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VARIETAL IDENTIFICATION OF HYDRANGEA USING RAPD MARKERS

Document prepared by experts from France

VARIETAL IDENTIFICATION OF *HYDRANGEA* USING RAPD MARKERS

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About 400 varieties of *Hydrangea* are known. Most varieties belong to the species *Hydrangea macrophylla* spp *macrophylla.* Even if there are only about 10 varieties currently commercialised, presently, consumers ask for new types of Hydrangea such as plants with flattened inflorescence and a greater number of varieties will appear soon in the market.

France is in charge of the description of the new varieties for UPOV and the reference collection is maintained and described by the ENITHP in Angers. The establishment of DUS is based on morphological characters which are often dependent on agroclimatic conditions (flower colour, leaf shape). The genetic basis of the varieties is not very wide. For these reasons, new additional characters need to be used to describe the varieties which will be applicant for protection in the future. We first used enzymatic markers and obtained a good discrimination of the varieties. However, the expression of some of the markers used was dependent on the stage of the plant. Therefore, we studied the potentiality of molecular markers.

This paper presents results on RAPD (Random Amplified Polymorphic DNA) markers and preliminary results on ISSR (Inter-Simple-Sequence-Repeats).

Material and methods

27 varieties of *Hydrangea macrophylla* spp *macrophylla* representing the 5 groups of flower colour and the 2 types of inflorescence and 2 varieties of *Hydrangea macrophylla* spp *serrata* (Diadem and Chinensis) have been analysed.

RAPD (Williams et al., 1990)

101 arbitrary sequence primers of 10 nucleotides (Bioprobe Systems) have been tested on a set of 8 varieties representative of the genetic variability. These 101 primers have been sorted out and 15 primers have been chosen on the following criteria: detection of polymorphism, amplification profiles of good quality, and primers giving bands unambiguously and easily scorable.

Reproducibility in the appearance of the bands have been tested for different amplifications of the same DNA extract and for 2 different individuals (different extractions and amplifications) which derived from the same motherplant.
Each band has been scored as present or absent, visually.

ISSR

7 minisatellite sequences (Jeffreys, M13, tri- and tetranucleotide repeats) used as primers of PCR have been tested. For these primers, only one amplification experiment has been carried out on one individual per variety. The reproducibility of the test has not yet been tested.

Results

RAPD Markers

Choice of the bands

The number of the bands varies from 3 to 15 according to the primer. Because this method should be possibly used for DUS testing, not all the bands have been considered but only the very clear and repeatable bands.

The bands showing too proximal electrophoretic mobilities have been eliminated. Only bands of strong intensity and therefore, which do not display variation in intensity throughout the set of the 29 varieties have been chosen (Figure 1). So, this avoids to attempt to define an intensity threshold to decide whether a band must be considered as present or absent depending on the global intensity of the pattern.
Among the 101 primers tested, 17 gave poor amplification, 56 showed polymorphic patterns and 15 only were selected yielding 37 bands which seemed reliable and which were used to describe the 29 varieties. The number of bands taken in account for each primer varies from 1 to 6, and is in average 3.

Distinction

The frequency of absence of the bands is very variable, from 2/29 to 28/29. Out of the 37 bands, 9 are specific of the subspecies *serrata*, 2 are specific of Rosea and 1 of Mme Mouillère. Of course, the selection of the bands is not quite neutral : specific bands are more likely to be selected because they are more easily detectable between different patterns. With only 4 primers (9 bands), it is possible to obtain the same efficiency for distinguishing the 29 varieties (Table 1). The specific bands are not needed for the distinction. This reflects that the two subspecies derived from a common genetic pool. These results are in favour of a geographic differentiation of a same species in two subspecies : spp *macrophylla* is located on the seaside, spp *serrata* in mountainous area.

The polymorphism observed between the varieties is relatively high : with the 37 bands, almost all the varieties are uniquely distinguished, excepted the pairs Altona and Europa, Merveille and Merveille Sanguinea, Vetchii and White Wave and the 3 varieties Iowa, Rotkehlchen, Möwe. Very similar results had been obtained using 3 enzymatic markers.

Among the couples or groups that remain undistinguished, the following are easily explainable :
The varieties Altona and Europa are originated from a same sowing.
The varieties Iowa and Rotkehlchen are probably the same variety.
The variety Merveille Sanguinea is a mutation of the variety Merveille for the colour of the leaves.

Although variety Möwe is morphologically very similar to variety G. Chadbund, the two varieties are not known to be genetically related. In our study, they differ by 8 bands (out of 37). Others varieties, non-genetically related, such as Elster and Pia Mina differ by 6 bands.

On the other hand, some results are not in good agreement with what is known on the varieties. For example, some varieties, although very different from each other, display the same RAPD patterns or differ by only one band : this is the case for Vetchii-White Wave and Bodensee-Tizz, respectively. On the contrary, Eugen Hahn and Kirsten which are close to each other, differ by 8 bands.

ISSR Markers

Out of the 7 primers tested, 3 ([GATA]4, [CA]8, [AT]8) have not yielded amplication products. Primer M13 was not very polymorphic. The primers [GTG]5 and [GACA]5 and 33.15 showed a high polymorphism between the varieties. The results seem very similar to what has been already observed but at least, one more experiment would be necessary to choose the repeatable bands according to the same criteria as for RAPD markers.

Conclusion

RAPD markers are powerful to distinguish and identify the varieties of Hydrangea. Although they reveal the similitude of varieties in some cases (Iowa-Rotkehlchen, Altona-Europa, Merveille-Merveille Sanguinea) RAPD markers do not reflect the genetic relatedness. It is not surprising because the choice criteria of the bands produce a bias and also because the markers are generally dominant.
At the subspecies level, the RAPDs were found very efficient to separate the two groups (macrophylla and serrata) and will be very useful to classify the varieties for which a doubt subsists.

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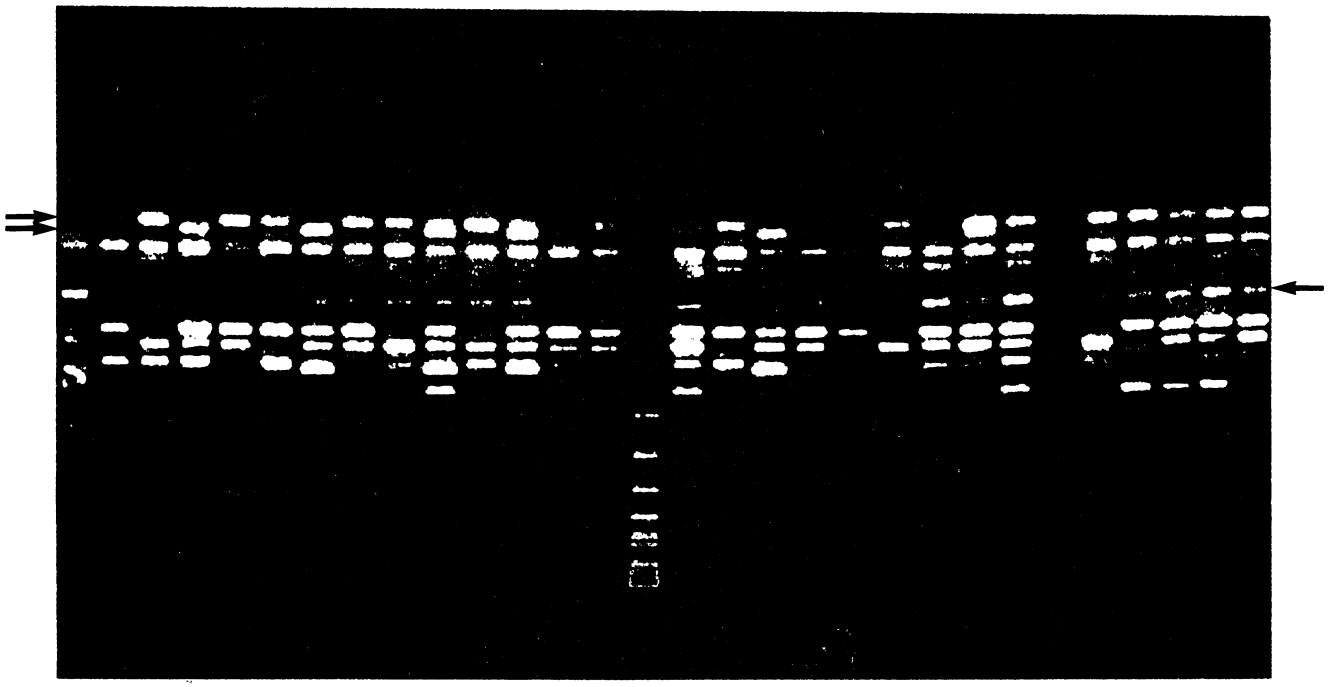

Figure 1

Table 1

	PRIMER								
	M2	B7	B7	B3	B3	B3	M2	B12	B7
	BAND (MW)								
	710	1060	555	630	580	350	610	530	790
Diadem	0	0	0	0	0	0	1	0	0
Chinensis	0	0	0	0	0	0	1	0	1
Kirsten	0	0	0	1	0	0	1	1	0
G. Chadbund	0	0	0	1	0	1	0	0	1
Brugg	0	0	0	1	0	1	1	1	1
Rabe	0	0	0	1	1	1	0	1	1
Rotkehlchen	0	0	0	1	1	1	1	0	1
Möwe	0	0	0	1	1	1	1	0	1
Iowa	0	0	0	1	1	1	1	0	1
Bodensee 1	0	0	0	1	1	1	1	1	0
Bodensee PB	0	0	0	1	1	1	1	1	0
Tizz	0	0	0	1	1	1	1	1	1
Eugen Hahn	0	0	1	0	0	1	0	0	1
Pia mina	0	0	1	0	0	1	0	1	1
Ramu	0	0	1	0	0	1	1	1	1
Müke	0	0	1	1	0	0	0	0	1
Libelle	0	0	1	1	0	0	1	1	1
Merveille	0	0	1	1	0	1	0	0	1
M. Sanguinea	0	0	1	1	0	1	0	0	1
Tovelit	0	0	1	1	0	1	0	1	0
Rosea	0	0	1	1	0	1	0	1	1
Deutschland	0	0	1	1	0	1	1	1	1
Boncuse	0	1	0	0	0	0	1	1	1
Altona	0	1	0	1	0	1	1	1	1
Europa	0	1	0	1	0	1	1	1	1
Elster	0	1	1	0	0	0	1	1	1
Mme Mouillère	0	1	1	1	0	0	1	1	1
White Wave	1	0	1	1	0	1	1	1	1
Veitchii	1	0	1	1	0	1	1	1	1

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