Distinction of melon genotypes using NIR spectroscopy

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Introduction

The cultivation and breeding of melon - musk- and watermelon - are traditionally of great importance in Hungary. The Department of Genetics and Horticultural Plant Breeding of the Szent István University has been working on the breeding and on the developing of biotechnological methods for melon breeding for decades. The Department takes also part in the supervising and upkeeping the official Hungarian melon gene bank. In our present study, we examined different melon genotypes using NIR spectroscopy from two aspects.

In the first approach, we characterized several representative, standard Hungarian cultivars with NIR spectroscopy. The identification of cultivars, breeding sources and lines by using molecular markers has a great significance from the point of view of upkeeping and protection of cultivars. NIR spectroscopy is a quick, non-destructive method and - what shouldn't be neglected from the aspect of environmental protection - does not need any kind of chemicals to be applied.

The second application we considered to be significant was the examination of hybridity. Seed production and hybrid breeding of melon have vast demand of labour and cost, because the sexual determination of melon is quite uncertain, it depends greatly on the climatic factors. Melons have the ability of xenogamy and of self-pollination also, and up to this time, there wasn't published any genetic system which could help the hybrid breeding of melon. Because of this, the clear distinction of hybrids and parent lines would be a great assistance for the breeder and the seed-producer. This can be carried out using DNA-based markers, but this way only the presence of non-hybrid seeds can be detected during representative sampling of a seed-bulk. Physical separation can't be achieved by DNA-based examinations, but the non-destructive NIR-technology fits for this task.

Summarized, in the present study our goal was the habitat- and vintage-independent distinction of the investigated melon genotypes using NIR spectroscopy.

Materials and methods

Plant material

We investigated bulk-seed samples of 5 watermelon varieties (accession numbers of the Hungarian melon gene bank: 2611, 2612 (variety2); 2511, 2512 (variety3); 2211, 2212 (variety5); 2251, 2252 (variety8); 2261, 2262 (variety 13)); 4 muskmelon varieties (accession numbers: 3221, 3222, 3223, 3224 (variety1); 3229 (variety7); 3421, 3422 (variety16), 3521 (variety17)); and a hybrid cultivar with its two parent lines. By most of the varieties two samples were tested, each with

different origin and from different vintage. From the muskmelon variety1, 4 samples were involved in the study. The different number of samples from a cultivar explains the different number of quality points in the figures.

NIR spectroscopy

The spectra of the investigated melon seed samples were recorded on a SPECTRALZYER 1025 scanning type spectrometer in the wavelength region of 1000-2500 nm with a spectral step of 2 nm. To filter the inhomogeneity of the bulk samples, each sample was filled in two times, and each measurement was repeated two times, thus for each sample belong six spectra, six quality points

Data evaluation

The spectra were analyzed with the software PQS32 v1.56, developed by Metrika R&D Co, Hungary. As a qualitative evaluation Polar Qualification System $(PQS)^1$ - using automatic wavelength range optimisation - was used. In all cases, the spectra were pre-treated by triangular smoothing (7 nm) and second derivative transformation (gap: 12 nm).

Results

The investigated muskmelon varieties were separated with a single optimal wavelength region between 1448 and 1576 nm, determined by automatic wavelength range optimization function of PQS (figure 1).

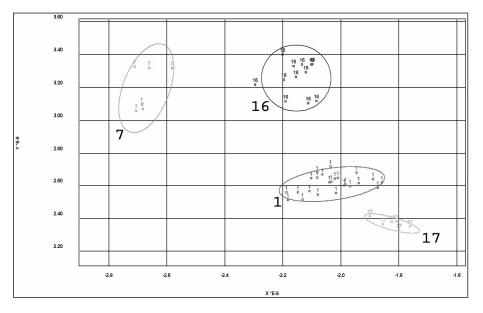


Figure 1. The quality points (centres of the polar spectra). Repeated measurements of the investigated muskmelon samples

The distinction of the investigated watermelon genotypes was possible only with a four step analysis. In the first step variety2 and 3 could be identified (figure 2a) in a wavelength range of 1014-1612 nm, the clusters of the three remaining varieties are overlapping. For these, further

optimum ranges were defined: 1274-1412 nm for variety5 (figure 2b) and 1552-1782 nm for variety8 (figure2c). For the optimal separation of variety13 was a doubled optimum range assigned: 1334-1344 and 1652-1688 nm.

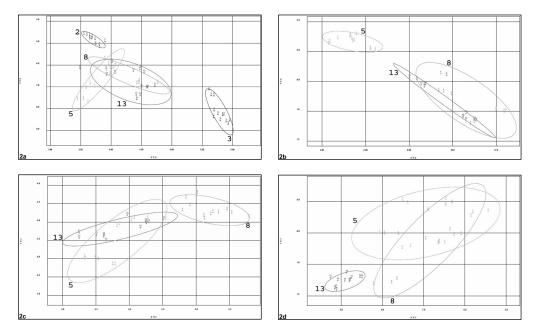


Figure 2a. The distribution of the quality points (centres of the polar spectra) of the five water melon varieties; 2b-d. The separation of the three varieties which were overlapped in Figure 2a

The hybrid and its parent lines could be distanced with a single optimum range of 1342-1410 nm (figure 3). The value of sensitivity (S) expressing the effectiveness of the classification was 12,32 which means that the distance between the quality points of the investigated samples was 12 times greater than the sum of their standard deviation.

Discussion

Both the musk- and the watermelon samples showed a significant difference in their spectra and in the location of the belonging quality points, because of the alternate origin and vintage of the seeds.

The investigated muskmelon varieties seem to be more polymorph than the watermelon ones. This could be explained by the fact that two cultivars (variety7 and variety17) were represented only by single samples. On the other hand, from variety1 four samples were investigated, and these showed a relatively small deviation in the location of the quality points (figure 1).

RAPD (random amplified polymorphic DNA) polymorphism of watermelon varieties is very limited,² and Hungarian cultivars can't be distinguished by this method.³ Similarly, the distinction of the investigated watermelon genotypes with NIR spectroscopy was more difficult (figure 2), than by the genetically more polymorph muskmelon varieties.⁴ There were several kinships among the investigated cultivars, which decreased the genetic polymorphism.

NIR spectroscopy mainly detects molecular phenotypic differences, which are very affected from the origin and the vintage. However, the high level of the standardization of NIR measurements and of the evaluation methods could raise the power of distinction.

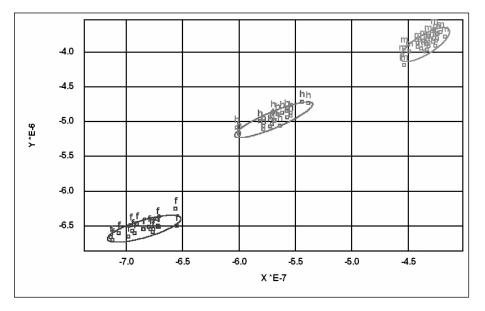


Figure 3. The quality points (centres of the polar spectra) of the hybrid water melon samples (f: father, m: mother, h: hybrid).

The distinction of the hybrid variety from it's parent lines was very effective (figure 3). This can be explained with the fact that in this case, the samples originated from the same habitat and vintage, so there were no phenotypic aberrations to sift.

Perspectives

The ultimate goal of our studies is to separate the hybrids and their parent lines form single-seed samples. We plan to involve further hybrids in our studies, and to investigate the hybrids from single-seed samples.

References

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