Evaluation of the Natural Variability in Iron Content and Selection of *Iris* sp. for Perfume Production

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Abstract. Rhizomes of *Iris* species used for perfume production do not contain scented irones immediately after harvest, precluding early selection of potentially high-producing genotypes. A recently developed technique involving a bacterial treatment (*Rahnella aquatilis* Izard, Gavini, Trinel, and Leclerc) of fresh rhizomes shortened the maturation time from 3 years to a few days. Variability in iron content among freshly harvested *Iris* species (76 clones) was evaluated, and three high-producing clones of *Iris pallida* Lam. were selected. Significant variability among clones was observed for iron content, growth, and yield.

Irones extracted from *Iris* species are aromatic principles used in the perfume and flavor industry to develop violet fragrance. Four main compounds (trans- α -, cis- α -, cis- γ -, and β -irones) have been described (Naves et al., 1947; Schinz et al., 1947) as responsible for the characteristic scent of iris oil. These terpenoid compounds originate from squalene (Jaenicke and Marner, 1990; Marner et al., 1988). The isomeric distribution, the absolute configurations, and the "sensory" properties of irones are dependent on the iris species from which they have been isolated (Galfre et al., 1993). The species that are cultivated for perfume production are *Iris pallida*, with a high content of (–) cis- γ -irone, and *Iris germanica* L. with high levels of (+) cis- α -irone. The levorotatory isomers are preferred in sensory tests. Extracts from freshly harvested rhizomes contain very low amounts of irones. Irones are synthesized very slowly after harvest and are usually extracted after 3 years of storage at room temperature (Garnero et al., 1978; Guenther, 1952). This gives a good compromise between iron content and storage cost, but iron content continues to increase even after 3 years of storage (Crabalona, 1959).

So far, only a few studies have been done on the selection of *Iris* species. Bezzi et al. (1993) reported significant differences between species in growth of rhizomes, but they were unable to evaluate the potential iron content of fresh tissue for lack of selection markers. No selection tools are currently available for such evaluation in freshly harvested rhizomes.

A strain of bacteria, *Rahnella aquatilis* isolated from the rhizosphere of the iris plant [(previously identified as *Serratia liquefaciens*, Grimes and Hennerty (Enterobacteriaceae)], can synthesize irones from precursors in freshly harvested rhizomes (Belcour et al., 1989). Transformation occurs within 7 d, so that rhizomes no longer need 3 years of storage prior to industrial extraction. This technique permitted the evaluation of natural variability, and the clonal selection of potentially high-iron producing clones. The first study reported using this technique involved 19 one-year-old clones (Jehan et al., 1994a). The results reported here were obtained from 76 iris clones representing four species.

Materials and Methods

Plant material. Four different iris species were used in this study: *I. pallida* (light-blue flowers, 69 clones collected from 12 different areas in France and Italy), *I. germanica* (dark-violet flowers, three clones), *I. florentina* L. (white flowers, two clones), and *I. variegata* L. (light-yellow flowers, two clones). *I. germanica*, *I. florentina*, and *I. variegata* were collected from private gardens in France.

Plants were divided and grown in a greenhouse for 1 year to obtain a sufficient number. Plants were set out in a field trial at Tours,

France, in Spring 1992, with three blocks in a randomized complete-block design. Five plants per clone were set 1 m apart in the row, with 1 m between rows, and harvested in Summer 1995. The rhizomes of the five plants per clone per block were combined for analysis (total of $76 \times 3 = 228$ samples).

Evaluation of growth. Just after harvesting, rhizomes of each group of five plants were weighed, then crushed and frozen at -20°C for subsequent analysis. An aliquot was dried in an oven at 110°C for 24 h to determine the dry-matter content.

Evaluation of iron content. The technique of Belcour et al. (1993), modified by Jehan et al. (1994b), was used to quantify iron content. A culture of bacteria (*Rahnella aquatilis*, previously determined as *Serratia liquefaciens*) grown on agar medium was transferred to a 50-mL conical flask containing 20 mL of nutrient broth (Diagnostics Pasteur, Paris) and incubated for 24 h at 26°C on a rotary shaker (100 rpm). Crushed tissue (8 g) was placed in 250-mL Erlenmeyer flasks and treated with 50 mL of the 1-day-old culture of *Rahnella aquatilis* (inoculation at 1%) in a mixed Murashige and Skoog (1962) and nutrient broth medium (90/10) adjusted to pH 2.5 with HCl (7.5 N). The incubation period was 7 d at 24°C on a rotary shaker (110 rpm). Three replicates were analyzed for each sample. Irones were extracted in 50 mL of tert-butyl methyl ether/ethanol (80/20, v/v) with an internal standard (anethol) and 20 mL of NaOH (1 N).

Analysis was performed by gas chromatography (FID detector) with a $25 \text{ m} \times 0.32 \text{ mm}$ WCOT capillary (CP-WAX-52CB) column with He as the carrier gas ($1 \text{ mL} \cdot \text{min}^{-1}$), and a temperature program of 60°C (4 min), then $12^\circ\text{C}/\text{min}$ to 150°C , and $3^\circ\text{C}/\text{min}$ up to 240°C . Injector and detector temperatures were 300 and 310°C , respectively. The iron content was expressed in $\text{mg} \cdot \text{kg}^{-1}$ DM, and the yield in mg iron per plant.

Statistical analysis. ANOVA and a multiple range test (Student–Newman–Keuls) were used to analyze the data for *I. pallida* clones with high yields of cis- γ -irone (Type A). Calculations were obtained by using the software STAT-ITCF (Gouet and Philipeau, 1989).

Results and Discussion

Determination of chemotype. Four iron isomers, trans- α -, cis- α -, cis- γ -, and β -irone, were detected by GC analysis. However, the content of β -irone was very low, so only the three isomers were quantified (Table 1). Although the phenotype of the 69 *I. pallida*

Table 1. Isomeric distribution of irones among clones of different *Iris* species.

Species (no. clones)	Proportion of each isomer (% of total iron content)		
	trans- α	cis- α	cis- γ
<i>I. pallida</i> (A) (59)	3.4 ± 1.7	33.2 ± 0.5	63.4 ± 1.3
<i>I. pallida</i> (B) (10)	0	81.4 ± 0.5	18.6 ± 0.5
<i>I. germanica</i> (3)	0	20.9 ± 0.8	79.1 ± 0.8
<i>I. florentina</i> (2)	0	18.9	81.1
<i>I. variegata</i> (2)	ND ^a	ND ^a	ND ^a

^aNot detectable.

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clones was strictly identical in the field, two chemotypes were detected. Chemotype A had close to the standard *I. pallida* composition, with a high content of cis- γ -irone. Chemotype B was quite different, with a high cis- α -irone content. The isomeric distribution was closely related to the species, as indicated by the standard deviation, and the differences between A and B led us to consider that the B group may not be *I. pallida*, but a possible mutant or hybrid. The interesting clones for perfume production are those of the A group.

The *I. germanica* clones presented an unusual chemotype characterized by high levels of cis- γ -irone. This contrasts with other data where cis- α -irone is predominant (Galfré et al., 1993). These clones have the same chemotype as *I. florentina*. This may strengthen the argument for the hypothesis of Kolheine (1986) that *I. florentina* is a variety of *I. germanica* with white flowers.

Growth characteristics of the rhizomes. Growth among species varied greatly (Table 2). The highest fresh mass was observed for *I. germanica* and was 4.8 times as high as that of *I. variegata*. The best growth was observed for *I. germanica*, whereas *I. pallida* (A group) and *I. florentina* were intermediate. The mean fresh and dry mass of the "unusual" *I. pallida* (B group) is close to that of *I. pallida* (A group). High variability in fresh and dry mass was observed in the population of *I. pallida* A clones, with $P < 0.001$.

Irone content. A broad variability (1- to 3-fold) was observed in the mean irone content of the four species (Table 2). The A clones of *I. pallida* contained twice as much irone per kg DM as the B clones. Irone contents in *I. florentina* and *I. germanica* were not significantly different and were similar to those of the B clones. *I. variegata* contained very low amounts of irones. Significant differences were found among the population of 59 *I. pallida* (clones A) tested separately ($P < 0.01$).

Yield of irone. Among species, irone yield varied from 1- to 8-fold (Table 2). The yield was highest for *I. pallida* clones A and the differences among clones were highly significant ($P < 0.001$). In this population of A clones, the correlation between irone content (mg·kg⁻¹ DM) and rhizome growth was significant (Fig. 1: $R = 0.302$ at $\alpha < 0.05$), suggesting that the industrial user must evaluate these two parameters.

These results describe for the first time the use of an experimental selection system designed to obtain high irone-producing clones of *Iris* sp. A significant variability between various clones of iris was demonstrated, at least in one location. Also for the first time, the technique used allowed the characterization of the chemotype of freshly harvested plants, and might therefore be used for phylogenetic studies. Three high-producing clones (IP1, IP2, IP3) were selected among the typical *I. pallida* clones A representing the best chemotype for the perfume producer. Potentially, these clones could yield 40% more irones in comparison with traditional genotypes. In vitro propagation of these clones is in progress (Firmin, 1998; Jehan et al., 1994b).

Table 2. Mean fresh and dry mass of rhizomes, irone content, and irone yields per iris plant of five species after 3 years of growth in the field.

Species	Fresh mass (g/plant)	Dry mass (g/plant)	Irone content (mg·kg ⁻¹ DM)	Total irone yield (mg/plant)
<i>I. pallida</i> (A)	866 ± 293 ^a	328 ± 111	1386 ± 153	461 ± 182
<i>I. pallida</i> (B)	1084 ± 146	372 ± 43	677 ± 73	252 ± 50
<i>I. germanica</i>	1342 ± 175	437 ± 85	595 ± 86	255 ± 38
<i>I. florentina</i>	819 ± 154	278 ± 46	673 ± 40	183 ± 46
<i>I. variegata</i>	279 ± 33	99 ± 17	ND ^b	ND ^b

^aStandard deviation.

^bSignificance of differences among *I. pallida* (A) clones by ANOVA at 0.01 (**) or 0.001 (***). No block effect was observed for any parameter.

^cNot detectable.

Fig. 1. Scatter plot with linear regression line showing relationship between growth of rhizomes and irone content for typical clones A of *I. pallida*. High-producing clones IP1, IP2, and IP3 are circled.

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