Breeding and cultivar identification of *Cichorium intybus* L. var. *foliosum* Hegi

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Abstract: *Cichorium intybus* var. *foliosum* Hegi (Belgian endives) is a leafy vegetable with a limited production area. Introduction of hydroponic forcing techniques has resulted in a demand for better performing cultivars. The genetic basis on which breeding is done, is narrow asking for input of new gene pools. Molecular biology techniques need to support breeding efforts. The creation of a molecular map and the development of QTLs will offer a higher efficiency in breeding. Cultivar identification will help to support breeders rights and will help growers to certify the seeds or roots obtained for production.

Keywords: *Cichorium intybus* L. var *foliosum* Hegi, RAPD, AFLP, QTL, cultivar determination,

Introduction: history of chicory breeding

Belgian endives have been produced since roughly the end of the 19th century. At that time this leafy vegetable was produced using soil coverage and artificial heating by means of an oil stove. In this way forcing of the taproot resulted in the typical etiolated crop. The major production area of endives at that time was located in the triangle between Mechelen, Leuven and Brussels (Belgium). As time went by growers started to select for themselves those plants that did well in production. Almost each grower had one or more (grower)selections.
For reasons of human labor and plant productivity, selection and breeding was started. In addition the introduction of hydroponics in the '70s asked for more performing selections. Selection and breeding started first at research stations like INRA, France (DeConick) and the KU Leuven, Belgium (Van Nerum). Later on seed companies became interested and took over the breeding work.

Selection work was dealing with (more) productivity and problems to keep the head of the endives closed since the soil pressure was responsible for this growth reaction. The genetic basis of the crop is very narrow; therefore one can expect that negative characters will show up as selection continues. It has been suggested that most of the "physiological problems" of endives (e.g. browning of the flowering stem, red color of the leaves) resulted from inbreeding depression (Van Nerum 1995, personal communications). As chicory cultivation continued to spread over the North of France, Flanders and The Netherlands commercial seed production was needed. Early and winter cultivars were developed and later the very late cultivars for summer production were introduced. More and more new F1 hybrids were developed.

These days year round production is made possible by the use of hydroponics culture techniques and the development of specific hybrids. This was a must since high investments were done in high technological hydroponics installations. By this the endive producers where given much more flexibility, productivity and quality compared to the laborious handwork of soil production. Breeders in France and The Netherlands are producing on a regular basis new cultivars. The average lifetime of a new cultivar on the market is limited. A good cultivar will survive 10 years but an average lifetime is no more than 3 to 5 years. This means that the breeding efforts are very big compared to the potential financial return of the crop.

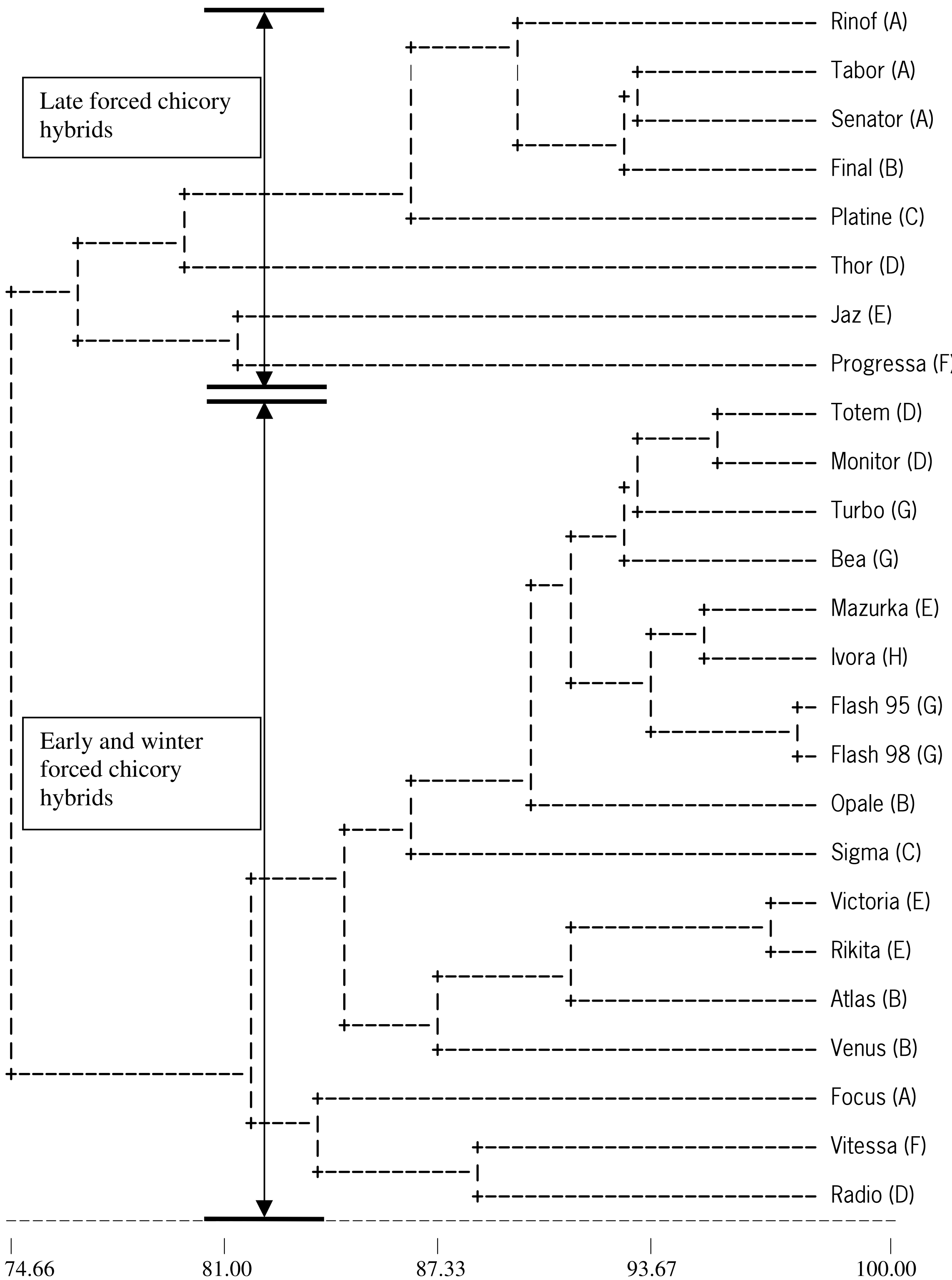

Figure 1. Dendrogram of Cichorium intybus L. var. foliosum Hegi F1 hybrids (1995-1996) based on RAPD data. An unweighted pair group average cluster analysis was performed on a similarity matrix constructed using ‘percent similarity’ indices. The seed company is given between brackets. (A) Nunhems, (B) Hoquet, (C) Vilmorin, (D) Bejo, (E) Clause, (F) Novartis, (G) INRA, (H) ENZA.

Technical innovations and the use of better cultivars (hybrids) pushed the production from 15 to 20 kg per m² up to around 60 and even 75 kg per m² these days.

All this supports the statement that breeders need to look for new methodologies and fresh genetic input to cope with the needs of the actual endive producers and consumers.

New research methods

Material and methods are detailed by Van Stallen 2003.

Cultivar identification

Different endive cultivars do possess different quality characteristics. But this is poorly used in a strategy to convince the consumer in eating endives. Different cultivars are actually only of importance to the grower and trader. The different endive cultivars are hardly recognizable by the human eye. To make more use of the different cultivars features, endives need to be sold to the consumers under the cultivar name, offering the expected crop quality aspects they wanted to buy. The potential of using RAPD and AFLP techniques to identify different endive cultivars was demonstrated (Van Stallen *et al*. 2000). In this study 24 different cultivars that were present on the Belgian market in 1995 and 1996 were used to come to a strategy offering us the possibility of cultivar identification. Using decamer primers (more than 1000 different primers were tested) a dendrogram could be constructed (Figuere 1).

It became clear that identification of a cultivar by using molecular techniques is very well possible. Both RAPD and AFLP techniques are suitable. The late cultivars were well separated from the early and winter cultivars. This is a clear indication that the forcing time for endive cultivars is genetically determined. The separation of cultivars is not related to the seed producers. In some cases very high similarity between cultivars were obtained (Rikita and Victoria). We were able to do the cultivar identification on the seed, seedling, root and harvested crop level.

Genetic homogeneity of endive hybrids

The homogeneity of a plant population can be tested. The number of decamer primers resulting in uniform banding indicates homogeneity. From table 1 it is clear that big differences can be observed between cultivars. This can be explained in a difference in cross compatibility of the parent lines or the homogeneity of these parent lines (Castano et al. 1997).

Table 1: Percentage of decamer primers tested (N=26) that generated polymorphic marker bands within chicory F1 hybrids.

Rinof 6.1%	Tabor 4.1%	Totem 14.3%	Victoria 4.1%	Bea 20.4%	Mazurka 8.2%	Focus 14.3%	Vitessa 28.6%	Final 10.2%
Rikita 20.4%	Sigma 16.3%	Jaz 14.3%	Monitor 22.4%	Atlas 16.3%	Progressa 6.1%	Flash95 14.3%	Opale 4.1%	Platine 0.0%
Radio 6.1%	Ivora 4.1%	Venus 20.4%	Turbo 10.2%	Senator 12.2%				

From these observations it became clear that the level of inbreeding of parent lines was very different between hybrids. Levels of almost nothing up to 30 % were observed. These results were a confirmation of what was found by other authors (Bellamy, 1995).

Using these molecular techniques one is able to select plants for specific characteristics and to clean up the parent lines.

Last but not least, to protect the cultivars by breeders rights it is important that for a crop like Belgian endives an efficient determination technique is available. Unforced roots have to be certified to give growers a guarantee for the cultivar they have bought.

Genetic situation of Belgian endive

Belgian endive is a *Cichorium sp* grown as an etiolated vegetable. The genetic similarity of this *Cichorium* species to other *Cichorium* species like green endives, root chicory, red Belgian endive and grower selections was analyzed. Based on the RAPD results a principal component analysis was performed. All *Cichorium* types were very clear separated. The red Belgian endive and the grower selections were separated from the endive hybrids. Clear separation of green endives and root endives were found (Figure 2).

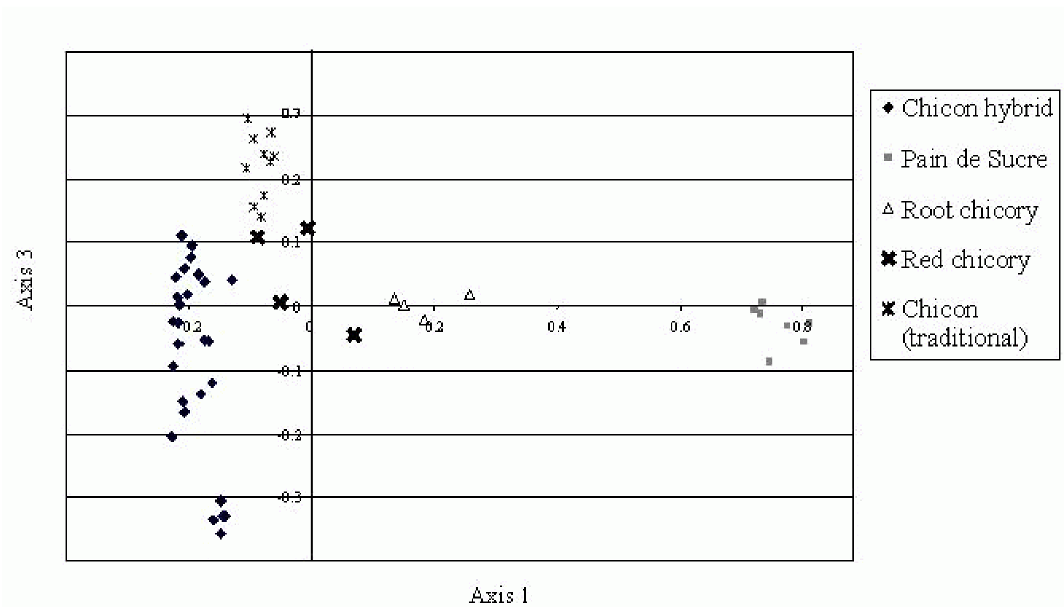

Figure 2. Principal coordinate plot (Axis 1-3). The principle components accounted for 43.02% respectively of the total variation.

Grower selections are of high potential for broadening the genetic basis for hybrid improvement. This will ask for an effort, not only to collect these selections but also to characterize them, eventually by the use of molecular markers. From figure 2 it is clear that the group of grower selections is well separated from the other groups of *Cichorium* species (Van Stallen *et al.*, 2001). To protect this bio diversity cryopreservation of seeds and apical meristems for *Cichorium* has been demonstrated (Vandenbussche *et al*. 2002).

Molecular mapping

Since it became clear that genetic technologies are very much applicable to endives the building of a genetic map was started with some technical support and advice of ENZA Zaden (The Netherlands). For this research two inbreed parent lines of Belgian endives were used. These lines differ considerably in their plant characteristics (Table 2).

The F2 population from these lines was used to find polymorphic bands using both RAPD and AFLP. We used 87 decamer primers and have found 129 polymorphic bands.

With 19 AFLP primer combinations, 296 markers were obtained. Genetic Linkage mapping was done using Joinmap (version 2.0).

Table 2. Chicory parent lines and their specific characteristics

	Wo-A	Wo-B
Field characteristics		
Vegetative force	Well	Medium
Leaf color	Dark green	Chlorotic
Erectness	Reasonable	Strong
Leaf incision	Strong	Some
Smoothness of leaf surface	Smooth/ Shiny	Somewhat rippled/pale
Root characteristics		
Shape	Conical/cylindrical	Conical
Ramification	Little sensitive	Sensitive
Branching	Little	Plenty
Cracking	Little sensitive	Sensitive
Crown	Ordinary	High
Chicory characteristics		
Shape (global)	Well	Reasonable
Firmness	Well	Moderate
Brown coloring of pith	Not sensitive	Very sensitive
Chicory preservation;		
Red coloring	Not sensitive	Very sensitive
Pith outgrowth	Weak	Very strong
Forcing period		
Early	Not suitable	Suitable
Winter	Moderate	Reasonable
Late	Suitable	Not suitable

At LOD threshold 5.0, 9 groups were found, offering a striking parallelism with the 9 chromosomes of the endive plant. In figure 3, an example of such a linkage group is presented indicating the location of some primers on a “chromosome”. Complete information on this topic is given by Van Stallen 2003.

Since the molecular marker groups obtained are splitting in 9 it is tempting to consider those groups as representative for the 9 know endive chromosomes. From these 9 groups we have calculated the size of the genome to be 730 cM by the method of Hulbert *et al.* (1988).

Compared to other crops like radicchio (1405 cM) (De Simone *et al.*, 1997) and lettuce (1950 cM) (Kesseli *et al.*, 1994) the genome of endive seems to be smaller. However, an interspecific AFLP map of lettuce, constructed by Jeuken *et al.* (2001), spanned 854 cM, what is more similar to our results. Our estimated map length for chicory was also in agreement with the estimated map length of *Tetramolopium* (781 cM) (Whitkus, 1998), another member of the Asteraceae family.

Markers with which the hybrid determination is possible are randomly sprayed over the 9 groups giving us comfort that the determination by using these molecular markers is robust.

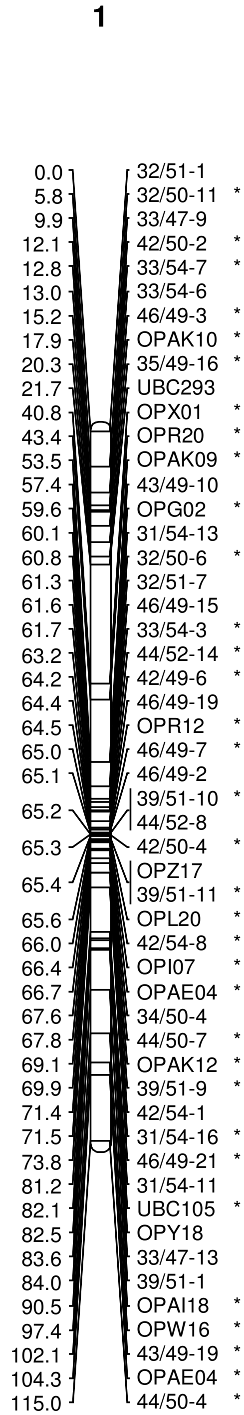

Figure 3. Genetic linkage map of *Cichorium intybus* L. var. *foliosum* Hegi based on RAPD and AFLP markers. Markers were grouped at LOD score 5. The linear order and relative distances (cM) of the molecular markers are presented. Markers specific for Wo-B are labeled with an asterix. RAPD markers are named by their decamer primer. AFLP marker bands are labeled by the primer combination from which they are produced (EcoRI primer/MseI primer), followed by a number indicating the relative position detected in the gel lane.

QTL analysis

Since a molecular map has been obtained for Belgian endives it was logic to continue this research to find out if these molecular markers can be linked with plant characteristics. Since we are interested in endive quality elements we have used some of these parameters in a QTL analysis.

QTLs for gross weight of the chicory were located on many linkage groups (Table 3). Three QTLs were detected using the interval mapping method. These QTLs explained 13.1, 8.9 and 12.7% of the total variance and were located on linkage group 1, 3 and 5. QTLs were also obtained for the characteristics chicory length, pith length, browning of the pith, taste etc.

Table 3. QTLs for the characteristic gross weight of chicory. QTL analysis was done using Interval Mapping. δ mean equals the mean value of the individuals carrying the marker genotype minus the individuals that do not have that marker genotype

	Marker	CM	δ mean (g)	LOD score	% exp
Group 1	OPY18	63.9-68.7	13.62	5.52	
		57.4-90.4		5.57	13.1%
Group 3	OPAJ09	0-18.5	17.49	3.62	
		0-18.5		3.62	8.9%
Group 5	OPAK20	73.9-83.2	16.35	4.05	
		71.8-82.4		5.4	12.7%

Concluding Remarks

Actually Belgian endive is not a world wide produced vegetable. The consumption is however very well introduced in many different countries. To meet the standards of different markets breeding has to continue. Since identification of cultivars is established we should take advantage of this. Chicory with specific characteristics should be certified for specific markets.

Having molecular markers for specific features of chicory, it will be possible to stimulate and support breeder's efforts for developing new cultivars in a more efficient way.

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