Industrial Crops & Products 240 (2026) 122730

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Exploring plant-environment interaction in *Iris pallida* Lam.

Annalisa Meucci [a,*], Giorgiana Chietera [b], Rita Maggini [c], Stefano Brizzolara [a], Andrea Raffaelli [a], Fabienne Bettini [b], Fernando Malorgio [c], Beatrice Pezzarossa [d], Irene Rosellini [d], Zhongqiang Chen [e], Anna Mensuali [a]

[a] Institute of Crop Science, Sant'Anna School of Advanced Studies, Piazza Martiri della Libertà, 33, Pisa 56127, Italy
[b] LMR Naturals by IFF (International Flavors & Fragrances SAS), 18/20 avenue Joseph Honoré Isnard, Parc Industriel des Bois de Grasse, Grasse 06130, France
[c] Department of Agricultural, Food and Agro-environmental Sciences, University of Pisa, Via del Borghetto, 80, Pisa 56124, Italy
[d] Research Institute on Terrestrial Ecosystems (IRET), National Research Council (CNR), Via Moruzzi, 1, Pisa 56124, Italy
[e] Health & Biosciences, IFF (International Flavors & Fragrances) Experimental Station, 200 Powder Mill Road, Wilmington, DE 19803, USA

ARTICLE INFO

Keywords:
Ecotypes
Rhizomes
Irones
Fragrance
Orris

ABSTRACT

Iris pallida essential oil, among the most expensive in perfumery, enriches many fragrances as a base note with its floral-woody nuances. Its violet-like scent is due to irones, ketone compounds produced during the storage phase of orris rhizomes, and originated from the oxidation of triterpenoid precursors called iridals. Orris, traditionally cultivated in Tuscany as an associated crop requiring minimal agronomic management, has experienced a progressive decline in Italian production over recent decades, despite the increasing demand from the perfume industry. To incentivize its cultivation, a deeper understanding of the critical aspects of the *I. pallida* production chain is necessary. A plant-environment interaction study was established to better understand the factors influencing rhizome quality and mass yield. Five *I. pallida* ecotypes traditionally cultivated for fragrance production were transplanted into five different areas and grown for three consecutive years. Biometric measurements were performed during the flowering stage and at the end of each annual cultivation cycle, and the analysis of iridal accumulation and irone production was carried out in every season. Results showed a strong environmental influence on ecotypes' growth. Iridal and irone concentrations were found not to be a convenient trait to discriminate ecotypes, while rhizome weight was found to be relevant for irone hectare^{-1} yield prediction. Moreover, rhizome growth rate seemed to be influenced by soil characteristics, opening the way to optimization and simplification of orris agronomic management. The obtained results can potentially be exploited to maximize rhizome quality and select the best-suited ecotypes for each cultivation area.

1. Introduction

Iris pallida Lam. is a minor perennial cultivated species in the Italian region of Tuscany with a high landscape and economic value, which is traditionally grown in the areas of Pratomagno (Arezzo) and Chianti (Firenze) for fragrances production. The most expensive *I. pallida* product is the absolute, essence that is sold at high prices and mostly used as a bottom note in perfume formulations (Belletti et al., 2013). What makes the absolute so precious is its irone content, as these ketone compounds are responsible for its characteristic violet-like scent. Irones are the result of the oxidation of triterpenoids called iridals, accumulated by orris rhizomes during the years of cultivation, and oxidized during the storage phase that lasts up to 3–4 years (Krick et al., 1983; Bicchi and Rubiolo, 1993). The agronomic management reserved for this plant is mainly based on a shallow tillage before orris transplantation and a periodical removal of weeds, while fertilization is administered in low quantities, as more consistent fertilizations have proven problematic effects for rhizome conservation. Among the elements traditionally supplied to plants, the one that showed the highest impact on rhizome quality is nitrogen (Landi and Nicolelli, 1970). The soil that is typically considered optimal for orris cultivation is a calcareous soil with a good quantity of clay, as it can provide both calcium and potassium to the plant, needful for macro-elements (Passerini, 1891). Rhizomes produced by Tuscan farmers are generally sold to local cooperatives that directly dialogue with French industries, which require raw material for essential oil production. In the last decades, *I. pallida* cultivation in Tuscany has drastically decreased as industries sourced raw material at a lower cost from Morocco and China (Belletti et al., 2013), and even if industry

* Corresponding author.
E-mail address: annalisa.meucci@santannapisa.it (A. Meucci).

https://doi.org/10.1016/j.indcrop.2026.122730
Received 3 November 2025; Received in revised form 15 January 2026; Accepted 16 January 2026
Available online 20 January 2026

demand for raw material has always been higher than the supply. Therefore, cultivating *I. pallida* still represents an interesting opportunity for farmers to earn an additional income, as it can also be cultivated on marginal areas or in association with other species, traditionally with olive and vine trees (Pezzarossa et al., 2016). The principal problems that discourage *I. pallida* cultivation are related to the difficulties in finding young plantlets for its plantation, traditionally propagated via rhizomes, and to the almost total absence of adapted mechanization solutions, during both cultivation and post-harvest phases, which increases labour costs. *I. pallida* is a slow-growing species, making it necessary to extend the cultivation cycle up to 4 years to compensate for the weeding costs at early stages of plant growth with a sufficient production of rhizome biomass per hectare. Another issue is related to the post-harvest time required to let iridals oxidize into irones, which drastically delays their sale time and results in reduced profits for both farmers and industrial actors. The increasing demand from perfumers, combined with the insufficient rhizome supply, sometimes pushes the distillation companies to extract rhizomes that are not fully aged, in which irones have not yet reached their maximum concentration potential. This, in turn, increases the costs of distillation per kilogram of irones. Despite these critical aspects, Italian rhizomes are still highly sought after since they are traditionally considered of higher quality compared to foreign supplies. However, the factors that influence iridals accumulation, rhizomes size and overall essential oil quality still need to be investigated and fully understood, as well as the best agronomic management and the most suitable climatic conditions for rhizomes yield, even if some comparisons among rhizomes cultivated in different areas were already reported in literature (Landi et al., 1994; Firmin et al., 1998, Pezzarossa et al., 2020).

To deepen the understanding of *I. pallida* agronomic behavior, a study was conducted on the interaction between plant characteristics and cultivation environments, by growing different *I. pallida* ecotypes across five diverse sites with significant latitude and pedoclimatic variation. The main objectives of this research were: i) to assess the effect of soil composition, climate and ecotype on the growth of *I. pallida*; ii) to explore the interaction between ecotype traits and environmental conditions at various cultivation sites; and iii) to measure the accumulation of iridals and the production of irones over multiple cultivation years, as well as to describe their temporal dynamics.

By addressing these objectives, the research aims to identify key factors that drive variability in yield and in iridals and irones production of *I. pallida*, thereby supporting the development of more efficient cultivation strategies.

2. Materials and methods

2.1. Experimental setup

Since *I. pallida* is mainly asexually reproducing, due to frequent seed sterility and low germination ability, it was suggested that the plant populations may not have undergone important genotypic changes (Simonet, 1932; McKey et al., 2010). Anyway, they may have evolved phenotypic differences under different environmental constraints, so in the text we referred to them as 'ecotypes'. Five *I. pallida* ecotypes traditionally employed for fragrance production were collected in their traditional areas of cultivation. Three ecotypes, named GR, BA and HE, were provided by LMR Naturals by IFF (Laboratoire Monique Remy, International Flavor and Fragrances group) based in Grasse (France), while the remaining two, named SP and VI, were provided by a commercial farm based in San Polo in Chianti (Firenze) and the Department of Agricultural, Food and Agri-environmental Sciences of the University of Pisa (DiSAAA-a.), respectively. The five selected ecotypes were all transplanted and cultivated for three years in five experimental areas during fall 2020: experimental fields of V. Victorine belonging to DiSAAA-a (I1), San Polo farm (I2), two agrarian areas in northern France (F1 and F2), and LMR field in southern France (F3) (Table 1). The latitude of Italian locations (I1, I2) and the southern French one (F3) is about 43°, while the latitude of the two northern areas (F1 and F2) is 47°.

Table 1
Schematic representation of the experimental plan.

Plants	5 ecotypes	French (GR, BA, HE) Italian (VI, SP)
Areas	5 fields	French (F1, F2, F3) Italian (I1, I2)
Plant density	30 plants/ecotype/row (150 plants per field)	30 cm on the row x 35 cm between rows
Agronomic management	Organic Conventional	I1, I2, F2 F1, F3
Sampling	At harvesting phase (September) Randomly selected on each row	1st year: 10 plants/ecotype/area 2nd year: 6 plants/ecotype/area 3rd: 4 plants/ecotype/area

2.2. Pedo-climatic characterization

Soil samples were taken from each selected area at a depth of about 20–30 cm, and physical and chemical analyses were performed by wet-sieving air-dried soil samples till the obtention of > 2 mm skeleton particles. Soil texture (sand, silt and clay) was determined by the pipette method according to Gee and Bauder (1986), pH was measured with a glass electrode (1:2.5 soil-water ratio) (Thomas, 1996) and cation exchange capacity (C.E.C.) was determined with barium chloride (pH = 8.1) (Sumner and Miller, 1996). The organic and inorganic carbon and nitrogen contents were determined by treating samples with dry combustion, according to the ASA-SSSA (1996) method, by using a Flash Smart CN Soil Analyzer (Thermoscientific, Waltham, MA, USA). The content of available potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) was determined by a sample predigestion with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2), followed by a microwave digestion (Milestone Ethos 900, Bergamo, Italy). Samples were then filtered using a Whatman® paper filter, diluted with milli-Q water, and their Ca^{2+}, Mg^{2+}, and K^+ content was analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES 5900, Agilent, Santa Clara, CA, USA). Soil analyses were performed right before plants transplantation at the beginning of the experiment, and every year at the end of the annual cultivation cycle.

Climatic data, such as T max (°C), T min (°C) and average annual rainfalls (mm), were collected from weather huts near the selected areas, specifically by the "Regional Hydrological Service" (http://www.sir.toscana.it/) for Italian sites and by "Historique meteo: archives meteo" (https://www.historique-meteo.net/) for French ones. Information on agronomic tillage, fertilization and treatments were also collected by interviewing the owners of each experimental site.

2.3. Biometric measurements at the flowering stage

Biometric measurements (number of flower buds, stem length, leaves length and width) were collected on *I. pallida* plants at the flowering stage (between 15th of April and 10th of May) in every selected cultivation area during the 2nd and the 3rd year, due to a partial lack of flowering on the 1st year. Moreover, some measurements in areas I2 and F3 were not performed (BA in the 3rd year and SP in the 2nd year) because some ecotypes did not flower due to cultivation problems (e.g., rodents).

2.4. Genetic analysis

2.4.1. DNA extraction, library preparation and sequencing

Genomic DNA was extracted from a single pooled biological replicate of 50 mg of lyophilized young *I. pallida* leaves per ecotype using the

DNeasy Plant Pro Kit (Qiagen, cat. no. 69204) according to the manufacturer's protocol. Samples were taken from each ecotype grown on the I1 site. The DNA concentrations were measured and normalized to 20 ng μL^{-1} for library preparation, using the Genomic tape and reagents (Agilent, USA) as directed. GBS libraries were prepared with the *Pst*I/*Msp*I two-enzyme system, as described in Poland et al. (2012). Post-PCR products were cleaned with AMPure XP beads (Beckman Coulter, USA) following the manufacturer's instructions. The final product concentrations were measured using the Tapestation D5000 tape and reagents (Agilent, USA) before sequencing on an Illumina NextSeq2000 platform.

2.4.2. Sequencing data processing, SNP Calling and genetic relationship

The GBSX pipeline (Herten et al., 2015) was used for demultiplexing of the barcodes, allowing a maximum of one mismatch. The demultiplexed reads were aligned to the *I. pallida* genome (Bruccoleri et al., 2023) with BWA (Li and Durbin, 2009) using default parameters. Genotype calls were conducted of Freebayes v1.3.8 (Garrison and Marth, 2012) and vcflib (Garrison et al., 2022), where the ploidy level was set to four and a minimum of two reads supporting an alternate allele was required to call a polymorphism. An in-house script was then used to filter out markers based on read depth (minimal 10 reads per site per genotype) and missing data (no missing data) and minor allele count (greater than 2). The genetic relationship among the *I. pallida* cultivars of interest was represented by a neighbour-joining dendrogram based on the hamming distance of the genotypes.

2.5. Biometric measurements at harvest time and Iridals content analysis

In all the selected areas, ten, six and four plants belonging to each *I. pallida* ecotype were harvested at the end of the vegetation period (September) of the 1st, 2nd and 3rd year of cultivation respectively, to allow for recovery of sufficient homogeneous material during the first year of cultivation; the number of replicates decreased over the years due to sampling constraints related to the increasing size of the plants, which made sample handling more complex. Measurements of leaves, roots, and fresh and dry rhizome weight (g) were therefore performed on every ecotype on every cultivation area, except for GR and BA ecotypes in I2, because of growth problems (e.g., rodents). Collected rhizomes were ground (grinder Moulinex, France) and put in an oven at 50 °C for ten days to analyse their water content, using the following formula:

Moisture (g_{H2O}/g_{fw}) = (fresh weight - dry weight)/fresh weight.

Then, according to the Pezzarossa et al. (2020) method, rhizomes were lyophilized for 48 h, and 0.2 g per sample was extracted with 2.5 mL acetone, transferred into a 10 mL test tube, kept under sonication for one hour in an ice bath (Starsonic 60 Symantec, 28–34 kHz), and stored overnight at −20 °C. The day after, the supernatant was collected, and the pellet was extracted again with 2.5 mL of fresh acetone and re-sonicated for an hour, stored again overnight at −20 °C. For each extract, the two supernatant aliquots were pooled and filtered with Chromafil® 0.20 μm PTFE membrane, 25 mm diameter syringe filters (Macherey-Nagel, Düren, Germany). Iridals analysis was carried out via Ultra-High-Performance Liquid Chromatography (Shimadzu Corporation, Kyoto, Japan) coupled to a triple quadrupole Sciex 5500 QTrap+ mass spectrometer (AB Sciex LLC, Framingham, MA, USA) equipped with atmospheric Pressure Chemical Ionization (APCI) source. The UHPLC system consists of two Exion LC AC pumps, an autosampler, a controller, a degasser, and a tray. Tandem Mass Spectrometry (MS/MS) measurements were carried out inside the collision cell of the mass spectrometer, using nitrogen as collision gas. Since pure standard references are not commercially available for the iridals, variation in both iripallidal and iriflorental among samples was evaluated by monitoring the protonated molecule in positive acquisition mode using single ion monitoring (SIM+) (ion: 487 *m/z*), and results were reported as area values. Chromatographic separation was performed using a Phenomenex Kinetex® 2.6 μm Biphenyl 100 Å LC column 100 × 2.1 mm (Phenomenex, Torrance, CA, USA). The elution gradient was carried out with acetonitrile containing 0.1 % formic acid (solvent A) and Milli-Q water with 0.1 % formic acid (solvent B). The elution followed a stepwise gradient: starting from 30 % solvent A, 30–100 % solvent A from 0 to 12 min, 100 % solvent A from 12 to 20 min, followed by 3 min of equilibration time at 30 % solvent A. The column oven temperature was set at 40 °C, the flow rate was 300 μL min^{-1} and the injection volume was 2 μL.

2.6. Rhizome oxidation and irones analysis

All the rhizome samples were also subjected to an innovative patented procedure (SKH GmbH, 2016): a specific protocol of controlled temperature and pressure maintained for five weeks in a reactor located at the IFF-LMR site allowed for the complete transformation of iridals into irones, then the samples were removed, ground in a bench mill, and extracted with the procedure described above for iridals. Irones LC-MS/MS analysis was carried out using the same equipment described for iridal analysis but using it in multiple reaction monitoring (MRM) acquisition mode. The reference standard of α-irone (Sigma Aldrich, Milan, Italy) has been used for identification purposes and for the quantification of α-irone and the semi-quantification of γ-irone by building an external calibration curve obtained by serial dilutions of a solution of 512 ng mL^{-1} of α-irone to a dilution of 1 ng mL^{-1}. Analytical parameters for α-irone were optimized by infusing a standard solution of 1 mg mL^{-1} in methanol into the source of the MS at a flow rate of 0.01 mL min^{-1}. The compound parameters (Declustering potential, DP; collision energy, CE; collision cell exit potential, CXP) were adjusted for the specific multiple reaction monitoring transition (Q1 mass: 207.2 Da; Q3 mass: 109.1 Da; DP: 94 V; CE: 27 V; CXP: 14 V). The MRM transition 207–109 was selected because it provides the highest signal intensity and yields an extremely similar response factor for the two isomers, whose spectra, still very similar, are shown in Figure S1. Irones concentration was calculated by interpolation using an "AutoPeak" algorithm with a regression method that uses the area as a parameter, "linear through Zero" as regression type, and "1/x" as weight type (average R2: 0.997). The signal intensity of irones in the samples and in the lowest point of the calibration curve (1 ppb) possessed S/N ratios much above 10, and, consequently, the study of LOQ and LOD values has not been performed. Irones peak qualitative confirmation was achieved using information-dependent acquisition (IDA) criteria, taking advantage of the ion trap functionalities of the 5500 + QTrap to switch from MRM to enhanced product ions (EPIs), obtaining the MS/MS spectrum using a CE of 35 eV with a CE spread of 15 eV.

2.7. Statistical analysis

All data were expressed as mean ± S.E. of the number of replicates.

One, two and three-way ANOVA tests were employed to determine the effect of different factors (Environment (En) in one-way ANOVA; Environment and Ecotype (Ec) in two-way ANOVA; Environment, Ecotype and Year (Y) in three-way ANOVA) and the effect of their interaction on selected variables. The mathematical model used for the three-way ANOVA is the following:

$$Y_{ijkl} = \mu + En_i + Ec_j + Y_k + (EnEc)_{ij} + (EnY)_{ik} + (EcY)_{jk} + (EnEcY)_{ijk} + \varepsilon_{ijkl}.$$

Normality of data and residuals was assessed using the Shapiro–Wilk test. Homogeneity of variances across groups was evaluated using Levene's test right before the application of the ANOVA tests. ANOVA analyses were performed by using XLSTAT Basic (version 2024.2.2.1422) with the following parameters: constraints an= 0, interactions/level: 1–2–3, confidence interval (%): 95, tolerance: 0.0001. Mean values were separated by the Tukey post-test ($p < 0.05$).

A correlation analysis of phenotypic trait parameters was carried out

by clustering the five ecotypes using the R program. To explore the relationships between the variables, a Pearson correlation matrix between the scaled parameters and ecotypes was calculated, using pairwise complete observations to handle missing data. The resulting correlation matrix was visualized using the "*pheatmap*" package in R Core Team (2024). The heatmap was color-coded to indicate correlation strength, ranging from blue (negative correlation) to red (positive correlation), with white representing no correlation. The dataset was standardized by scaling each variable to have a mean of zero and a standard deviation of one.

Redundancy analysis (RDA) was performed with CANOCO 5 (Šmilauer and Lepš, 2014). The dataset was standardized by scaling each variable to have a mean of zero and a standard deviation of one. Multicollinearity was handled by removing parameters that showed a strong correlation through a preliminary PCA analysis. The data used related to observations performed during the 3rd year (commercial timing) are: Rhizome moisture (RhiMoisture), iridals concentration (Iridals), rhizome dry weight (DWRhi), number of shoots (n°shoots) and rhizome on total plant fresh weight ratio (Rhi/TOTFW); soil parameters used are: Cation Exchange Capacity (C.E.C.), magnesium (Mg), calcium (Ca), potassium (K), nitrogen (N), carbon (C), and organic carbon (Corg) content; climatic parameters used are: average annual rainfall (Rainfall), average maximum temperature of the warmest month of the year (Tmax), and average minimum temperature of the coldest month of the year (Tmin).

3. Results

3.1. Pedo-climatic and agronomical characterization of the cultivation areas

The characterization of the different areas in which *I. pallida* ecotypes were cultivated was fundamental to understanding the main differences in terms of chemical and physical properties of the soil. The analysis of texture revealed that all areas were characterized by loamy-sand and sandy-loam soils, as classified by the USDA-NRCS (1996). Moreover, all the areas were characterized by a calcareous soil, showing the same pH value of about 8 on average (Table S1). F3 belongs to the Bouche du Rhône area, which is typically characterized by clay and calcareous soils, draining and not deep, particularly suited for vine trees. In F3, *I. pallida* is traditionally cultivated by farmers who sell their rhizomes to the IFF industry to produce essential oil. The northeast areas (F1 and F2) are usually characterized by clay-calcareous deep soils, as confirmed by our results. *I. pallida* is not traditionally cultivated in these areas since the plant has been traditionally used to complement agricultural revenues by growing in marginal lands. In Italy, the soils that are considered suitable for *I. pallida* cultivation are those of the Chianti (Florence) and Pratomagno (Arezzo) areas. I2 is based in Chianti, and it is characterized by a particular type of soil called "Galestro", a kind of soil traditionally considered the best for *I. pallida* cultivation, as it contains a high amount of calcium. The I1 site belongs to the DiSAAA-a department of the University of Pisa, where *I. pallida* is traditionally cultivated for experimental purposes.

Regarding the chemical composition, the soil electrical conductivity and macro-elements content were measured right before the transplantation and at the end of every annual cultivation cycle, for three years. The overall results are reported in Tables S2 and S3, while Table 2 summarizes the data collected during the 3rd year of cultivation, since that corresponds to the most common cultivation period for the commercial crop of *I. pallida* rhizomes.

The electrical conductivity (E.C.) showed no significant differences before plant transplantation in the field, then it increased in every area, showing the highest values in F2 one year after the transplantation phase (H1) and in F3 on the 3rd year of cultivation (H3) (Tables 2, S2). At the end of the experiment (Table 2), F1 showed the lowest E.C. in comparison with the other areas. The area with, on average, the highest calcium (Ca^{2+}) values over the years was I2, while the area with the lowest values of Ca^{2+} content was F1, even if on the last harvest it reached similar values to those recorded for I1 and F2 areas (Tables 2, S2). Regarding K^{+} content, I2 showed the lowest values when compared to the other areas at the beginning and at the end of the cultivation (Tables 2, S2). Mg^{2+} content remained stable along the years in I2 and F1 and fluctuated in I1, F2 and F3, showing the highest value in I2 and the lowest value in I1 at the last harvesting (Tables 2, S2). Concerning nitrogen (N), organic carbon (C org) and cation exchange capacity (C.E.C.), F2 showed on average the highest values during all the years of cultivation. On the contrary, F3 showed the lowest values. The carbon (C) content was higher in F2, while it was lower in F1 when compared to all the other areas (Tables 2, S3).

Data of climatic parameters, recorded once per year during all the duration of *I. pallida* cultivation (Table S4), showed an evident difference in terms of average maximum temperature of the warmest month (T max) between northern French areas (F1, F2), for which 23–24 °C were recorded, and the other three areas, which had values ranging between 29 and 31 °C on the 1st year. In the following years, temperatures drastically increased in all areas, with F3 showing the highest value during the 2nd year. The average minimum temperature of the coldest month (T min) remained almost stable throughout the years in northern France and in Italy (I1 and I2), while it changed during the 2nd year in the southern France location F3, decreasing from 6 °C to 0 °C. The average annual rainfalls were higher at higher latitudes (F1, F2) during the 1st year, while they decreased during the last two years, going from 830 mm on average, down to 270 mm. On the contrary, in Italy, rainfall increased during the last two years. In F3, a lack of rainfall was detected; this area usually receives around 737 mm, as reported in the Historical meteo French database (https://www.historique-meteo.net/), while during the three years of cultivation, the average rainfall was 200 mm. In general, northern French areas (F1, F2) showed lower

Table 2
Electrical conductivity (E.C., μs cm^{-1}), macro-elements content (K^{+}, Ca^{2+}, Mg^{2+}, g kg^{-1}), percentage of nitrogen (N %), Carbon (C %), Organic Carbon (C org%) and Cation Exchange Capacity (C.E.C. cmol(+) kg^{-1}) of soil samples collected in the experimental areas at harvesting phase during the 3rd year of cultivation. Data, reported as mean values ± S.E., were subjected to analysis of variance performed within each parameter (one-way ANOVA), and different letters indicate significant differences among means (Tukey post-test, $p \leq 0.05$).

Area	E.C. (μs cm^{-1})	Ca^{2+} (g kg^{-1})	K^{+} (g kg^{-1})	Mg^{2+} (g kg^{-1})	N (%)	C (%)	C org (%)	C.E.C. (cmol(+) kg^{-1})
I1	630 ±2 **b**	8 ±0.2 **b**	0.23 ±0.01 **ab**	0.05 ±0.01 **c**	0.3 ±0.00 **b**	4.5 ±0.1 **a**	4 ±0.3 **a**	17 ±0.2 **c**
I2	566 ±2 **d**	11 ±0.2 **a**	0.1 ±0.01 **d**	0.08 ±0.01 **a**	0.2 ±0.01 **c**	3.7 ±0.2 **b**	1.8 ±0.1 **b**	18 ±0.1 **b**
F1	531 ±1 **e**	8 ±0.1 **b**	0.24 ±0.02 **a**	0.08 ±0.01 **b**	0.2 ±0.01 **d**	1.5 ±0.1 **c**	1.5 ±0.1 **bc**	20 ±0.2 **a**
F2	580 ±15 **c**	8 ±0.2 **b**	0.18 ±0.00 **bc**	0.07 ±0.01 **b**	0.4 ±0.01 **a**	4.8 ±0.1 **a**	4 ±0.1 **a**	21 ±0.1 **a**
F3	688 ±4 **a**	7 ±0.1 **b**	0.17 ±0.01 **c**	0.08 ±0.01 **b**	0.1 ±0.00 **e**	2 ±0.01 **c**	0.8 ±0.01 **c**	19 ±0.6 **b**

temperatures compared to the others, and the rainfall pattern was extremely variable.

Information collected from interviews with farmers on the adopted agronomic management (Table 3) showed the application of nearly the same techniques, based on manual or mechanical soil preparation and a periodical weeding done manually (I1, F1, F2), mechanically (I2), or chemically (F3). In I2, a green manure was also performed before *I. pallida* transplantation, and a clod breaking was executed in the French areas (F1, F2 and F3). All the areas differed in terms of the previous crop cultivated on the experimental plot that was designed, except for F1 and F3, which both cultivated wheat before orris transplantation. Fertilization was not performed in I1 and F2; it was carried out organically in I2 and F1, and synthetic fertilization was applied in F3 (Table 3). In all cases, performing it right before the transplantation.

3.2. Ecotypes characterization

It is known that anatomical characteristics could be significant for the description of Iris species (Mitić and Pavletić, 1999). Within the Iris genus section Iris, one of the most interesting series is the Pallidae one. This series was named on the basis of *I. pallida* Lam. species, characterized by dry-skinned and snow-white spathes and pale blue flowers (Lamarck, 1789). *I. pallida* is a vigorous perennial rhizomatous plant that can reach 1.2 m in height, with narrow, grey-green leaves (https://www.rhs.org.uk/). According to some post-Lamarck botanists, this species comprises all the cultivated or naturalized species that show the common characteristics of Lamarck's *Iris pallida*. Therefore, it was determined that a group of species, called Pallidae series, shows complexity in morphological and anatomical characteristics (Trinajstic, 1976).

A preliminary genetic analysis to confirm the effective diversity among the ecotypes chosen for this study was carried out by GBS technology and the genetic relationship was represented by a neighbour-joining dendrogram based on the hamming distance (Fig. 1). The five ecotypes are clearly distributed into three clusters, with the French BA showing a closer genetic relationship to the two Italian ecotypes VI and SP than to the other two French ecotypes, GR and HE. Those latter are well distinguished from each other and from the cluster where the three other ecotypes belong. The resulting genetic distance legitimates the chosen investigation approach.

Looking at the biometric measurements collected at the flowering stage, a significant difference for both ecotype and selected area can be seen, as well as for their interaction (Table S5). Concerning stem length and number of buds, the ecotypes cultivated on the same area behaved similarly, with lower values detected in F3. Differences among the ecotypes could be seen in leaf parameters: in the F1 area, leaf length and width of the GR ecotype were higher than the other ecotypes in the 3rd year, while the ecotype that showed the highest development in F3 was HE (Table S5).

Table 3
Information on agronomic management, previous crop cultivated, and fertilization administered on the areas cultivated with *I. pallida* ecotypes.

Area	Soil tillage and treatments	Previous crop	Fertilization
I1	Manual soil preparation with a hoe, periodical manual weeding	-	no
I2	Green manure, pre-transplant refinement, harrow for weeds	Field bean	Organic P and K during the year of rest
F1	Plowing and milling in autumn, clod breaker in December, periodical manual and with tiller weeding	Wheat	Organic N21 -P22 -K13
F2	Plowing and milling in autumn, clod breaker in December, periodical manual weeding	Fallow	no
F3	Plowing and milling in autumn, clod breaker in December, chemical weeding in autumn and spring and emergency irrigation during August	Wheat	N12-P09-K18

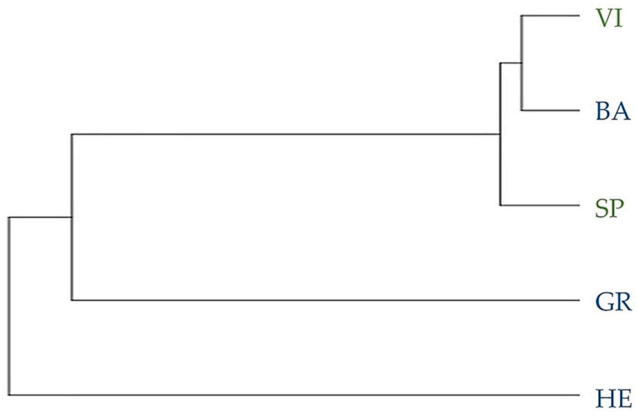

Fig. 1. Genetic relationship among the five ecotypes represented by a neighbour-joining dendrogram based on the hamming distance of the genotype. Ecotypes of French origin are shown in blue, while those of Italian origin are shown in green.

The results of the statistical analysis on data collected at the end of every annual cultivation cycle, in accordance with the monitoring carried out in the flowering phase, showed significant differences both among the ecotypes and the cultivation areas (Table S6). Considering plants' total fresh weight, all the ecotypes performed better if cultivated in F1 and F2 areas, with GR being the most voluminous one in comparison with the other ecotypes. In I2, plants of all the ecotypes (except for GR and BA not grown because of rodents) grew less than in the other areas. Biometric measurements performed on roots and leaves (Table S7) showed no significant differences among the ecotypes cultivated in the same area, except for GR, which had, on average, significantly higher values than the other ecotypes in F1 in the 3rd year of cultivation. I1, F1 and F2 were the areas where all the ecotypes showed the highest values in terms of total biomass, while the worst performances were obtained in I2 and F3. The results related to rhizome dry weight measurements confirmed the aforementioned observations. Rhizomes gained weight over the years, showing the best performances in F1, where GR increased in weight on average 17 times, while the other ecotypes increased on average 7.5 times. No significant differences were detected in terms of rhizome moisture among the ecotypes cultivated on the same area (Table S8).

Therefore, based on the aforementioned phenotypic trait parameters, the five ecotypes were clustered based on the correlation analysis, focusing on data related to the 3rd year of cultivation that corresponds to the conclusion of the commercial cultivation cycle of this species (Fig. 2): the results showed an evident separation of the GR ecotype from all the others, mainly influenced by parameters linked to leaves characteristics (length, width and dry weight) and to rhizome and roots dry weights, that showed a strong correlation (red shades). The two Italian ecotypes proved to be similar, as were the BA and HE ecotypes, generating two different clusters.

3.3. Characterization of ecotypes productivity

To evaluate rhizome quality at harvesting time, iridals content is usually measured, as irones are accumulated during the post-harvest storage. The natural conversion of iridals into irones is a very long-lasting process (2–3 years), which, added to the years of cultivation necessary to obtain rhizomes worth being harvested, makes the *I. pallida* production chain one of the longest in essential oils production field. Being able to accelerate this procedure could represent an interesting step forward in reducing and optimizing rhizome oxidation. Different

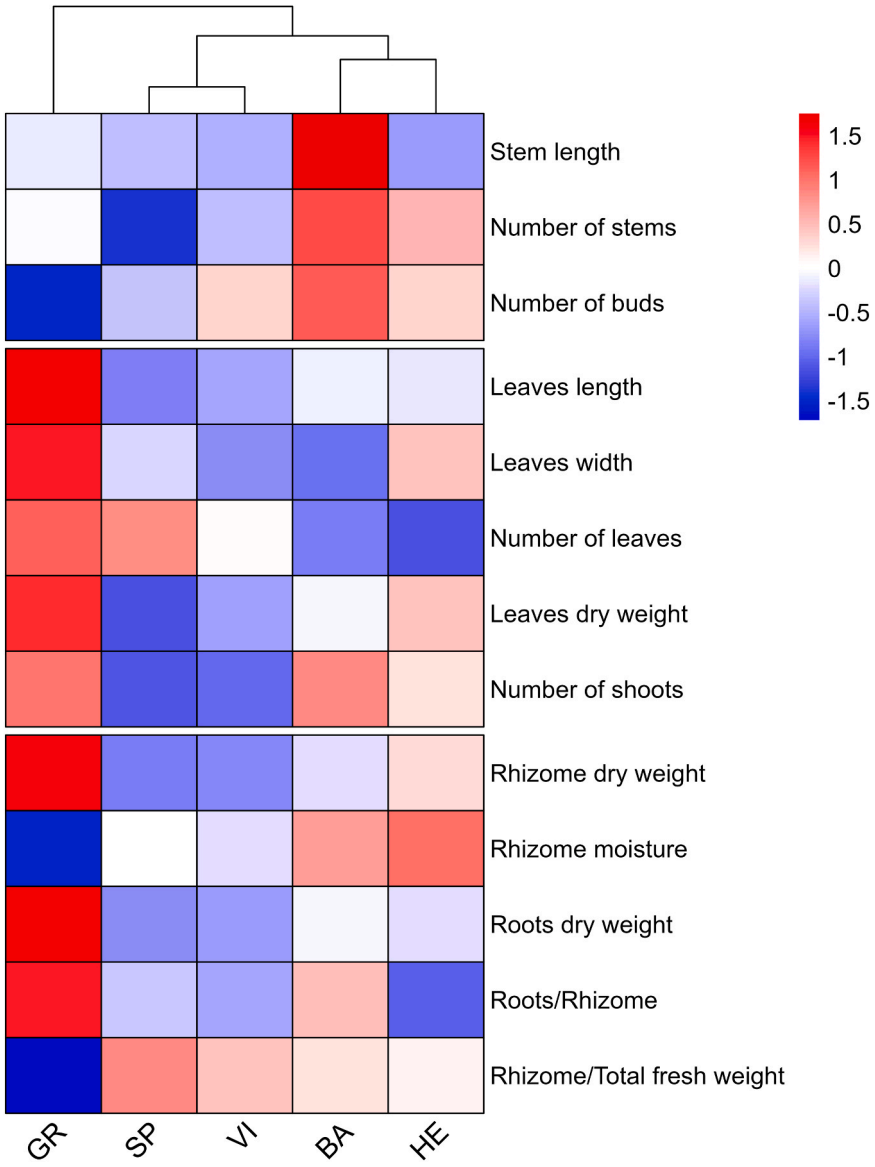

Fig. 2. Correlation analysis between phenotypic trait parameters (monitored at the 3rd year of cultivation) and ecotypes (GR, HE, BA, VI, SP). The data used for each ecotype correspond to the mean across all the areas of cultivation. The dataset was standardized by scaling each variable to have a mean of zero and a standard deviation of one. The heatmap is color-coded to indicate the strength of correlation, ranging from blue (negative correlation) to red (positive correlation), with white representing no correlation.

approaches were studied to solve this problem, mostly focused on the application of enzymes capable of catalyzing the oxidation process, but also conducted by using isolated bacteria.

An industrial protocol exists that forces the full oxidation of iridals by the use of chemical oxidants like potassium permanganate and allows for the estimation of the "irones potential" in rhizomes. This method is historically used to estimate the final quality of a raw material before purchase, but some discrepancy in the results obtained from chemically-oxidised rhizomes and naturally aged ones pushed us to adopt a more natural process of fast aging using the patented method described above.

A forced oxidation process can therefore be applied onto rhizomes, allowing for to completely oxidize iridals into irones. It is therefore possible to quantify irones instead of iridals, thereby avoiding variations due to the instability of iridals over time and the lack of reliable reference standards for building a calibration curve for iridals. From the analysis performed both on iridals and irones content, in both fresh and oxidized samples, it was possible to see the effectiveness of the forced oxidation. Fig. 3 shows the overlapping of chromatograms of the same sample before (Fresh) and after (Oxidized) the treatment: iridals were present in the fresh sample, and not in the oxidized one; on the contrary, irones were not present in the fresh sample and appeared in the oxidized one.

Based on these results, the productivity of the different ecotypes was determined in terms of irones production: rhizomes were collected for three years, subjected to forced oxidation, extracted and therefore analysed. The most relevant trait to describe ecotype productivity is the total content of irones per plant, which was calculated based on the irones concentration in rhizome tissue (Table S8) multiplied by the average dry mass of the rhizome of a plant (Tables S9).

Fig. 4 reports the mean values of irone concentrations in the whole dry rhizome of a plant, for each ecotype cultivated in the five areas at the 3rd year of cultivation. The best overall performances were observed in F1 and F2, located at the highest latitudes, with GR and HE ecotypes showing the highest irones yield in F1 and SP in F2. Significant differences in ecotypes' productivity could be seen in each cultivation area except for the I2 area. The results showed that the productivity of each

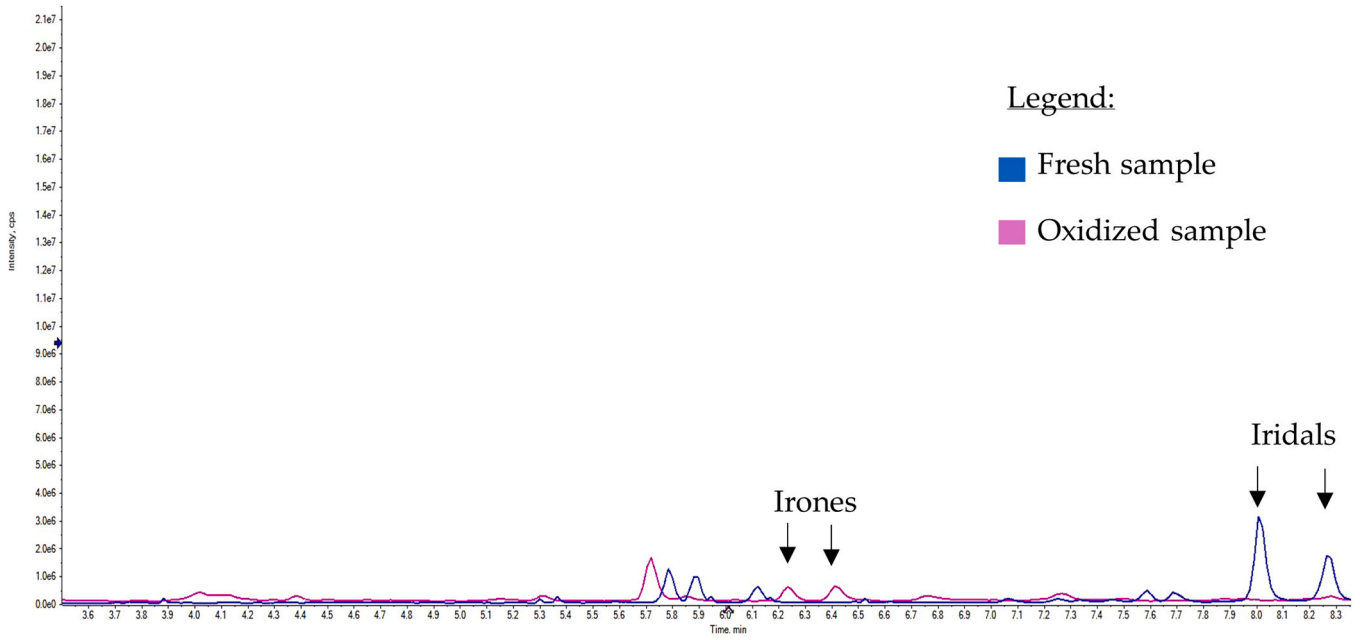

Fig. 3. TIC chromatograms of the extracts obtained from rhizomes of *I. pallida* before (Fresh) and after forced oxidation (Oxidized).

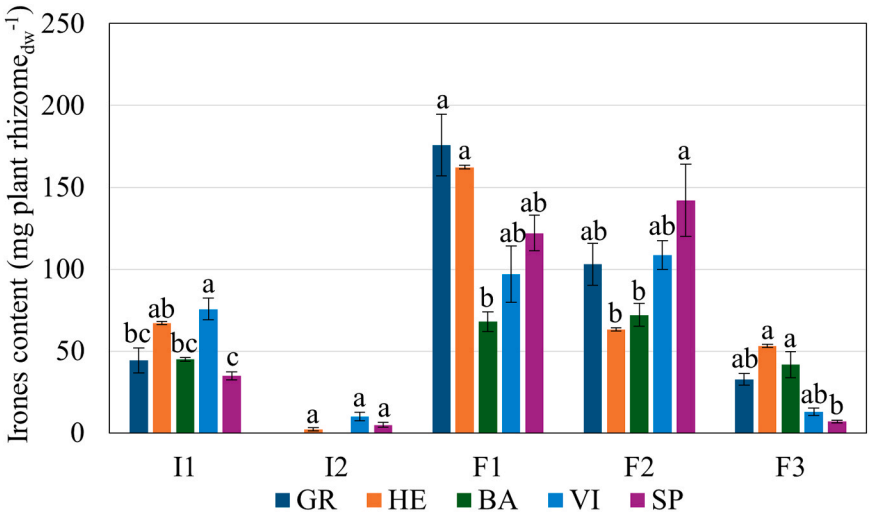

Fig. 4. Irones yield per whole plant rhizome (mg rhizome$_{dw}^{-1}$) of *I. pallida* ecotypes (GR, HE, BA, VI, SP) cultivated on the selected production areas (I1, I2, F1, F2, F3). Data, reported as mean values ± S.E., were subjected to analysis of variance performed within each cultivation area (one-way ANOVA) and different letters indicate significant differences among means (Tukey post-test, $p \leq 0.05$).

ecotype varies according to the growing area, and that in many areas it is possible to identify a more productive ecotype.

The irones yield varied over time in all the studied ecotypes, depending primarily on the growth pattern of the rhizome biomass during the three years of cultivation (Table S8).

In Fig. 5, the trend of irones production of the Italian ecotype SP and the French ecotype BA was reported as an example, showing a significant increase in yield already in the 2nd year of cultivation, followed by a stabilization of the values between the 2nd and the 3rd year.

It was also evident how the two ecotypes responded differently to cultivation environment and conditions, showing in both cases a better yield in the French areas F1 and F2.

3.4. Multivariate analysis on plant-environment interaction data

An RDA analysis was performed to find out correlations between the environmental parameters of the cultivation areas and the accumulation of secondary metabolites at the end of the traditional cultivation cycle of orris plants (3rd year). For this reason, we considered all together the parameters related to rhizome growth and structure (dry weight, moisture and number of shoots) and the iridal levels, directly influenced by environmental factors. Fig. 6 shows a positive correlation among iridals concentration, rhizome moisture (RhiMoisture) and rhizome fresh weight/total plant fresh weight ratio (Rhi/TOTFW) parameters. Moreover, the number of shoots and rhizomes dry weight showed a slight or no correlation with iridals concentration, respectively. A positive correlation of both n° of shoots and rhizomes dry weight (DWRhi) with some soil characteristics could be pointed out, such as with organic carbon (Corg), nitrogen (N), potassium (K) and cation exchange capacity (CEC), whose values resulted at the highest level in F1 and F2 cultivation areas. A strong positive correlation was also detected between potassium (K^+) and DWRhi, whilst the iridals concentration was not influenced by

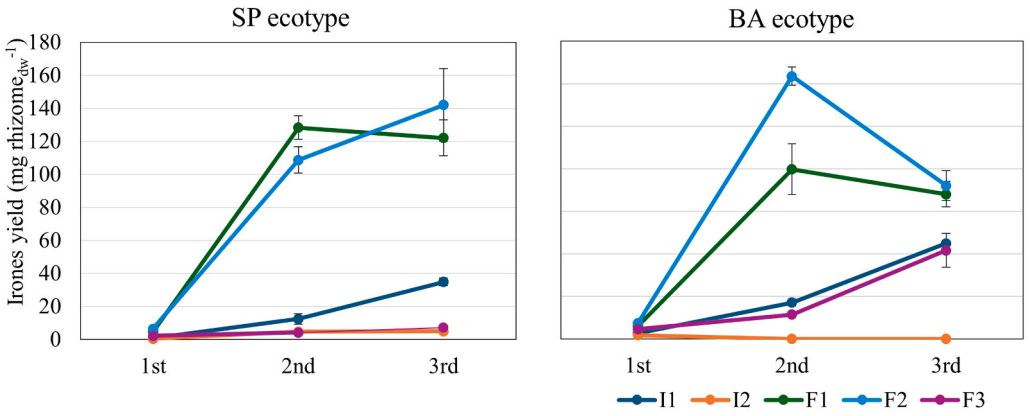

Fig. 5. Total irones yield (mg rhizome$_{dw}^{-1}$) of SP and BA *I. pallida* ecotypes at 1st, 2nd, 3rd year of cultivation in the selected areas (I1, I2, F1, F2, F3). Data are reported as mean values ± S.E.

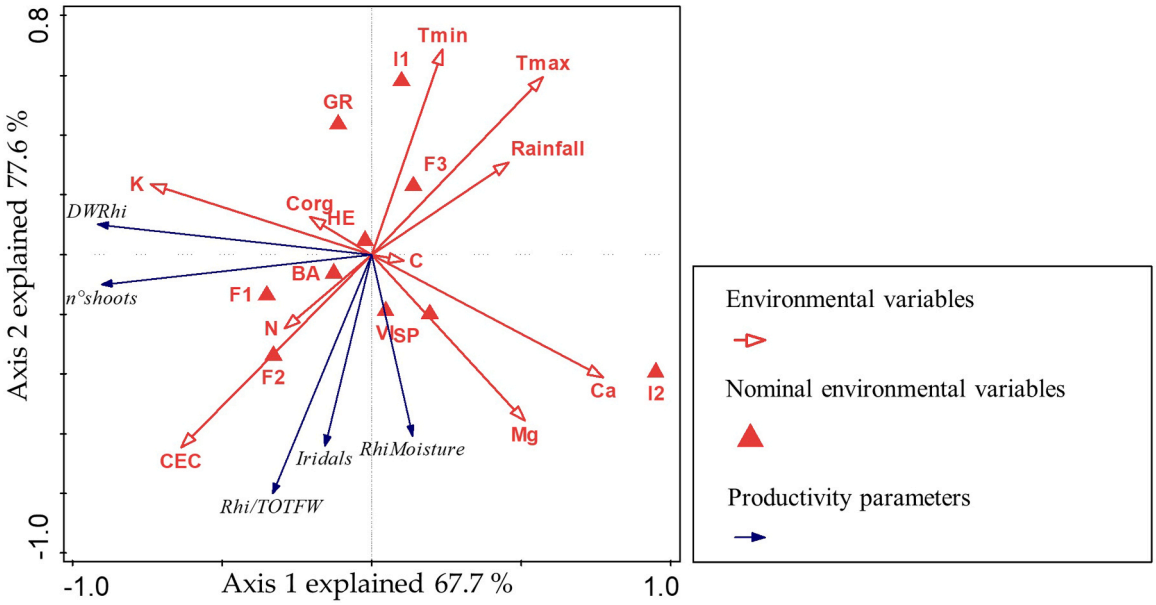

Fig. 6. Redundancy analysis (RDA) for the first two principal dimensions of the main parameters of *I. pallida* characterization. The plant parameters used are Rhizomes moisture (RhiMoisture), iridals concentration (Iridals), rhizomes dry weight (DWRhi), number of shoots (n°shoots) and rhizome on total plant fresh weight ratio (Rhi/TOTFW). Soil parameters used are Cation Exchange Capacity (C.E.C.), magnesium (Mg), calcium (Ca), potassium (K), nitrogen (N), carbon (C), and organic carbon (Corg) content. Climatic parameters used are Average annual rainfall (Rainfall), average maximum temperature of the warmest month of the year (Tmax), and average minimum temperature of the coldest month of the year (Tmin). Nominal variables are indicated as red triangles and correspond to the cultivation areas selected for the study (I1, I2, F1, F2, F3) and the ecotypes cultivated (GR, BA, HE, VI, SP). The data used are related to the 3rd year of cultivation (commercial harvesting time).

K^+. Moreover, a slight positive correlation could also be seen between iridal level and calcium (Ca^{2+}) and magnesium (Mg^{2+}) soil content. I2 was the area where the highest values of Ca^{2+} and Mg^{2+} could be detected. Additionally, a negative correlation could be observed between rainfalls and both rhizome dry weight and iridal amount, as well as between iridal amount and maximum and minimum temperatures (Fig. 6).

Looking at the distribution of the cultivation areas, three clusters could be observed: one composed of F1 and F2 areas, a second grouping I1 and F3, and a third one including only I2. This pointed out the differences among the cultivation areas, showing that F1 and F2 were the areas where iridal accumulation appeared to be enhanced.

To better elucidate the effects of ecotype, production cycle (1st, 2nd, 3rd year), and overall characteristics of the cultivation environment (area I1, I2, F1, F2, F3) on the three most important *I. pallida* productivity traits, irones concentration (μg g_{dw}^{-1}), irones content (μg plant^{-1}), rhizome dw (g_{dw}plant^{-1}), a three-way ANOVA was performed, and the results were reported in Table S10, S11, and S12. All the displayed factors and their interactions showed high significance for all three parameters considered, while for irones concentration only the Ecotype*Environment*Year interaction showed no statistically significant difference (Tables S10, S11, S12).

In Fig. 7, the observed effects of the independent variables were summarized as percentages of the total variance. Cultivation areas and year of cultivation explained most of the observed variability for rhizome biomass and irones yield per plant, accounting respectively for 40 % and 35 % of the explained variation of rhizome dry mass and 32 % and 35 % of the explained variation of irones yield per plant. Factors characterizing the ecotypes and their interaction with the environment explained observed variation to a lesser extent. A different scenario explains instead the variability for the irone concentrations, where 65 % of the trait variability was linked to the year of plant development;

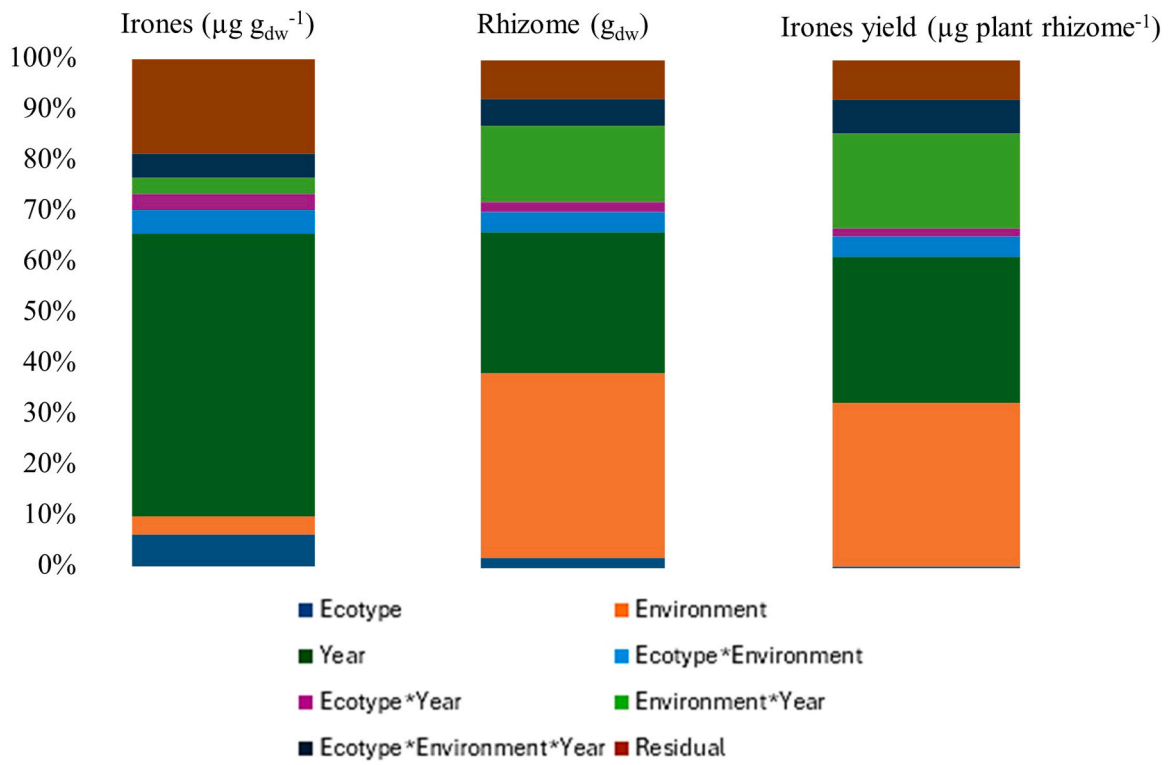

Fig. 7. Histograms showing the effects due to ecotype (GR, HE, BA, VI, SP), production cycle (1st, 2nd, 3rd year), environment of cultivation (area I1, I2, F1, F2, F3) and their interaction (Ecotype*Year, Ecotype*Environment, Environment*Year and Ecotype*Environment*Year) on the three most important productivity traits: irones concentration (Irones µg g_{dw}^{-1}), rhizome dry weight (Rhizome g_{dw}) and total irones yield (Irones µg plant rhizome $^{-1}$), percentages of the total variance. Data were subjected to the analysis of variance (three-way ANOVA).

factors directly linked to the ecotypes themselves account for 10 % of the variability, while 20 % of the total variation is attributed to residual unexplained variability.

4. Discussion

4.1. Pedo-climatic characteristics of the cultivation areas

One of the main environmental factors that contributes to crop productivity is soil physicochemical characteristics. Soil characterization of the areas traditionally used for *I. pallida* cultivation was investigated only in a few papers limited to the Tuscan region (Landi, 1994; Landi and Nicolelli, 1970; Pezzarossa et al., 2020). Our results agree with Pezzarossa et al. (2020) study concerning nitrogen and potassium content, while they slightly differ in terms of calcium, magnesium and organic carbon, both in Italian and French areas. In particular, the potassium and nitrogen contents result suitable for orris cultivation in all the areas selected for the project, considering that *I. pallida* is a potassophilic plant and requires a non-excessive nitrogen administration (Landi and Nicolelli, 1970; Aiello et al., 1996). Moreover, the areas are characterized by Mediterranean climates (I1, I2, F3) with warm and dry summers and mild winters, moderate rainfalls, or continental climate (F1 and F2) with cold winters and mild summers. *I. pallida* can adapt to different types of environments, being a rustic plant, and it can also cope with cold weather (Florio, 2016). Our RDA results confirm its adaptation to cold temperatures, showing no correlation between rhizome dry weight and the average minimum temperature of the coldest month; on the contrary, the rhizome dry weight shows a slight negative correlation with the average maximum temperature of the warmest month. This could be put in relation to the high rhizome production in F1 and F2 areas, where temperatures remained lower in comparison to the other three areas, mostly during the 1st and the 2nd years.

4.2. Ecotypes characterization

According to Mitić et al. (2000), plant stem length is a stable morphological characteristic among the different groups that make up the series. This evidence was confirmed by our data, which showed no significant differences among ecotypes cultivated on the same area. In other species belonging to the *Pallidae* series, it was pointed out a differentiation in the morphometric features of the leaves, demonstrating that this parameter can be considered to discriminate different populations. Our results show some significant differences in terms of leaf length and width among the ecotypes cultivated on the same area. More specifically, GR showed to behave differently if compared to the other ecotypes in terms of leaf dimensions in the two northern areas (F1, F2). The number of buds is not reported by literature as a discriminating feature because of its inconstancy (Pampanini, 1909), with previous data reporting 2–5 buds per stem (Mitić and Pavletić, 1999), while in our study, the number of buds goes from 6 to 11. The different attitude of the GR ecotype can also be observed in terms of root dry weight and total plant fresh weight, showing significant differences in terms of dimensions, resulting in bigger plants in comparison with the other ecotypes.

Rhizomes obtained in I1 and F3 show dry weights (on average 328 g per plant) in line with those reported by Firmin et al. (1998), of on average 302 g. This last study consisted of an evaluation of 69 clones collected from twelve different areas in France and Italy that were grown in Tours (France) for three years. The result obtained for *I. pallida* was compared by Firmin et al. to other Iris species, specifically *I. germanica,* which showed to be bigger, and *I. florentina,* which has, on average, the same weight. However, high variability in fresh and dry mass was observed in the population of *I. pallida* clones (Firmin et al., 1998). This was also confirmed by our results, as the five ecotypes selected for the study, when cultivated in I2, grew less than in the other areas, and, on the contrary, those cultivated in F1 and F2 areas showed particularly

high dry weights if compared to the traditional weights, reaching on average 773 g per plant.

The results of the preliminary description of the genetic distance between ecotypes confirmed the observed sensitive divergence. This might reflect the origin of the differences inside *I. pallida*, a complex species, native to north-eastern Italy, domesticated and widespread in Tuscany (Colasante, 2014) and in other Mediterranean areas, including France. Among the French ecotypes, BA is known to be one of the most "traditional", likely brought to France in the past century by Italian farmers. This ecotype is closely related to the Italian ecotypes SP and VI according to the genetic analysis. Overall, we hypothesized that the five ecotypes of *I. pallida* exhibit different rhizome growth rates and diverse irones productivity, and that this could be associated with both genetic differences and phenotypic adjustments due to the environmental conditions of the cultivation areas.

4.3. Iridal accumulation

The chromatograms of extracts from fresh and oxidized rhizomes confirm, as reported in a previous study (Krick et al., 1983), that iripallidal and iriflorental are the direct precursors of α and γ-irones, which developed after rhizome oxidation occurring during the postharvest phase. Over the last years, the geographical and botanical origin effect on orris butter composition has been the object of a few studies, mostly based on iridal content (Guenet, 1993; Masson et al., 2014). In Masson et al. (2014) study, a comparison between *I. pallida* chromatograms belonging to Moroccan, Chinese and Italian rhizomes pointed out that Italian ones are characterized by a greater abundance of iripallidal and iriflorental if compared to the others. In a study from Bezzi et al. (1993), six *I. pallida* Italian types, from the Tuscany and Veneto regions, were cultivated for four years to determine their rhizome productivity and quality. The results showed a significant difference in two of the six types that appeared more performative than the others (San Polo and Greve types) and which possessed higher iridal concentrations. Their productivity was on average 140 mg of iridals per 100 g of rhizomes dry weight, which is in line with the iridal content of our ecotypes (120 mg per 100 g of rhizomes dry weight on average).

From our study, a relationship between iridal productivity and cultivation area emerged: all the ecotypes performed better in the two areas at the highest latitude (F1 and F2), showing a production of iridals per plant of 700 mg on average. These results were not in agreement with Pezzarossa et al. (2020) study, where no relevant differences were detected in iridals concentration among some Tuscany farms. This discrepancy could be due to the slight differences among the environmental conditions of the farms, belonging to the same geographical area, in comparison to the areas selected for our study.

4.4. Ecotypes productivity

The forced oxidation method performed in our study is a new effective technique applied by LMR Naturals by IFF based on a licenced patent (SKH-GmbH, 2016), and it represents an advantage from a commercial point of view as well as from a research point of view, as it allows to evaluate a batch of rhizomes by rapidly and effectively oxidizing all the iridals after the harvesting and drying process, in comparison to the natural process or other techniques (Belcour et al., 1993; Bicchi and Joulain, 2025; de Bonneval et al., 2020; Gil et al., 1992). Regarding the chromatographic profile of the extracts obtained from fresh and oxidized rhizome samples, our results showed to be in line with those reported in previous studies focused on the same species (Bicchi et al., 1996; Masson et al., 2014; Mykhailenko, 2018; Pezzarossa et al., 2020). The forced oxidation technique described above allowed us to evaluate the productivity of orris plants in terms of irones content, differently from what could usually be found in the above-cited studies based on iridal content (Bezzi et al., 1993; Masson et al., 2014; Pezzarossa et al., 2020).

Annual monitoring of the quantity of both irones extractable from rhizomes and their growth during the three-year cultivation cycle highlighted the possibility of determining, for each ecotype and for each cultivation area, the best time to obtain the maximum yield in irones per plant, a fundamental parameter for the extractive industries. In some crops it may be necessary to cultivate the plants for at least three years to increase irones yield per hectare while when there is no increase in rhizomes mass between the 2nd and 3rd year, orris can be cultivated for only two years to accelerate crop turnover and farmers earnings, contrarily from what has been reported by Bezzi et al. (1993), where it is suggested to prolong orris cultivation until 4 years to obtain the maximum yield but only in term of rhizome growth.

4.5. Plant-environment interaction

From the literature, we assume that the existing relation between environmental characteristics and *I. pallida* productivity has not been sufficiently investigated yet. The effect of a single environmental factor (light intensity and quality) on phenolic production of *I. variegata* was investigated by Živković et al. (2021), while the effect of agronomical factors (plant density and harvest time), and the influence of the soil type on the yield of secondary metabolite extract (orris butter; Palchetti et al., 2025) and rhizome growth (Dorman et al., 2009) were investigated, without taking into consideration the overall environmental characteristics of the cultivation area.

From the RDA analysis performed putting in relation iridals concentration, number of shoots, rhizome dry weight and moisture with environmental parameters, it emerges that iridal concentration is positively correlated with rhizome moisture. This could be related to the role that iridals play in membrane integrity, as membrane constituents that can partially replace sterols, which incentivizes cells' turgor (Bonfils and Sauvaire, 1996). Moreover, in tissues exposed to dehydration and water stress, a lower concentration of iridals was detected (Bonfils and Sauvaire, 1996; Bonfils et al., 1994). Focusing therefore on environmental variables, a positive correlation of iridal content with soil cation exchange capacity (C.E.C.) confirmed the results previously detected by Pezzarossa et al. (2020). Furthermore, C.E.C. and nitrogen (N) and organic carbon (Corg) contents in soils result positively correlated with rhizomes dry weight and the number of shoots, as for $K+$, confirming the potassiophilous attitude of this species (Landi and Nicolelli, 1970; Aiello et al., 1996); all these parameters showed higher values in North-east France (F1 and F2). This evidence could lead to optimizing agronomic management to increase rhizome yield. Secondary metabolites formation in many plant tissues is strictly related to the environmental conditions to which the plants are subjected, and the modulation of these factors could be used to influence the accumulation of bioactive compounds in commercial crops (Verma and Shukla, 2015; Li et al., 2020). From our results, iridal accumulation in orris seems to be negatively correlated with maximum and minimum temperatures, as well as with average annual rainfall, which confirms the inverse correlation between rainfall and essential oil production detected in other wild species (Mehalaine and Chenchouni, 2021). The three-way ANOVA results reported in Fig. 7 suggest that the variability observed in rhizomes dry mass of the studied crops could be mainly determined by the different cultivation areas, and to a lower extent by the ecotype and year of cultivation, with direct consequences on productivity, which is based on the quantity of biomass constituted by the rhizome of each plant. Further investigations are required to elucidate changes in irones concentration that occur in plants over the years of cultivation: the irones concentration is maximal in the 2nd year, to further decrease during the 3rd year (Table S9) in favour of the mass increase (Table S8), with a sort of partial dilution effect over time. Moreover, as about 10 % of the variability of irones concentration is linked to the ecotype, it could be possible to select ecotypes more effective at accumulating iridals. This could help improve the economic convenience of essential oil distillation, particularly reducing the required energy consumption per kg of

distilled irones. It is also of interest to note that 20 % of the total variation seats under residual unexplained variability, supporting the hypothesis that there might be unexplored parameters impacting the concentration of these secondary metabolites and that it would be worth further investigating on a reduced set of ecotypes.

5. Conclusions

This study investigated the effects of soil composition, climate, and ecotype on the growth, development, and productivity of *I. pallida*, as well as the accumulation of iridals and the production of irones over multiple cultivation years. By cultivating five ecotypes in five different environments, it was possible to clearly discriminate the respective influences of ecotype and environmental conditions, highlighting the key role of soil composition, particularly organic carbon, potassium, and nitrogen, in rhizome production. Morphological differences among ecotypes were also assessed, supported by preliminary genetic analyses. Additionally, this study demonstrated a reduction in the time required for iridals-to-irones conversion, providing a valuable tool for industrial quality assessment of harvested rhizomes. Overall, the results contribute to the optimization of *I. pallida* rhizome production through improved, site-specific cultivation strategies and expand its cultivation area beyond the traditional ones, thereby benefiting the agricultural industry and contributing to the availability of this valuable plant resource. Further research is required to better understand the regulation of secondary metabolite synthesis, the iridal biosynthetic pathway and its oxidation into irones, as well as to achieve a definitive genetic characterization of the ecotypes.

CRediT authorship contribution statement

Fernando Malorgio: Methodology, Conceptualization. **Fabienne Bettini:** Formal analysis. **Andrea Raffaelli:** Investigation, Formal analysis. **Giorgiana Chietera:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Data curation, Conceptualization. **Annalisa Meucci:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Stefano Brizzolara:** Writing – review & editing, Investigation, Formal analysis. **Rita Maggini:** Writing – review & editing, Investigation, Formal analysis. **Irene Rosellini:** Investigation, Formal analysis. **Beatrice Pezzarossa:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Anna Mensuali:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Zhongqiang Chen:** Investigation, Formal analysis.

Funding

This study was carried out within the Agritech National Research Center and received funding partially from European Union Next-GenerationEU (PNRR) AGRITECH, PNRR M4C2, id CN00000022 (CUP J53C22001610007), and partially from LMR Naturals by IFF (International Flavors & Fragrances SAS. This manuscript reflects only the authors' views and opinions, and neither the European Union nor the European Commission can be considered responsible for them.

Websites

http://www.sir.toscana.it
https://www.historique-meteo.net/
https://it.climate-data.org/europa/francia/provenza-alpi-costa-azzurra/trets-8337/
https://www.climieviaggi.it/clima/francia
https://www.rhs.org.uk/

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

A special thanks goes to Irene Kahline, Jena Dryden, Aubrey Otis for their precious work on the genomic DNA extraction protocol optimization, and to Dave Seliger, PhD for the time spent on the genetic data interpretation, while at IFF (International Flavors and Fragrances) Health & Biosciences (Experimental Station, Wilmington DE 19803, USA); to Cristina Ghelardi, technician at CROPLAB of Sant'Anna Advanced School, for her precious support in laboratory and field activity.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2026.122730.

Data Availability

Data will be made available on request.

References

Aiello, N., Bezzi, A., Clementel, F., 1996. Effetti della concimazione organico-minerale sulla resa quanti-qualitativa di rizomi di *Iris pallida* Lam. ISAFA Comun. di Ric. 96/3.
ASA-SSSA, 1996. Methods of soil analysis, part 1 and 3 physical and chemical methods.
Belcour, B., Courtois, D., Ehret, C., Petiard, V., 1993. Rapid production of irones by maturation of orris rhizomes with two bacterial strains. Phytochemistry 34 (5).
Belletti, G., Fani, E., Marescotti, A., Scaramuzzi, S., 2013. The role of traditional products in the valorisation of marginal rural areas: the case of *Iris pallida*. Span. J. Rural Dev. 4 (4), 11–22.
Bezzi, A., Aiello, N., Villa, S., Bicchi, C., Rubiolo, P., 1993. Productivity and quality of rhizomes of some different types of *Iris sp*. Int. Symp Med. Aromat. Plants 344, 98–109.
Bicchi, C., Joulain, D., 2025. A Comprehensive review on essential oils and extracts from Iris rhizomes. Phytochem. Rev. 24 (2), 1629–1665.
Bicchi, C., Rubiolo, P., 1993. Analysis of constituents of iris rhizomes. Part I: high performance liquid chromatographic-particle beam-mass spectral analysis of iridals from *Iris pallida* rhizomes. Phytochem Anal. 4, 171–177.
Bicchi, C., Rubiolo, P., Fresia, M., 1996. Analysis of constituents of iris rhizomes. Part III—packed column supercritical fluid chromatography and high pressure liquid chromatography of iridals from rhizomes of *Iris pallida* L. Phytochem. Anal. 7 (1), 37–41.
Bonfils, J.P., Sauvaire, Y., 1996. Localization of iridals in *Iris germanica* rhizomes. Phytochemistry 41 (5), 1281–1285.
Bonfils, J.P., Sauvaire, Y., Baissac, Y., Marner, F.J., 1994. Iridal levels in Iris rhizomes—effects of wounding and dehydration. Phytochemistry 37 (3), 701–705.
de Bonneval, E., David, O.R.P., Doré, J., Hojlo, A.S., Inrig, W., Muller, C., de Swardt, D. Iris: l'iris en parfumerie; Coédition Nez éditions/Laboratoire Monique Rémy, San Diego, USA: 2020; p. 96.
Bruccoleri, R.E., Oakeley, E.J., Faust, A.M.E., Altorfer, M., Dessus-Babus, S., Burckhardt, D., Oertli, M., Naumann, U., Petersen, F., Wong, J., 2023. Genome assembly of the bearded iris, *Iris pallida* Lam. GigaByte. https://doi.org/10.46471/gigabyte.94. PMID: 37829656; PMCID: PMC10565908.
Colasante, M.A., 2014. Iridaceae Present in Italy. Sapienza Universitaria editrice, Roma, Italy.
Dorman, M., Sapir, Y., Volis, S., 2009. Local adaptation in four Iris species tested in a common-garden experiment. Biol. J. Linn. Soc. 98 (2), 267–277.
Firmin, L., Courtois, D., Pétiard, V., et al., 1998. Evaluation of the natural variability in Irone content and selection of *Iris* sp. for perfume production. HortScience 33 (6), 1406–1407.
Florio, F.E. Propagazione *in vitro* via embriogenesi somatica di *Iris pallida* Lam. (Giaggiolo). 2016, University of Pisa, thesis.

Garrison, E., Kronenberg, Z.N., Dawson, E.T., Pedersen, B.S., Prins, P., 2022. A spectrum of free software tools for processing the VCF variant call format: vcflib, bio-vcf, cyvcf2, hts-nim and slivar. PLoS Comput. Biol. 18 (5).
Garrison, E., Marth, G. Haplotype-based variant detection from short-read sequencing. 2012, arXiv:1207.3907 Available from ⟨https://arxiv.org/abs/1207.3907⟩.
Gee, G.W., and Bauder, J.W. Particle-size analysis. 1986, Methods of soil analysis: Part 1 Physical and mineralogical methods, *5*, 383-411.
Gil, G., Petit, J.L., Seris, J.L. Process for obtaining irone by enzymatic route. US Patent 5100790A, 1992.
Guenet, A. Comparaison des beurres d'iris de diverses origines botaniques etgéographiques, in: 12èmes Journées Internationales Huiles Essentielles, Digne, 1993, 492.
Herten, K., Hestand, M.S., Vermeesch, J.R., Van Houdt, J.K., 2015. GBSX: a toolkit for experimental design and demultiplexing genotyping by sequencing experiments. BMC Bioinforma. 16 (1), 73.
Krick, W., Marner, F.J., Jaenicke, L., 1983. Isolation and Structure Determination of the Precursors of a-and y-Irone and Homologous Compounds from *Iris pallida* and *Iris florentina*. Z. F. üR. Naturforsch. C. 38 (3-4), 179–184.
Lamarck, J.B. Encyclopédie méthodique, botanique, 3. Paris. 1789, pp: 294.
Landi, R. Population Performance, Fertilization Effects, and Environmental Factors on Orris Production (*Iris pallida* Lam.). Int. Conference "Coltivazione e miglioramento di piante officinali", Trento (Italy), 1994, 47-70.
Landi, R., Nicolelli, M., 1970. Due valide ragioni per coltivare il Giaggiolo. Floric. Inf. Agrar. 16655–16658.
Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25 (14), 1754–1760.
Li, Y., Kong, D., Fu, Y., Sussman, M.R., Wu, H., 2020. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol. Biochem. 148, 80–89.
Masson, J., Liberto, E., Brevard, H., Bicchi, C., Rubiolo, P., 2014. A metabolomic approach to quality determination and authentication of raw plant material in the fragrance field. Iris rhizomes: a case study. J. Chromatogr. A 1368, 143–154.
McKey, D., Elias, M., Pujol, B., Duputié, A., 2010. The evolutionary ecology of clonally propagated domesticated plants. N. Phytol. 186 (2), 318–332.
Mehalaine, S., Chenchouni, H., 2021. Quantifying how climatic factors influence essential oil yield in wild-growing plants. Arab. J. Geosci. 14, 1257.
Mitić, B., Nikolić, T., Liber, Z., 2000. Morphological and anatomical relationships in alpine-dinaric populations of the genus *Iris* L. Pallidae series (A. Kern) Trinajstić. Acta Soc. Bot. Pol. 69 (4), 285–291.
Mitić, B., Pavletić, Z., 1999. Comparative morphological analyses of the genus *Iris* L., Pallidae series (A. Kern.) Trinajstić (Iridaceae). Nat. Croat. Period. Musei Hist. Nat. Croat. 8 (4), 369–384.
Mykhailenko, O., 2018. Composition of volatile oil of *Iris pallida* Lam. from Ukraine. Turk. J. Pharm. Sci. 15 (1), 85–90.
Palchetti, E., Brilli, L., Padovan, G., Mariani, G., Marini, L., Moretta, M., 2025. Effect of Planting Density and Harvesting Age on *Iris pallida* Lam. Biomass, Morphology and Orris Concrete Production. Agronomy 15 (7), 1719.
Pampanini, R., 1909. L' *Iris cengialti* Ambr. e le sue forme. Nuov. Giorn. Bot. Ital. 16 (1), 63–96.
Passerini, V. Ricerche sulla composizione del giaggiolo/*Iris germanica*, «Le Stagioni Sperimentali agrarie Italiane», vol. XXI, 1891, 4, 565.
Pezzarossa, B., Borghesi, E., Pini, R., Bretzel, F., Maggini, R., Malorgio, F., 2020. Influence of pedo-climatic conditions on the quality of *Iris pallida* rhizomes. Eur. J. Hortic. Sci. 85 (2), 100–109.
Pezzarossa, B., Bretzel, F., Malorgio, F., Borghesi, E., Maggini, R., Scaramuzzi, S., 2016. La coltivazione del Giaggiolo in Toscana: dalla marginalità alla valorizzazione. Colt. Prote 7–8.
Poland, J., Endelman, J., Dawson, J., Rutkoski, J., Wu, S., Manes, Y., et al., 2012. Genomic selection in wheat breeding using genotyping-by-sequencing. Plant Gen. 5 (3), 103–113.
R Core Team, 2024. R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria.
Simonet, M. Bull Biol France et Belgique, 1932, 105, 255-444.
Šmilauer, P., Lepš, J., 2014. Multivariate Analysis of Ecological Data Using CANOCO 5. Cambridge University Press, Cambridge.
Sumner, M.E., Miller, W.P., 1996. In: Sparks, D.L. (Ed.), Cations exchange capacity and exchange coefficients. Methods of Soil Analysis, Part 3 – Chemical Methods, Soil Science Society of America Book Series. Soil Science Society of America Inc, Madison, USA, pp. 1201–1230.
Thomas, G.W., 1996. In: Sparks, D.L. (Ed.), Soil pH and soil acidity. Methods of Soil Analysis, Part 3 – Chemical Methods, Soil Science Society of America Book Series. Soil Science Society of America Inc, Madison, USA, pp. 475–490.
Trinajstic, I., 1976. Beitrag zur Taxonomie des *Iris pallida* Komplexes. Biosistematika 2 (1), 67–78.
USDA, 1996. Soil Survey Laboratory Methods Manual. Soil Survey Investigations Report No. 42. U.S. Department of Agriculture, Natural Resources Conservation Service, Washington, DC. Version 3.0.
Verma, N., Shukla, S., 2015. Impact of various factors responsible for fluctuation in plant secondary metabolites. J. Appl. Res. Med. Aromat. Plants 2 (4), 105–113.
Živković, U., Avramov, S., Miljković, D., Barišić Klisarić, N., Tubić, L., Mišić, D., Tarasjev, A., 2021. Genetic and environmental factors jointly impact leaf phenolic profiles of Iris variegata L. Plants 10 (8), 1599.