Europ.J.Hort.Sci., **73** (3). S. 97–103, 2008, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Novel Breeding Strategies for Ornamental Dahlias II: Molecular Analyses of Genetic Distances between *Dahlia* Cultivars and Wild Species

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Summary

Genetic distances between nineteen *Dahlia* cultivars and three genotypes of wild *Dahlia* species and a hybrid were analysed with 1432 AFLP-Markers generated by 10 Hind/Mse primer combinations. Markers were transformed into a 0/1-matrix and relative distances were computed by means of the Jaccard-coefficient. From these data a dendrogram was constructed by the UPGMA-method. In the dendrogram the cultivars cluster together into one group, while the wild species and the hybrid branched off at a larger genetic distance. The level of genetic similarity of the cultivars averaged between 0.68 and 0.77. Only two varieties displayed a similarity of 0.92 to each other. This relatively large genetic distance between the cultivars indicates a vast potential for further variety development. Within the cultivars different clusters are formed which do not show any relation to phenotypic characteristics like inflorescence morphology or breeding origin. This lack of correlation indicates that a classification of dahlias into horticultural groups does not reflect genetic relationships between cultivars. Therefore, this classification should not be used to select parents for further breeding apart for the few characters it is built on. With knowledge about the genetic background additional information for the selection of genotypes as parent in breeding is available. Genotypes can be choosen for their low level of relatedness or for their genetically characteristics independent from the horticultural type.

Key words. *Asteraceae* – Heliantheae – Coreopsidinae – AFLP – UPGMA – dendrogram

Introduction

Dahlia cultivars are very popular ornamental plants and show a high variation in flower colour and morphology. The present common ornamental types (*Dahlia variabilis)* are assumed to originate from crosses between the wild *Dahlia* species *Dahlia coccinea* (n=16 or 32) and natural *Dahlia* hybrids at the end of the 18th Century after the import of species and hybrids described by Cavanilles from Mexico to Europe (Meyer 1991; von Hansen et al. 1996). The natural *Dahlia*-hybrids were named *Dahlia pinnata* Cav. and *Dahlia rosea* by Cavanilles. von Hansen et al. (1996) postulated that *D. rosea* is a synonym for *D. pinnata*, because of the common hybrid character. The hybrids originated from hybridisation and backcrosses between *D. sorensenii* and different types of *D. coccinea* (von Hansen et al. 1996). The chromosome number and the ploidy level of the species and hybrids primarily involved in the evolution of cultivars remains unclear. All present day cultivars share a chromosome number of n=32 and are polyploids of unknown ploidy level (Gatt et al. 1998). About one hundred years later another wild *Dahlia* species, *D. juarezii*, hybridised to garden forms leading to the cactus dahlias (Meyer 1997). Due to this complex history and several decades of further hybridisations between varieties and species genetic relationships between present day cultivars remain elusive. Most of these cultivars originated from uncontrolled open pollinations between the predominantly outbreeding *Dahlia* varieties. The offspring are tested for the quality of their major horticultural characteristics and superior genotypes are vegetatively propagated as cultivars. Modern cultivars are grouped into 10 different horticultural classes. This classification is based on morphological characters but no information about their genetic background is available. In the present investigation 19 *Dahlia* cultivars from the colour groups white, yellow, red and pink, representing different flower morphologies and three genotypes of wild *Dahlia* species and -hybrids were analysed with AFLP-markers to obtain first information about the genetic diversity within *Dahlia* cultivars and between the cultivars and their potential ancestors. Furthermore, we analysed potential correlations between the horticultural classification of dahlias based on morphological characters and the genetic relatedness of the cultivars. This information should serve to improve breeding strategies for cultivated dahlias.

Material and Methods

For the analysis three genotypes of wild *Dahlia*: – *D. coccinea* (collection number of the Botanical Garden Copen-

hagen: HJ7128, n=32), *D. sorensenii* and *D. spec* (x *pinnata*) – and nineteen *Dahlia* cultivars were selected. The wild species were obtained from the collection of P. Ambrosius, Marbach and from a Mexican collection of the Botanical Garden Copenhagen. These wild *Dahlia* genotypes are assumed to be involved in the development of cultivated dahlias (*D. variabilis*). The other *Dahlia* species probably involved in dahlia evolution, *D. juarezii* was not available. The nineteen cultivated dahlias used here comprised sixteen cultivars from the 20th century ('Doxy', 'Vollmond', 'Holly', '229/82', 'Maria', 'Wiehler Gold', 'Arabian Night', 'Requiem', 'Highness', 'Bach', 'Primaner', 'Piper`s Pink', 'Schneewittchen', 'Butterfly', 'Rotkäppchen', 'Rosenrot') and 3 cultivars from the 19th century ('White Aster', 'Kaiser Wilhelm', 'Stolze von Berlin'). All the cultivars differ in inflorescence morphology (Fig. 1) or -colour (white, pink, yellow, red) and their origin (e.g. the breeder). The cultivars were developed by different breeders from different countries. Most of the breeders use a restricted number of genotypes as basic breeding stocks and so their cultivars should only represent a very restricted gene pool. Our selection of cultivars was made to ensure, that genetic similarity measures were not biased by selection from only a single breeding pool (Fig. 3). Only cultivars 'Rosenrot' and 'Wiehler Gold' originated from the same breeder as other cultivars with theses characteristics were not available.

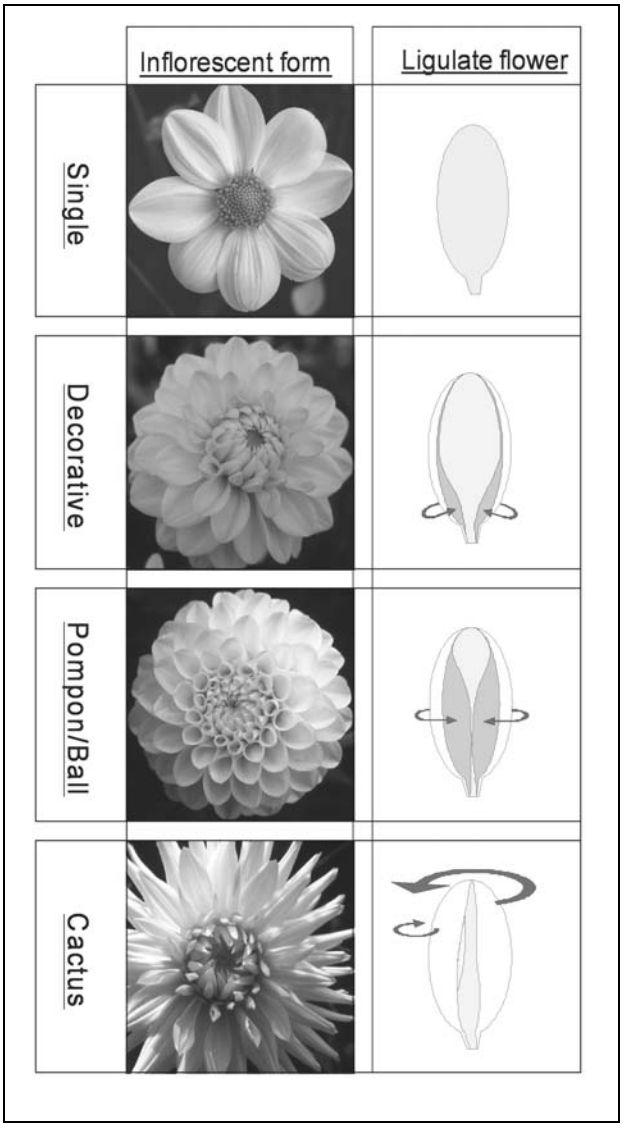


Fig. 1. Different inflorescence morphologies commonly used to classify dahlias into different horticultural groups. The column ligulate flowers describes the morphological modification of the outer, ligulate flowers through inward coiling in different dahlia horticultural classes.

: uncoiled ligulate flower : coiling direction

DNA was isolated from young leaves as described by Behr and Debener (2004). DNA-fragment patterns were generated by the AFLP-method (Vos et al. 1995) modified by von Malek et al. (2000) using 10 selective primer combinations. For the final amplification an IRD700-labelled HindIII-Primer (5'-GACTGCGTACCAGCT-NNN -3') and an MseI-Primer (5'- GATGAGTCCTGAGTAA-NNN-3') with three selective bases were used (Table 1). The combinations were scored twice independently. Marker fragments were separated over 6 % Polyacrylamide-gels and visualized on a LICOR Gene ReadIR 4200 automated sequencer (LI-COR Biosciences GmbH, Bad Homburg, Germany). The AFLP-patterns (Fig. 2) were manually transformed into a 0/1-matrix. Genetic distances and phenograms were calculated by the NTSYspc software (NTSys.PC Version 2.10 s, Applied Biostatistics). The Jaccard-coefficient was used to calculate relative genetic distances and the dendrogram was computed via the UPGMA method implemented in NTSYSpc. For bootstrap analysis 100 Bootstraps (35 % substitution) were generated from the 0/1-matrix and out of these 100 distance matrices were computed in Phyltools (Phylogenetic Computer Tools, Version 1.32 for windows). The consensus tree and bootstrap levels were computed in PHYLIP (*PHYL*ogeny *I*nference *P*ackage, Version 3.6; www.evolution.genetics.washington.edu/phylip.html) by the Neighbour-Joining and UPGMA method respectively.

Table 1. Combination of selective Bases in selective Amplification and developed markers.

Primer combination	Hind III-IRD Primer	MSE I Primer	Number of developed markers
1	AAC	ACC	191
2	AAC	ACG	193
3	AAC	AGG	178
4	AAC	ATG	162
5	AAT	ACA	34
6	ACA	ACA	67
7	ACA	ACC	192
8	ACA	ACG	166
9	ACA	AGC	158
10	ACA	ATG	91

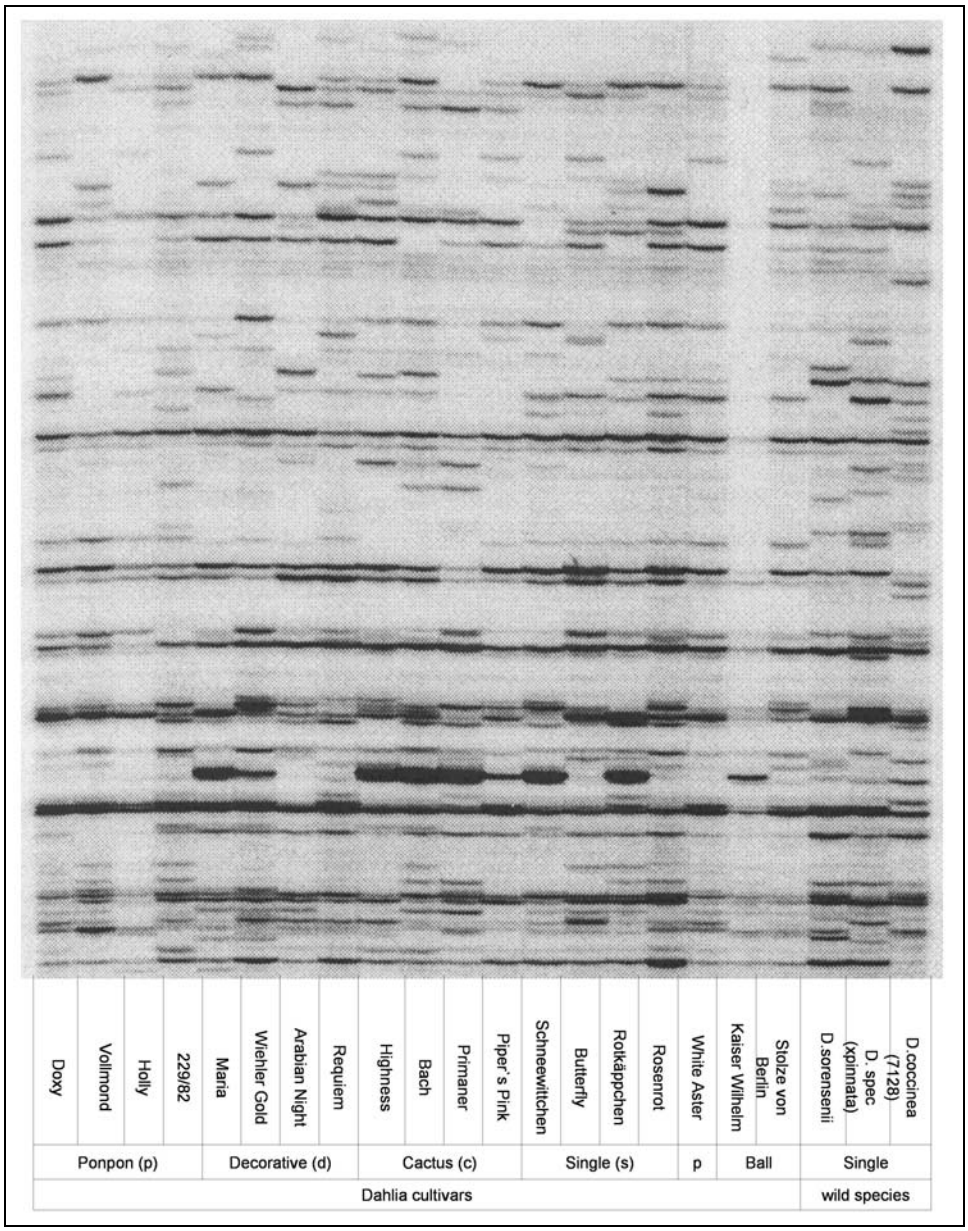
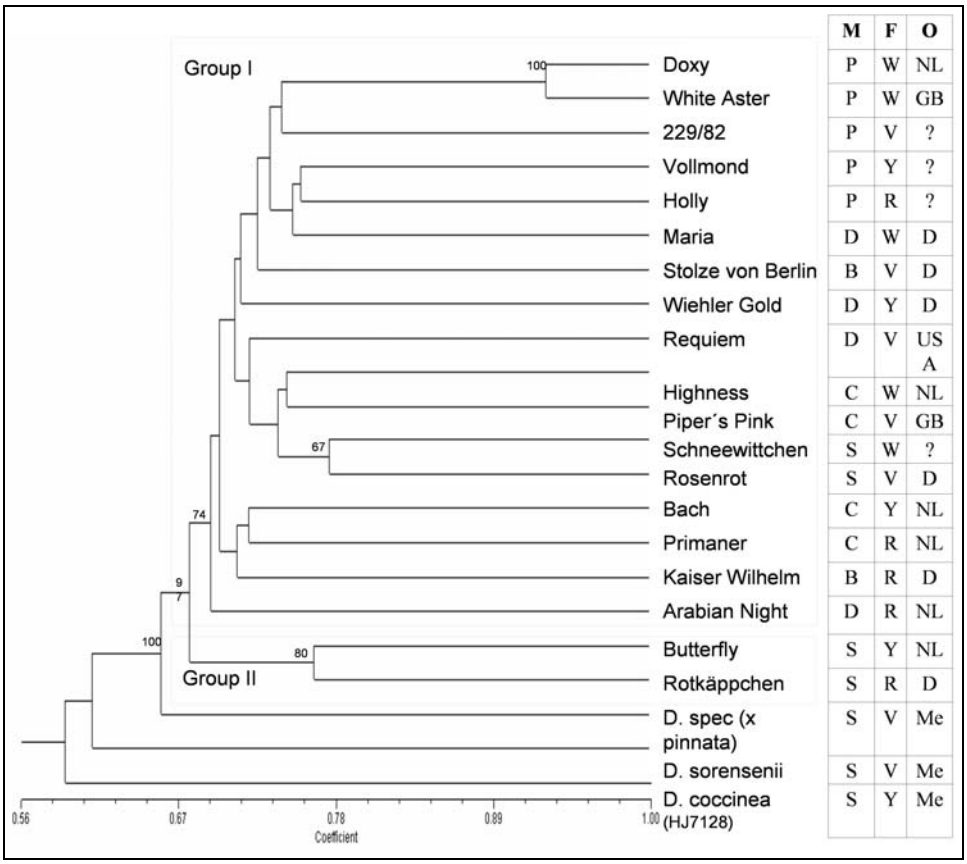


Fig. 2. AFLP-pattern in *Dahlia* (primer combination Hind AAC/Mse AGC).

Results

Based on 10 primer combinations a total of 1432 markers could be detected by the primer combinations. The number of AFLP-fragments generated ranged from 34 up to 193 markers per primer combination and are comparable to results from other plant species (Table 1, Vos et al. 1995; Von Malek 2000; Debener 2000). Of these 1432 markers 1096 (76.5 %) were polymorphic between at least two of the genotypes analysed. In the group of the cultivars 156 (14.2 %) of the markers showed monomorphic and 940 (85.8 %) polymorphic character, in the wild species 444 (40.5 %) monomorphic and 652 (59.5 %) polymorphic markers could be observed. 245 (22.6 %) of the markers were only detected in the cultivars, the wild species showed no bands at the according position and 117 markers (10.68 %) were only detected in the wild species (s. Fig. 4). From these data a matrix of relative similarity between the genotypes was computed (Table 2). The wild species and the cultivars could be clearly differentiated by their AFLP-patterns. The similarity values of all investigated *Dahlia* genotypes vary from 0.59 to 0.92.

The dendrogram computed by the UPGMA-method (Fig. 3) displays long branches indicating large genetic distance between most of the genotypes. The *D. coccinea* genotype, branches off first with a similarity of 0.59 to all other genotypes, followed by the wild species *D. sorensenii* with 0.61 and the wild hybrid *D. spec (*x *pinnata)* at a similarity level of 0.66. The branch point between wild species and cultivars is supported by a bootstrap value of 100 %. The cultivars are grouped into one cluster with similarity values from 0.68 to 0.77 between most cultivars except 'Doxy' and 'White Aster' with a similarity value of 0.92. This cluster can be subdivided into two groups. Group 2 separates at a similarity level of 0.68 with a bootstrap level of 97 % and includes only two gen-



	M	F	O
Doxy	P	W	NL
White Aster	P	W	GB
229/82	P	V	?
Vollmond	P	Y	?
Holly	P	R	?
Maria	D	W	D
Stolze von Berlin	B	V	D
Wiehler Gold	D	Y	D
Requiem	D	V	USA
Highness	C	W	NL
Piper´s Pink	C	V	GB
Schneewittchen	S	W	?
Rosenrot	S	V	D
Bach	C	Y	NL
Primaner	C	R	NL
Kaiser Wilhelm	B	R	D
Arabian Night	D	R	NL
Butterfly	S	Y	NL
Rotkäppchen	S	R	D
D. spec (x pinnata)	S	V	Me
D. sorensenii	S	V	Me
D. coccinea (HJ7128)	S	Y	Me

Fig. 3. Dendrogram of dahlia cultivars and wild species based on molecular markers. M : inflorescence morphology (P = pompon, B = Ball, D = decorative, C = Cactus, S = single) F: flower colour (W = white, V = violet, Y = yellow, R = red), O :origin (D = Germany, NL = Netherlands, GB = Great Britain, Me = Mexico, ? = unknown).

otypes. These two cultivars 'Butterfly' and 'Rotkäppchen' are separated at a similarity level of 80 % with a bootstrap value of 80 %. The other cultivars group out at a similarity level of 0.69 with a bootstrap value of 74 %. From the clusters in the cultivar group only the cluster with 'Schneewittchen' and 'Rosenrot' (67 %) and the one with 'Doxy' and 'White Aster' (100 %) show a high bootstrap value. All other clusters show bootstrap values of less then 35 % with less support. Comparing the position of the varieties within the dendrogram to the phenotypic characteristics like different inflorescence morphology, colour or origin no match could be observed (Fig. 3). In group one the whole range of inflorescence morphologies can be observed and there is no clustering based on inflorescence structure. Cactus cultivars like 'Highness' group together with a decorative cultivar like 'Requiem' or 'Piper`s Pink' together with a single flowering cultivar like 'Rosenrot'. In group two with only two genotypes there are only single flowering cultivars, an inflorescence type, which is also observed in a cluster together with other forms in group one (Fig. 3). The same lack of correlation is obvious if the colour of the ray flowers is used as a discriminating character (Fig. 3).

Discussion

Knowledge about the genetic diversity of a crop is of critical importance for future breeding strategies. The advent of molecular markers more then two decades ago provides the means to infer genetic distances among cultivars even of minor crops as for example ornamental species (Debener et al. 1996, 2002; Ben Meir and Vainstein 1994; Linde et al. 2007). Most marker systems as AFLPs and RAPDs are multilocus systems with individual marker fragments distributed over the whole genome and are much better indicators of genetic relationships then individual morphological characters (Debener et al. 2003). Marker analyses in ornamental species revealed different degrees of similarities among cultivars. Whereas some crops as e.g. garden roses or *Dieffenbachia* display large genetic variability among cultivars other crops as e.g. *Aglaonema* and *Osteospermum* show little genetic variation among most cultivars (Debener et al. 1996; Chen et al. 2004a, b; Gawenda 2006). In some cases like *Aglaonema* and garden roses the observed molecular variation within a limited breeding gene pool is also higher as expected and like in dahlias no correlation between morphological characters and genetic variation could be observed (Debener et al. 1996; Chen et al. 2004a, b) the species show also no correlation between the genetic variability and colour or flower structure This may be due to differences in reproductive biology and genome stability between some species. However, this demonstrates the utility of thorough analyses of cultivars with molecular markers.

All the results in this investigation are based on one genotype per combination and only on three wild dahlia genotypes available. The species were used as out-group to get an impression about the genetic background and the variation in *Dahlia* cultivars. Because of the limited number of investigated genotypes the data may not

Table 2. Similarity of genotypes shown by a Matrix of relative similarity computed by Jaccard-coefficient.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
A: 'Doxy'	1.00																					
B: 'Vollmond'	0.74	1.00																				
C: 'Holly'	0.75	0.76	1.00																			
D: '229/82'	0.74	0.70	0.74	1.00																		
E: 'Maria'	0.73	0.75	0.75	0.73	1.00																	
F: 'Wiehler Gold'	0.69	0.71	0.72	0.71	0.74	1.00																
G: 'Arabien Night'	0.69	0.67	0.68	0.71	0.71	0.67	1.00															
H: 'Requiem'	0.70	0.69	0.68	0.71	0.70	0.70	0.72	1.00														
I: 'Highness'	0.70	0.69	0.73	0.74	0.70	0.72	0.70	0.72	1.00													
J: 'Bach'	0.70	0.68	0.68	0.73	0.69	0.72	0.69	0.69	0.73	1.00												
K: 'Primaner'	0.68	0.66	0.66	0.71	0.67	0.69	0.69	0.68	0.71	0.72	1.00											
L: 'Piper's Pink'	0.71	0.68	0.70	0.74	0.70	0.72	0.71	0.74	0.75	0.74	0.71	1.00										
M 'Schneewittchen'	0.68	0.68	0.68	0.73	0.70	0.68	0.67	0.68	0.72	0.70	0.71	0.72	1.00									
N: 'Butterfly'	0.68	0.67	0.67	0.66	0.66	0.65	0.67	0.68	0.67	0.66	0.64	0.67	0.67	1.00								
O: 'Rotkäppchen'	0.68	0.67	0.68	0.68	0.68	0.66	0.67	0.68	0.70	0.67	0.66	0.69	0.70	0.76	1.00							
P: 'Rosenrot'	0.73	0.72	0.73	0.75	0.73	0.72	0.72	0.74	0.75	0.71	0.71	0.76	0.78	0.73	0.77	1.00						
Q: 'White Aster'	0.93	0.73	0.75	0.74	0.73	0.71	0.69	0.70	0.72	0.71	0.68	0.72	0.69	0.70	0.70	0.74	1.00					
R: 'Kaiser Wilhelm'	0.70	0.70	0.70	0.69	0.70	0.70	0.69	0.70	0.71	0.71	0.71	0.70	0.69	0.68	0.67	0.74	0.73	1.00				
S: 'Stolze von Berlin'	0.72	0.75	0.74	0.71	0.71	0.71	0.69	0.70	0.71	0.68	0.66	0.72	0.67	0.66	0.66	0.75	0.73	0.69	1.00			
T: *D. sorensenii*	0.60	0.62	0.61	0.61	0.61	0.59	0.62	0.60	0.60	0.60	0.59	0.60	0.60	0.61	0.62	0.65	0.62	0.64	0.60	1.00		
U: *D. spec* (x *pinnata*)	0.68	0.64	0.67	0.68	0.65	0.65	0.63	0.65	0.65	0.66	0.64	0.65	0.63	0.66	0.65	0.68	0.68	0.67	0.66	0.59	1.00	
V: *D. coccinea* (7128)	0.59	0.59	0.59	0.58	0.60	0.59	0.61	0.60	0.58	0.58	0.58	0.57	0.58	0.59	0.60	0.63	0.59	0.60	0.59	0.60	0.59	1.00

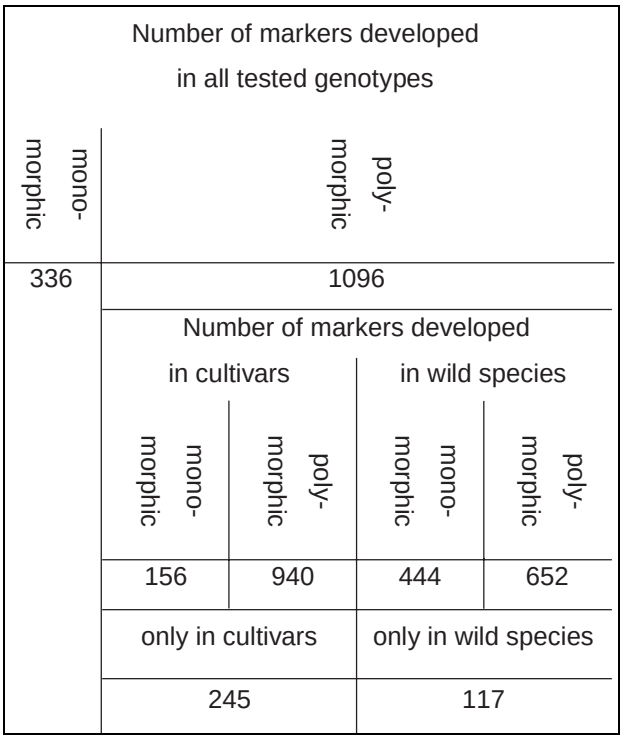


Fig. 4. Number of developed mono-and polymorphic AFLP-markers.

represent the variability of the whole dahlia cultivar spectrum. However, it gives a first estimate about genetic similarities among dahlia cultivars selected from different gene pools.

In this study we could show that levels of similarity with values down to 0.68 are relatively low in dahlias compared to cultivars of other ornamental species from the *Asteraceae* (Chen et al. 2004b; Gawenda 2006). This can be explained by multiple hybridisations between species and species hybrids at the beginning of dahlia breeding about 200 years ago and the common practise of most breeders to select open pollinated seedlings from larger populations of diverse genotypes. As the polyploidy, genetically highly diverse dahlias are predominantly outbreeding (Behr and Debener 2004) this will maintain high levels of genetic diversity within breeding gene pools. Another reason may be that the dahlia cultivars are often exchanged between the breeders so that the gene pools are largely overlapping. This high level of diversity is also shown by the high level of polymorphic markers (85.8 %). One exception of this is the close relationship between cultivars 'Doxy' and 'White Aster' with a similarity of 0.92. Having clearly distinct AFLP marker patterns this close relationship may be explained by an origin from the same progeny or even further by the derivation of one from the other due to sport mutations and some clonal instability as observed in some other crops as e.g. in Syngonium (Chen et al. 2006). In this crop sports can be distinguished by AFLP patterns and Jaccard indices of 0.98 and 0.99 resulting from somaclonal variation. As expected, the wild species genotypes are separated from the cultivars with larger genetic distances. The *Dahlia coccinea* genotype (HJ7128 n=32), branches of first. This genotype has the largest genetic distance to the other genotypes followed by *D. sorensenii* which clusters closer to the cultivars. It has been proposed that the cultivars originated by hybridisation between *Dahlia coccinea* and a wild hybrid of *Dahlia sorensenii* and *D. coccinea* (von Hansen 1996). Under this assumption the *D. sorensenii* genotype occupies the expected position in the dendrogram. It is located at a larger distance to the cultivar as compared to the wild hybrid *D. spec (*x *pinnata)* genotype. The natural hybrid shows a higher similarity to the cultivated dahlias. Therefore, the topology of the dendrogram is in agreement with hypothesis on the development of cultivated dahlias as described by von Hansen et. al. (1996). However, the distances of the wild species and the species hybrid genotypes to the cultivars imply a continuum which is also supported by some of the similarity values of the species to the cultivars. The value of the *Dahlia coccinea* genotype (HJ7128) reaches 0.63, the one of *D. sorensenii* genotype 0.65 and the value of the wild hybrid *D. spec (*x *pinnata)* genotype 0.68 (Table 2).

The low level of similarity (Table 2) in the cultivars may due to the low bootstrap values in the group of the cultivars. The cultivars with a similarity level above 0.76 (Fig. 3) show bootstrap levels higher than 67.

In contrast to this, the present study displays a lack of correlation between the clusters of the dendrogram constructed by the UPGMA-method and the phenotypic characteristics currently used for the horticultural classification of *Dahlia* cultivars. The major horticultural characteristics like e.g. the structure of the inflorescence or flower colour (American Dahlia Society 2006; Deutsche Dahlien-, Fuchsien-, und Gladiolen- Gesellschaft e.V. 2006) are probably due to monogenic or oligogenic factors which would be consistent to knowledge about these traits in other, well characterised species (Coen et al. 1991; Kim et al. 2005). Although no information about the genetics or the location of those factors in the dahlia genome is available it can be assumed that the genetic complexity is similar to those species investigated up to date. The AFLP data, leading to the tree in this investigation represent the whole genetic background and the factors leading to the horticultural characteristics are only a very small section of the genome. In computing the trees, the weight of the factors for horticultural characteristics is low compared to the rest of the genetic background of dahlias.

The following conclusions for dahlia breeding can be drawn from our observations:

Though morphological characters need to be considered for the choice of parents in variety breeding genetic backgrounds should not be selected an overall morphological similarity.

As the genetic similarity among cultivars is relatively low compared to their distance to wild species, particular traits absent from the gene pool of cultivars as e.g. disease resistances may be introgressed from unadapted germplasm without drastic effects on trait complexes.

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Received July 10, 2007 / Accepted March 4, 2008

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