traits and the production of secondary metabolites in *Nicotiana tabacum* plants. Then, an in-vitro experimental study was carried out to corroborate the proposed model. The relationship between the morphology and distribution of the different types of trichomes in the upper and lower leaves with the contrasting profiles of the chemical composition (diterpenes and sugar esters) of the leaf exudates between different lines of tobacco were found. We determined the influence of each trichome type with secondary metabolites production and the necessary concentration to achieve antimicrobial and antioxidant activity.

1. Introduction

Plants are a supreme source for the isolation and discovery of new drugs (Veeresham, 2012). The humans depend on plant secondary metabolites, to develop medicines, fragrances, foods and other plant natural products for different industries (Fomum and Nsahlai, 2017). Plant secondary metabolites constitute an enormously rich reservoir of chemical biodiversity. Plant natural products can be divided into several groups by its chemical structures. The terpenoids are one of plant metabolites more studied. Probably, they are the biggest group of secondary metabolites in plants also with some primary roles. Showing a vast structural diversity derived from the joining of five-carbon isoprene units and subsequent modifications as cyclization, dimerization, hydroxylation and others. The terpenes or terpenoids are classified by the number of the five-carbon units they contain as monoterpenes, sesquiterpenes, diterpenes, triterpenes, and so on (Kutchan et al., 2015).

Terpenes have a wide number of properties. Many of them are sticky, smelly, oily and toxic. All this, with the aim to attract animals that disperse seeds and pollen, to defend plants against insects, pathogens, herbivores and affect the growth of competing plants. They also protect the plants against abiotic stress (Harada et al., 2010a). Essential

oils and their major components (terpenes) have a broad spectrum of biological activity, in particular as antimicrobial. Various monoterpenes such as geranylgeraniol, geraniol, geranylgeranyl acetate, citronellol and nerol, showed activity vs *M. tuberculosis* (Edris, 2007), (Dadalioglu and Evrendilek, 2004). The essential oil of *Lemon grass* has considerable amounts of α -citral, β -citral, citronellol, citronellal, linalool and geraniol which have shown to have antimicrobial activity against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Vik et al., 2007). Another example is presented by the essential oil of *Melaleuca alternifolia* (Tea tree), which is composed, among other things, of α -terpineol, linalool and terpinen-4-ol and demonstrates effective antimicrobial activity (Carson et al., 2011).

Most of terpenes are synthesized and stored in trichomes (hairs on the surface of many plants) and they have been implicated in protection against various biotic and abiotic stress conditions. The morphology of these epidermal protuberances varies greatly, depending on the tissue and the species. It is well-known that Glandular Trichomes (GTs) have the capacity to synthesize, store and secrete large amounts of specialized metabolites, many of which are commercially important, therefore these structures are considered biofactories for the production of natural compounds. Then, they are translocated through transporters as one of the defense mechanisms of plants to handle highly toxic

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compounds (Amme et al., 2005).

In tobacco leaves (*Nicotiana tabacum*), the presence of GTs is involved mainly in the secretion of a variety of diterpenoids and other metabolites (Cui et al., 2011). However, the relationship between the production of natural products, the morphological structures involved in the process and the biological activity is still unknown. This is due, fundamentally, to the fact that there is no direct and bidirectional correspondence that allows to easily establish this relationship (Van Cutsem et al., 2011). Requiring of an extensive *in-silico* experimentation and information processing.

Under the hypothesis that there is a correlation between the morphology of glandular trichomes and their chemical composition, in this article, a study of the possible connections between the morphological features of the tobacco leaf and the production of six secondary metabolites is presented. The experimental design was conducted in the following order: (1) the characterization of the shape, distribution and density of Glandular trichomes in leaves of selected tobacco accessions; (2) *in-silico approach*, involving machine learning algorithms able to learn the relationship between trichomes and the production of metabolites; (3) *in-vitro assays*, here the *in-silico* model was contrasted with profiles of diterpenes and sucrose esters as a major secondary metabolites of the leaf exudates.

The relationship between the morphology and the distribution of the different types of trichomes in the upper and lower leaves with the contrasting profiles (chemical composition of diterpenes and sugar esters) of the leaf exudates between different lines of tobacco were determined. As well as, the influence of each type of glandular trichome and the terpenes concentration necessary to achieve antimicrobial and antioxidant activity were studied.

This article is organized as follows: section 2 shows the materials and methods, describing the tobacco accessions used in the experimentation, as well as the machine learning algorithms employed in the *in-silico* study and the *in-vitro* assay configurations. The results obtained and the discussion of them can be found in section 3. Finally, the main conclusions reached are shown, highlighting our main contributions and future work.

2. Materials and methods

Study of the antimicrobial effect of different accessions of tobacco evidence the relationship that exists between the leaf morphology, the chemical composition and the biological activity. For this reason, we evaluate the activity of lines with different terpenoids profiles (accessions Nic.1061, Nic.1016, Nic.1015).

2.1. Vegetal material

To perform the extraction of the plant material, eight *Nicotiana tabacum* accessions (Nic1012, Nic 1019, Nic 1015, Nic 1006, Nic 1003, Nic 1033, Nic 1017 and Nic 1016) were taken (Fig. 1). The plants used for the investigation at the time of extraction of the samples were 3 months old grown in field conditions.

To study the trichome morphology and distribution, two leaves of each plant per line of culture were used. Young leaves fully unfolded, coming from the second position on the stem after the small leaves in formation. With a length of approximately 15–18 cm long. These leaves were fixed and prepared for observing and photographing in the scanning electron microscope. A total of 12 images of different areas of the upper leaf surface and 12 images of different areas of the lower surface were obtained. The tobacco accessions were obtained from the IPK-Genebank of Gatersleben, Germany.

2.2. In-silico studies

Data preparation: For *in-silico* experimentation, six data sets were used, one for each natural product. Each set contains 72 objects with 15

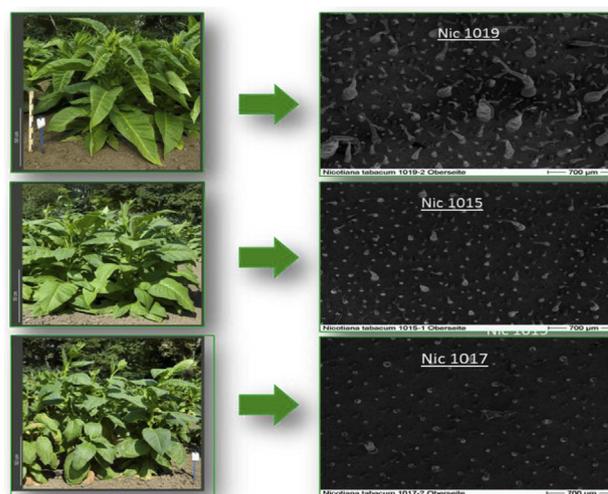


Fig. 1. Image of three contrasting accessions of tobacco. (Left) Image of the tobacco plant in field conditions. (Right) Representative Image of upper side leaf obtained from scanning electron microscopy showing the differences in trichomes density and trichomes types between the accessions.

different measurements of phenotypic traits and one class. The measurements and the class present numerical data and do not present incomplete data. (For more detailed information, please refer to section 1 “Experimental data”, of the Supplementary materials).

The input vector has a fixed length. It includes the information of the phenotypic traits (attributes) and the measurement of one of the compounds (class) for each plant. The traits are divided into three groups of five. Groups one and two correspond to the beam and the underside of the sheet respectively. In these two groups, four of the traits correspond to the counting of a specific trichome type, which is common in the upper side and the reverse of the leaf, and the fifth trait is the sum of the four previous ones. In the third group the first four attributes correspond to the total of trichomes by types and the fifth attribute is the sum of the totals of the two previous groups (Fig. 4). The class refers to the measurement of one of the secondary metabolites studied.

Attribute selection methods: The feature selection process consists of two main components: the evaluation function and a search method. The evaluation function allows to calculate the quality of a subset of traits; while the search method, usually heuristic, is responsible for generating the subsets of traits.

The “CfsSubsetEval” (CSE) algorithm is used as evaluation function. This one calculates the correlation of the class with each attribute and eliminates attributes that have very high correlation as redundant attributes. Subsets of features that are highly correlated with the class by having low inter-correlation are preferred (Alibeigi et al., 2011).

The search methods to be used are: “BestFirst” (BF) (Alibeigi et al., 2011), which traverses the space of subsets of attributes by means of the avid hill climbing method, employing a general recursive algorithm (backtracking) (Alibeigi et al., 2011), which attempts to build an incremental solution, eliminating those solutions that do not satisfy the restrictions of the problem at any time; and, “GeneticSearch” (GS) performs a search using simple genetic algorithm (Velarde et al., 2016).

Prediction techniques: supervised or predictive algorithms will be used in the process of prediction. Which predict the value of an attribute (class) from a set of attributes (features). Inducing the relationship between the attributes and the class to which they belong.

This process requires a training stage (construction of a model using a subset of data with known class) and a test stage (testing the model on the rest of the data). For our experimentation we have selected Regression Tree (Santesteban-Toca et al., 2015). An algorithm able to process continuous variables and to generate a decision tree where each

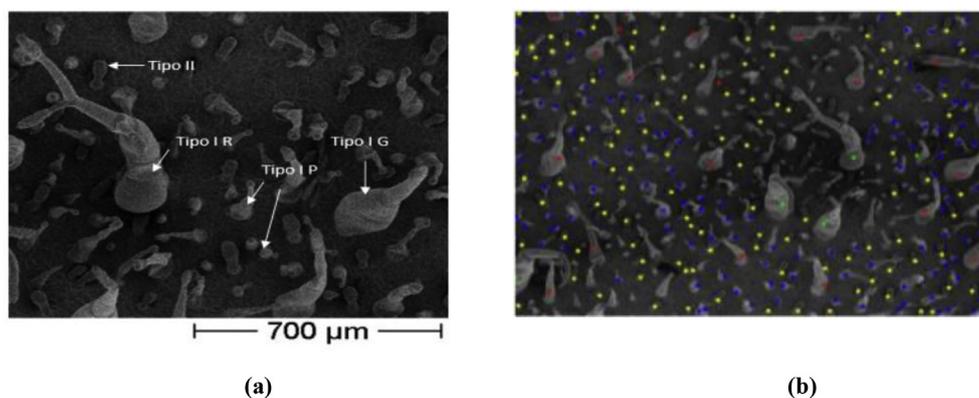


Fig. 2. (a) Classification of trichomes from *Nicotiana tabacum* leaves. (b) Counting of glandular trichomes by colors according to their types and morphologies observed. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

decision node contains the values of predicted output variable.

2.3. In-vitro studies

Tobacco leaves are covered with glandular trichomes at different developmental stages. Their morphological and mechanical features (size, shape, density, orientation) influence many aspects of the plant physiology and ecology, like their functions (biosynthesis and excretion of secondary metabolites). And its density can vary depending on the species, culture, tissue and environmental conditions (Kang et al., 2010). For these reasons, the glandular trichomes of the genus *Nicotiana* of the family Solanaceae have been classified into two groups: a group in which the trichomes are long with glands in the form of a screw (Type I) and another group in which they are short with spherical glands (Type II). They also differ in the presence of chlorophyll in long trichomes. All these trichomes can have unicellular or pluricellular stems, as well as unicellular and pluricellular glands (Shafee-Hajiabad et al., 2015).

Additionally, other classifications were made according to the observed morphology. The classification was done manually for the 18 images with defined colors for each type of trichome (Fig. 2a). Made for the counting and analysis of the morphology, and distribution of trichomes in the upper and lower leaves of 8 lines of *nicotiana tabacum* with contrasting metabolic profiles of their leaf exudates. Type I G: Large, Type I R: Branchized, Type I P: Small and Type II. Type IG, marked in red, are long pluricellular stems and gland with a size $\geq 300 \mu\text{m}$. Type IR, green, are long branched pluricellular stems. Type IP, in blue, are long pluricellular stems usually small with size $< 300 \mu\text{m}$. At last, Type II were marked in yellow identified as short stems of uni or pluricellular spherical glands.

Fig. 2b, shows the types of trichomes classified by colors, process performed by a specialist in the field. The count of trichomes was performed using software developed by the Bioinformatics Department of the Bioplant Center for color counting of trichomes, after preliminary identification and classification. The following variables are evaluated: total trichomes per line, total trichomes in the beam, total trichomes in the back, and total trichomes by type in both the beam and the underside of the leaf.

Leaf trichomes of *Nicotiana tabacum* represent a biologically active and stress-responsive tissue, as evidenced by gene expression analysis (Harada et al., 2010b). For these reasons, to profiling of diterpenes and sucrose ester, the leaf exudates of tobacco accessions were extracted by using dichloromethane (DCM) according to Severson et al. (1984). All leaves of three plants were dipped in 1,5 L of DCM, 4 times during two seconds. The solvent was evaporated and the residue dissolved in 100 ml of DCM. The extract is stored at 4 °C for further analysis.

Sample preparation and quantification method: Diterpenes profiling: 100 μl of dichloromethane extract were collected and dried to constant

dry weight. The value of the dry weight of the extracts was compared between accessions to assess metabolite concentration in exudates. In order to simplify the diterpenes profile a hydrolysis reaction to remove the sucrose esters were performed. The reaction is carrying out adding to the sample 1 ml of sodium hydroxide solution (2 mol l^{-1}), after 10 min of sonication, the supernatant containing the soluble fatty acids and sugars is discarded. Diterpenes were not affected by the reaction and remain in the precipitate. After the reaction, the supernatant was dissolved in 1 ml of methanol 90% by sonication during 10 min. The injection volume of the sample was 10 μl and three replicates for each sample were injected.

Sucrose esters profiling: 20 μl of each sample were used, and dried to constant mass. The dry extract was dissolved in 200 μl of Acetonitrile (ACN 75%) and 5 μl of injection volume was used in the UPLC-MS.

Diterpene profile analysis: 100 μl of the dichloromethane extract was taken and dried to constant dry mass. The value of the dry mass of the extracts (3 replicates) was compared between lines to evaluate the metabolites concentration in general of the exudates.

The sugar esters interfere in the sample, so for the analysis of diterpenes it is necessary to submit the sample to a hydrolysis with a solution of 1 ml of 2 N NaOH for 10 min of sonication. The supernatant containing the soluble fatty acids and the sugars is discarded. The diterpenes were not affected by the reaction and remain in the precipitate. After the reaction the supernatant was redissolved in 1 ml of 90% methanol for 10 min of sonication, a suitable and inexpensive solvent for the diterpenes. 10 μl of sample was injected and 3 replicates for each sample were realized.

Statistical analysis: The results were processed using the Statistical Package for Social Sciences SPSS version 15 for Windows, SPSS inc. Parametric (one factor ANOVA, Tukey $p \leq 0.05$) and nonparametric (Kruskal-Wallis, C Dunnet $p \leq 0.05$) tests were performed, previously the data showed the normal distributional and homogeneity of variance assumptions, according to the Kolmogorov-Smirnov (5%) and Levene (5%) tests, respectively. In some cases, data transformation was necessary. The details of the statistical analysis are shown in each figure or table of results. Correlation analysis between variables were performed using bilateral bivariate correlations test and determining the Pearson coefficient and Kendall Tau_b for a significance of $p \leq 0.05$.

3. Results and discussion

3.1. In-silico studies

In the analysis of the attributes, using CfsSubsetEval with BestFirst (CSE-Bf) and CfsSubsetEval with GeneticSearch (CSE-Gs), similar results were obtained. Both algorithms selected the same traits for the six data sets used (Table 1). In the first column, the data sets to which the machine learning methods were applied appear. In the second column

Table 1
Selection of phenotypic traits applying machine learning.

Database	Traits
Ds-AGd	Type I L upper side, Type I S underside, Type II upper side
Ds-Aco	Type I S upper side
Ds-BCd	Type I L upper side, Type I S upper side, Type II underside
Ds-BCo	Type I S upper side
Ds-Ca	Total trichome upper side, Type I S upper side
Ds-La	Type I S underside

Table 2
Representative profiles of sucrose esters of leaf exudates from eight tobacco accessions. The content of sucrose esters for the six groups (I–VI) and the accessions having these profiles.

Sucrose esters profiles	Groups of sucrose esters per mass						Accessions
	I	II	III	IV	V	VI	
Profile 1	++	++	++	++	++	++	Nic 1012, Nic 1019, Nic 1006, Nic 1033, Nic 1003, Nic 1016
Profile 2	++	++	++	++	++	-	Nic 1017
Profile 3	++	++	+	+	+	-	Nic 1015

the selection of features made by the CSE-Bf and CSE-Gs algorithms.

As it is observed in Table 2, the traits selected in their great majority belong to the underside of the leaf of *Nicotiana tabacum*. It is also appreciated that the trichomes more influential are upper side. These results differ completely from those obtained by statistical techniques. However, the high level of correlation and the inclusion of the level of expression of the metabolites studied in the model give credibility to these results.

3.1.1. Relationship between phenotypical traits and the production of secondary metabolites studied

The RT (regression trees) algorithm, implies a set of rules extracted from regularities observed in the learning data. This rule-based decision technique has the ability to provide an interpretable knowledge model to describe the relationship that could exist between phenotypical traits and the production of secondary metabolites.

As a result, a regression tree is generated for each of the six diterpenes. Where, each sheet shows the amount of the metabolite to be produced with a level of confidence that is dependent on the number of times the pattern is observed. In Fig. 3, we depict the regression tree that describe the α -CBTdiol production in function of traits. This tree shows the way in which the production of a given metabolite will occur, based on the features that best predict it, with a certain confidence

level. The paths from the root to each of the leaves make up the rules that describe this production (See epigraph 2.1. of section “Experimental results”, in Supplementary Material, for more information about the *in-silico* analysis of secondary metabolites production in function of traits).

The ability of Regression Trees to generate a small collection of rules with high confidence represents a great advantage for its interpretation mechanism. Providing a set of rules ordered in terms of the level of confidence and coverage. This facilitates the work of researchers, responsible for raising hypotheses about how these metabolites are produced, and their subsequent biological explanation. Another interesting observation is, on a first impression it might seem that not all trichomes are involved in the production of the metabolites. But this is a statement that is not completely correct. It is due to the high degree of correlation that exists between several traits.

A more exhaustive analysis can be seen in Fig. 4a, which shows the influence of the traits on the expression of the metabolites studied. Where it is possible to appreciate that all the traits present a similar behavior, they only differ in the magnitude with which they affect the production of the metabolite. On the other hand, Fig. 4b shows, in green color, the high degree of correlation that exists between the different kinds of trichomes. Which causes the machine learning algorithm to focus on those features that are more representative (influential), in the production of the metabolite.

3.1.2. Main remarks

- The total number of trichomes is similar in the upper side and the underside of the leaf but this distribution is not the same for each type of trichomes. There is a higher number of trichomes Large and Branched type I and type II in the upper side than in the underside for most of the accessions analyzed.
- There is a quantitative-relationship between the chemical composition of exudates and the shape and distribution of glandular trichomes of the upper side and the underside of tobacco leaves.
- As a result, one regression tree for each of the 6 diterpenes is proposed. Generating a small collection of rules with high confidence, where each leaf of the decision tree shows the amount of the metabolite to be produced according to its phenotypical traits.

3.2. In-vitro studies

Analysis of trichomes morphology of leaf from eight tobacco accessions: Total trichomes on the upper side and under side of the tobacco leaves (Fig. 5). Values represent the mean of 12 counts from the upper side and 12 counts from the underside images respectively corresponding to different areas of the leaf. Different letters correspond to significant

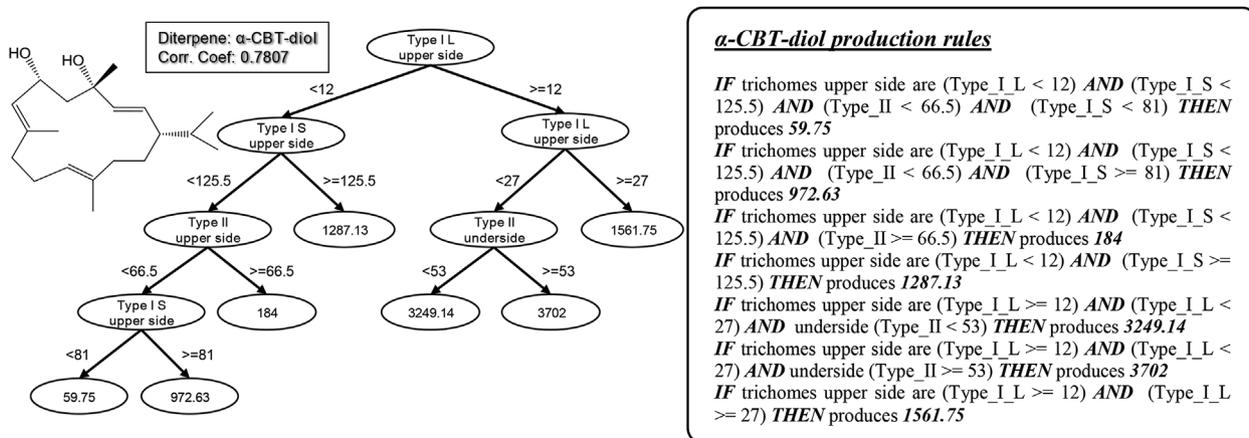


Fig. 3. Regression tree that describe the α -CBTdiol production in function of traits. On the right the set of rules generated by the decision tree.

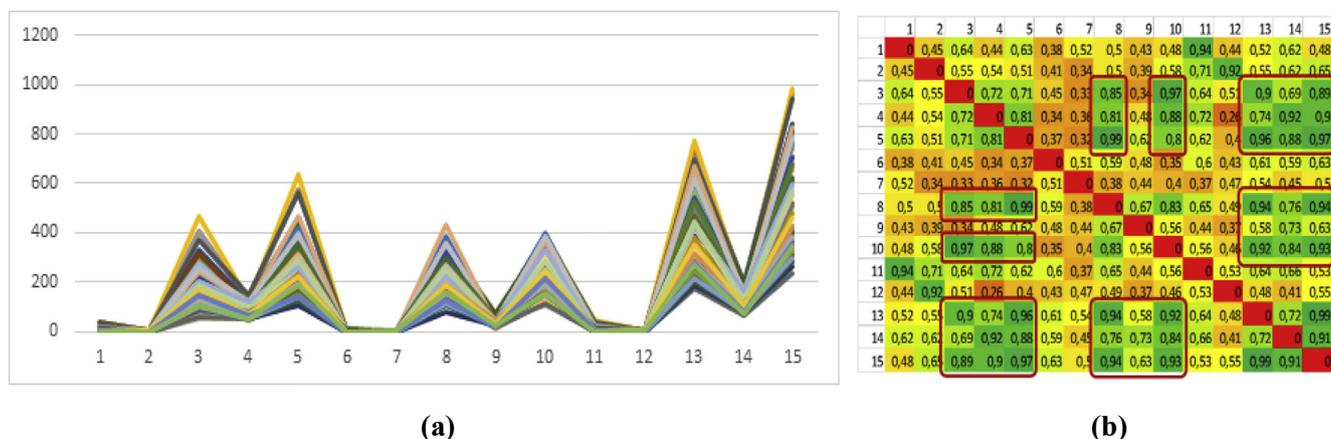


Fig. 4. Trait profile: (a) influence of the traits on the expression of the metabolites studied, where, we represent the 15 traits analyzed in the abscissa (x axis) and their expression levels in the ordinates (y axis); (b) Spearman correlation matrix using feature measurements, where dark red represents no correlation and dark green high correlation, where, both abscissa and ordinate represent the traits and the cells their correlation level. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

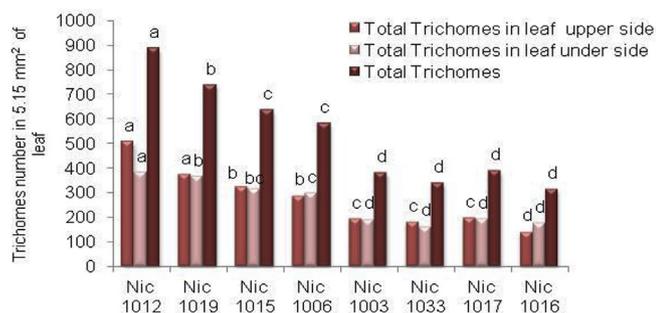


Fig. 5. Total number of trichomes found in an area of 5,15 mm² of the leaf on upper side and the underside of eight tobacco accessions.

differences when comparing between lines for each independent variable according to Kruskal Wallis and Dunett C for $p \leq 0,05$.

Number of trichomes by type and distribution in the upper side and the underside of tobacco leaves: Fig. 6 shows values represent the mean of 12 counts from the upper side and 12 counts from the underside images respectively corresponding to different areas of the leaf. Means with different letters represent significant differences between lines for each trichome type (one-way ANOVA, Tukey $p \leq 0,05$) of transformed data ($X_{trans} \sqrt{X} + 0,5$). Asterisks above bars represent significant differences between the upper side and the underside for each accession.

3.2.1. Relation between trichome morphology and chemical composition of leaf exudates

Determination of the profile of diterpenes as major compounds present in foliar exudates from different accessions of *Nicotiana tabacum*. For this, the expression of the P450 hydroxylase gene related to the synthesis of the major diterpene CBT diol in the foliar exudates of selected lines was analyzed.

Chromatograms analysis, resulting from the separation of the five diterpenes of interest (α -CBTdiol, β -CBTdiol, α -CBTol, β -CBTol, Cis-Abienol) corresponding to 40 tobacco accessions (Fig. 7). Five profiles that group the accessions evaluated are shown. In the chromatograms, the accession that corresponds to it is indicated and to the right, the group of accessions with that profile is indicated in a box.

Fig. 8 shows the content of diterpenes α -CBTdiol, β -CBTdiol, α -CBTol, β -CBTol, Labdeniol, Cis-Abienol, present in the dichloromethanic leaf exudates of eight tobacco accessions of three months grown in field conditions. Different letters represent significant differences between accession for each diterpene (One-way ANOVA and Tukey, $p \leq 0,05$). The profiles of sucrose esters of leaf exudates from

eight tobacco accessions is shown in Table 2.

The correlation between variables related to trichomes and variables, related to chemical composition of leaf exudates of eight tobacco accessions grown in field conditions, can be observed in Table 3. The Pearson correlation coefficient is shown to bilateral correlation between Trichomes and dry weight of the crude extract (second column), whereas for all other variables the correlation coefficient is shown for Kendall Tau_b test in both cases significant for $p \leq 0,05$ and is represented by an asterisk (*).

3.2.2. Determination of the biological activity (antibacterial, antifungal) of foliar exudates of selected accessions against phytopathogens

The *in vitro* analysis of antibacterial effect of different concentrations of terpenoid extracts of tobacco leaves of selected accessions for their antibacterial activity against the phytopathogenic bacteria *Xanthomonas campestris* tomato (Xc.tom) and *Pectobacterium carotovorum* (Pc), is shown in Table 4. The area of inhibition includes the disc area (0,4 cm²), K: Kanamycin as a positive control (500 μ g) and neither: no inhibition (See epigraph 2.2. of section “Experimental results”, in Supplementary Material, for more information about of *in vitro* analysis of antibacterial effect of terpenoid extracts).

As a summary, in Table 5, we show the *in vitro* antimicrobial activity of terpenoids extracts of leaf tobacco exudates against phytopathogens: (+) active in the inhibition of microbial growth; (–) not active in the inhibition of microbial growth; (*) more active extract for that pathogen.

3.2.3. Main remarks

- There is a greater number of large and branched type I trichomes in the beam of most of the accessions analyzed and they were related to large amounts of CBT diols and more complex diterpene profiles. There is a greater number of types II trichomes in the beam than in the lower part of most of the accessions analyzed, but no direct relationship was found with the presence of any specific diterpene. A high density of trichomes is related to a high concentration of terpenes in extracts from foliar exudates.
- The presence of five major diterpenes in the foliar exudates of 40 accessions of *N. tabacum* L. studied allowed to select seven representative accessions of five contrasting diterpene profiles. The extracts of 10 accessions studied showed chemical diversity in concentration and composition. The accession Nic 1015 presented the highest amount of α -CBT diol and Nic 1003 the highest amount of cis Abienol.

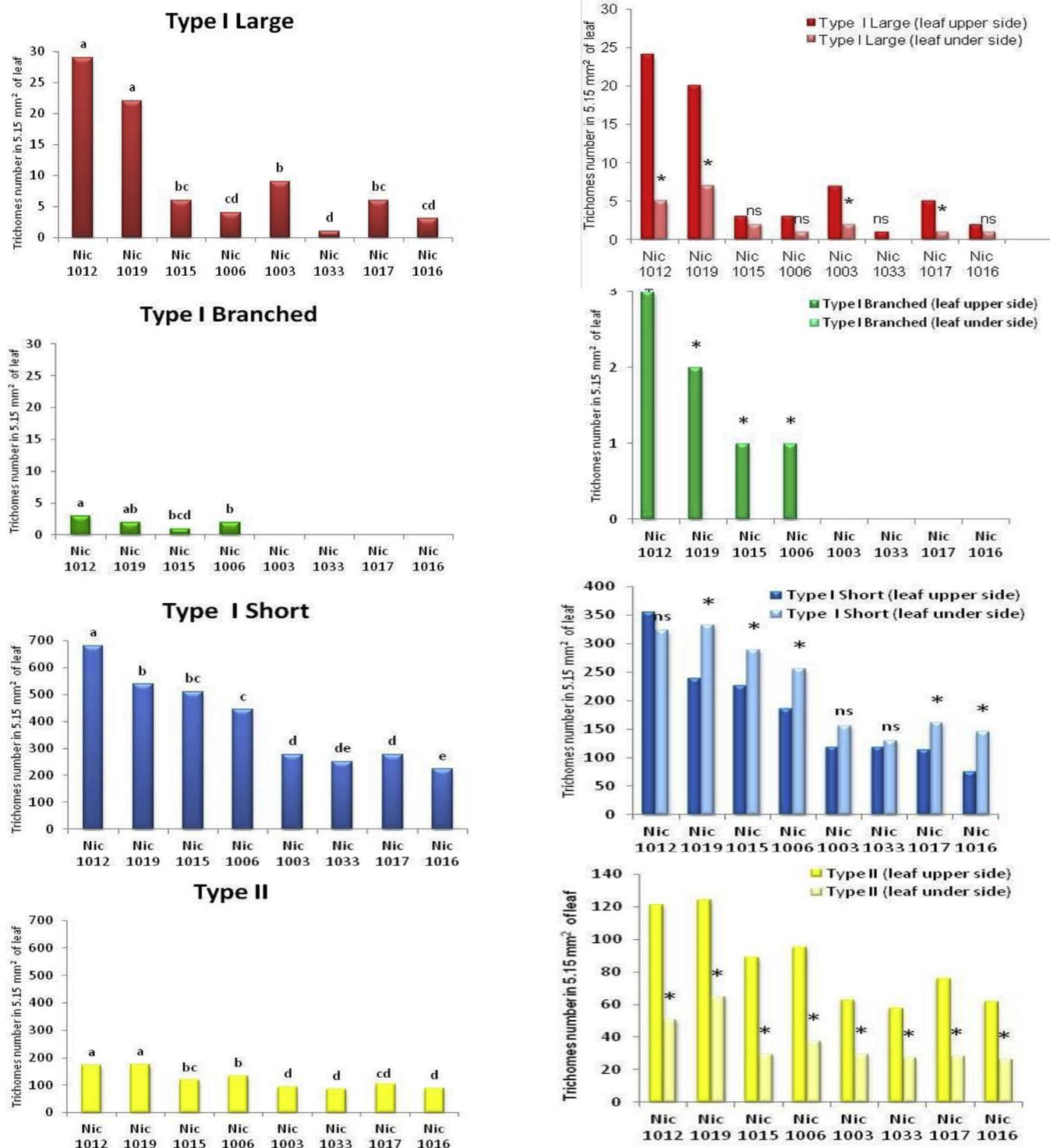


Fig. 6. Comparison between the tobacco accessions in the number of trichomes by type and distribution in the upper side and the underside of the tobacco leaf.

- The results suggest the potential use of extracts obtained from foliar exudates of *Nicotiana tabacum* L. of the accessions Nic 1015, Nic 1061, CE, BHmN, SNN and Nic 1019 against bacteria, fungi and oomycete evaluated, as effective agents for the protection of crops, standing out the extract of the accession Nic 1015 as of greater spectrum of action. The diterpenes cis abienol and sclareol inhibit the *in-vitro* growth of most of the fungal pathogens evaluated.

4. Conclusions

In this article a multiple disciplinary study was presented, were: (1) we relate the morphological features of the tobacco leaf with the production of six secondary metabolites, by the employment of *in-silico* experimentation; (2) we establish the responsibility of the morphology of glandular trichomes and their chemical composition, in his antimicrobial activity. The *in-silico* analysis allowed to determine phenotypic traits that are directly related to the production of each metabolite

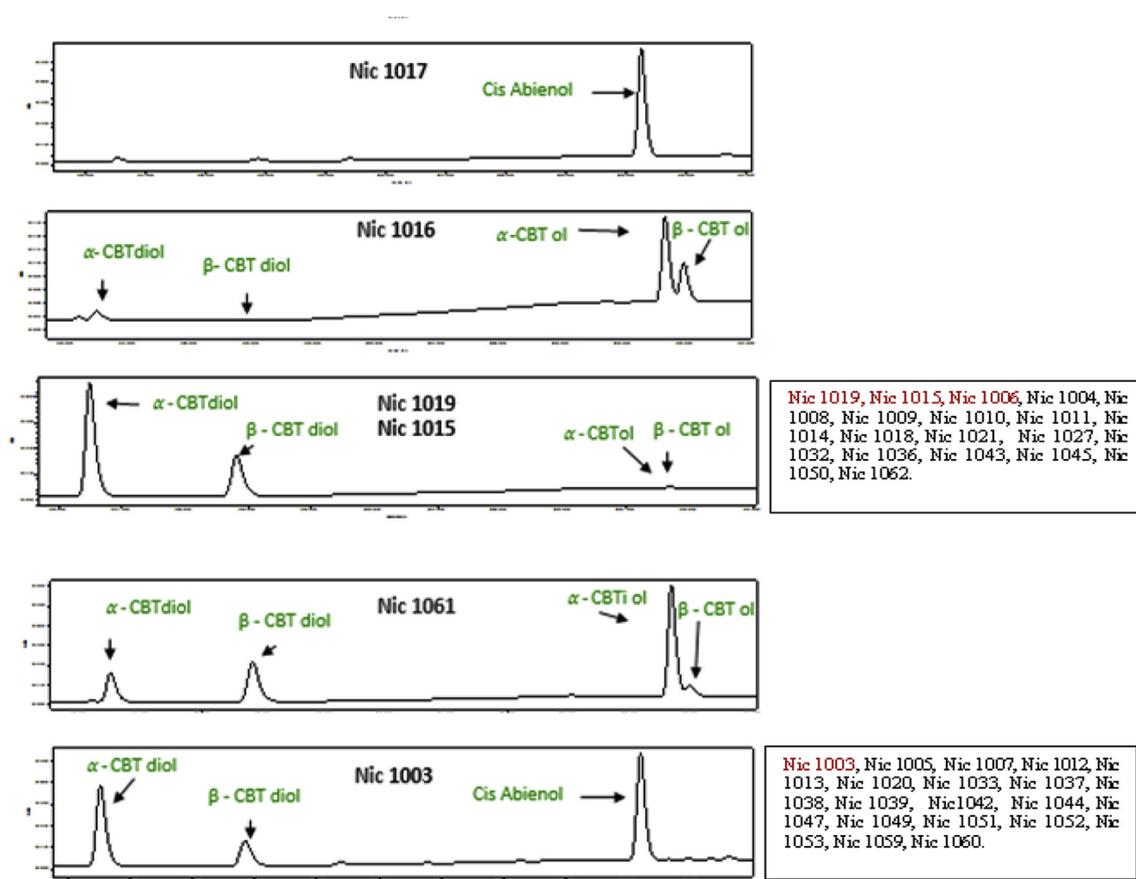


Fig. 7. Chromatograms resulting from the separation of the five diterpenes of interest.

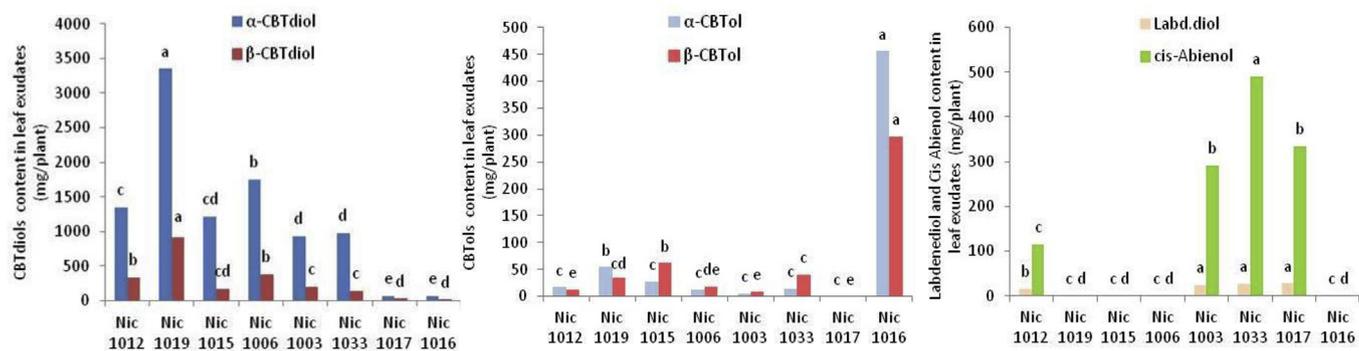


Fig. 8. Content of six diterpenes present in the dichloromethanic leaf exudates of eight tobacco accessions.

Table 3

Correlation between variables related to trichomes and variables related to chemical composition of leaf exudates of eight tobacco accessions grown in field conditions.

	Dry weight of extract	Complexity diterpenes profile	CBT diols content	CBT ols content	Cis Abienol content	Labdene-diol content	Complexity SE profile
Total of Trichomes	0,853 *	0,214 *	0,6 *	-0,220	-0,229 *	-	0,061
Trichomes leaf upper side	0,851 *	0,222 *	0,617 *	-0,167	-0,218 *	-	0,085
Trichomes leaf under side	0,803 *	0,176 *	0,575 *	-0,057	-0,267 *	-	0,320
Trichomes Type I (L)	0,741 *	0,312 *	0,508 *	-0,660	-0,410	-	0,202
Trichomes Type I (B)	0,612 *	0,087	0,462 *	-0,217	-0,113	-	0,017
Trichomes Type I (S)	0,831 *	0,241 *	0,606 *	-0,111	-0,205 *	-	0,062
Trichomes Type II	0,813 *	0,104	0,516 *	-0,115	-0,241 *	-	0,057

studied, with a high level of correlation and precision, compared with traditional statistical methods. Facilitating the *Nicotiana tabacum* plants characterization, in function of its ability to produce these

natural compounds. The obtained decision trees, are able to correlate the plant capacity to produce the secondary metabolites observing its phenotypic traits, with an average accuracy of 86% for Alpha_CBT_diol

Table 4

Antibacterial effect *in vitro*. Means values with different letters represent statistically significant differences between solvent concentrations (in a statistical analysis using one-factor Anova and Tukey test for $p \leq 0,05$ and $n = 3$).

Amount of extract applied to each disc of filter paper (μg) and the area of inhibition of bacterial growth (cm^2)											
Bacteria	Accessions	5	25	50	75	100	200	300	400	500	K
<i>(Xc.tom)</i>	BHmN	ni	0,78 c	0,95 c	1,54 bc	1,80 bc	1,54 bc	2,00 b	2,00 b	2,00 b	3,0 a
	Nic.1061	0,64 c	0,64 c	1,33 bc	1,54 bc	1,80 bc	1,80 bc	1,80 b	1,80 b	1,80 b	3,1 a
	Nic.1016	ni	0,50 c	1,13 bc	1,33 bc	1,80 b	2,00 b	1,80 b	1,80 b	2,00 b	2,9 a
<i>(Pc)</i>	Nic.1015	0,50 c	0,64 bc	0,78 bc	0,64 bc	0,78 bc	0,78 bc	0,78 bc	1,54 b	1,54 b	1,9 a

Table 5

Summary of the *in vitro* antimicrobial activity of terpenoids extracts of leaf tobacco exudates.

Pathogens	Nic 1015	Nic 1017	CE	SNN	Nic 1019	Nic 1006	BHmN	Nic 1003	Nic 1016	Nic 1061
<i>X. campestris</i>	+	-	-	-	-	-	-	-	-	-
<i>E. carotovora</i>	+	-	-	-	-	-	-	-	-	-
<i>E. amylovora</i>	+	-	-	-	-	-	-	-	-	-
<i>Ps. tabaci</i>	+	-	-	-	-	-	-	-	-	-
<i>Ps. glycinea</i>	+	-	-	-	-	-	-	-	-	-
<i>Ps. phaseolicola</i>	-	-	-	-	-	-	-	-	-	-
<i>Ps. tomato</i>	-	-	-	-	-	-	-	-	-	-
<i>Cladosporium</i> sp	+*	+	+	+	+*	+	+	+	-	-
<i>A. alternata</i>	+	+	+*	+	+*	+	+	+	+	+
<i>R. solani</i>	+	+*	+	+	+	-	-	-	+	+
<i>F. graminearum</i>	+	-	+	+	-	-	-	+	-	+*
<i>F. oxysporum</i>	+	-	-	-	+	-	+	-	-	+
<i>B. oryzae</i>	+	-	-	-	+	-	+	-	-	+
<i>C. gloesporioides</i>	+	-	-	-	+	-	+	-	-	+
<i>S. oryzae</i>	+	-	-	-	+	-	+	-	-	+
<i>P. infestans</i>	-	-	+	-	+*	-	+	+	-	+

and up to 91% for Alpha_CBT_ol. With the ability to generate understandable models for researchers, as added value. The *in vitro* results demonstrate the potential use of extracts obtained from foliar exudates of *Nicotiana tabacum* L. of the accessions Nic 1015, Nic 1061, CE, BHmN, SNN and Nic 1019 against bacteria, fungi and oomycete evaluated, as effective agents for the protection of crops. In addition, we found that diterpenes cis abienol and sclareol inhibit the *in vitro* growth of most of the fungal pathogens evaluated. In future researches, for a better explanation of action mechanisms of this isolated compounds, bacteria's such as *Xanthomonas axonopodis* pv. *vesicatoria* and *Pseudomonas syringae* pv. *Syringae*, might be selected.

Conflicts of interest

The authors declare no conflict of interest, financial or otherwise.

Author contributions

Study conception and design:

- Hans Peter Mock (served as biological scientific advisor).
- Martha Hernández de la Torre (served as biological scientific advisor).
- Cosme E. Santiesteban-Toca (served as bioinformatics scientific advisor).

Acquisition of data:

- Yanelis Capdesuñer (field data).
- Karel Vives Hernández (field data).

Analysis and interpretation of data:

- Yanelis Capdesuñer (*in-vitro* data analysis and interpretation).
- Karel Vives Hernández (*in-vitro* data analysis and interpretation).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plaphy.2019.04.015>.

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