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Genetic diversity of *Brassica oleracea* var. *capitata* gene bank accessions assessed by AFLP

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Abstract The genetic diversity of 20 cabbage (*Brassica oleracea* var. *capitata*, including sub.var. *alba* and *rubra*) cultivars and landraces from the Gene bank of Crop Research Institute was estimated using amplified fragment length polymorphism (AFLP) marker technology. Two cultivars of *Brassica pekinensis* (syn. *Brassica rapa* var. *pekinensis*) were used as outliers in the study. Thirty AFLP primer combinations produced a total of 1084 fragments. A total of 806 fragments, 364 (45%) of them polymorphic, were found across 20 *Brassica oleracea* var. *capitata* accessions. The accessions were clustered into two main groups. Special subgroups, reflecting place of origin, were observed within these groups. Ten selective primer pairs were found to be most informative because each of these uniquely identified all of the accessions used. Furthermore, two accessions of *Brassica pekinensis* were clearly differentiated from the *Brassica oleracea* var. *capitata* accessions. AFLP is an efficient tool for determination of genetic diversity of cabbage gene bank accessions.

Keywords: amplified fragment length polymorphism, *Brassica oleracea* var. *capitata*, cabbage, discriminatory power, genetic diversity

INTRODUCTION

Brassica oleracea L. var. *capitata* (cabbage) is one of the most important crop plants of the species *Brassica oleracea* L., which has a long growing tradition in the Czechia. It is an herbaceous, biennial, dicotyledonous flowering plant with leaves forming a characteristic compact head. The cabbage is differentiated into white head cabbage (*Brassica oleracea* var. *capitata* sub.var. *alba*) and red head cabbage (*Brassica oleracea* var. *capitata* sub.var. *rubra*). The influence of cabbage consumption on human health is evident and is, in addition to being a source of vitamins and fibre, connected with secondary metabolites called glucosinolates, which are known to possess anticarcinogenic properties (summarised by Sarikamiş et al. 2009). Some variation in active substances between cultivars has been described. Singh et al. (2006) found variability in antioxidant phytochemicals (ascorbic acid, lutein, β-carotene, DL-α- tocopherol and phenolics) in 18 cabbage cultivars. Variation in terms of glucosinolate profiles and levels were observed in different cabbage genotypes (Sarikamiş et al. 2009; Kabouw et al. 2010). For white cabbage, samples derived from different regions of Europe had some differences in glucosinolate and protein profiles (Kusznierewicz et al. 2008). The plants with high glucosinolate contents will be further utilised as a potential genetic source for breeding.

The same importance as identification of differences in active substances has determination of genetic variability between cultivars. The identification of genetic markers that strictly differentiate single cultivars is helpful for effective conservation of plant material in gene banks and for breeders. Only a few studies (Kresovich et al. 1992; Phippen et al. 1997; Van Hintum et al. 2007) have investigated genetic variation within cabbage cultivars. Furthermore, characterisation of diversity in genetic resources and genotype fingerprinting are important for managing gene banks. Different methods were used for this characterization, including the AFLP (Amplified Fragment Length Polymorphism) technique developed by Vos et al. (1995). This method has been successfully used for the assessment of genetic diversity in many plant species (*e.g.*, *Zea mays* L.- Hartings et al. 2008; *Solanum*

lycopersicum- Van Berloo et al. 2008; *Gossypium* spp.- Murtaza, 2006; *Olea europaea*- Sanz-Cortés et al. 2003; *Lolium perenne* L.- Guthridge et al. 2001). In the case of the genus *Brassica*, Negi et al. (2004) demonstrated the great value of AFLP markers in the management of gene banks by using them to estimate the genetic diversity of 18 *Brassica nigra* cultivars. AFLP-based genetic diversity has also been described in *Brassica juncea* (Srivastava et al. 2001), *Brassica carinata* (Jiang et al. 2007), *Brassica rapa* (Zhao et al. 2005; Liu and Meng, 2006; Takuno et al. 2007; Warwick et al. 2008), *Brassica campestris* (syn. *Brassica rapa*- Das et al. 1999; Huh and Huh, 2001) and *Brassica napus* (Čurn et al. 2002; Liu and Meng, 2006). The detection of polymorphisms by AFLP is more efficient than with other marker systems (Das et al. 1999- *Brassica campestris* L. cultivars, Lombard et al. 2000- *Brassica napus* L. cultivars).

The aim of our study was to find AFLP markers for fingerprinting and estimating genetic diversity within Czech cabbage accessions deposited in the gene bank.

MATERIALS AND METHODS

A group of 20 cabbage accessions (cultivars and landraces) (*Brassica oleracea* var. *capitata*) (Table 1) were acquired from Crop Research Institute Prague, Gene bank Olomouc. Young leaves collected from 20 accessions of cabbage seedlings and from 2 cultivars of *Brassica pekinensis,* 'Hilton' and 'Nozaki' were homogenised in liquid nitrogen and genomic DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Germany).

The AFLP assay was carried out according to the Perkin-Elmer protocol (Part number 402083, Rev.A, November 1995). In brief, DNA was restriction digested with EcoRI (Fermentas) and MseI (New England Biolabs) and AFLP ligation was carried out using an AFLP Ligation and Preselective Amplification Kit for Regular Plant Genomes (Applied Biosystems). T4 DNA ligase (Fermentas) was used for ligation. Next, 5 µl of the restriction ligation product was visualised on 1.5% agarose and 9 µl of the rest of the ligation mixture was diluted with 91 µl $TE_{0.1}$. The preselective amplification reaction was prepared using 2 µl of the diluted restriction-ligation DNA prepared earlier, 0.5 µl of the preselective primer pairs and 7.5 µl of AFLP Core mix. A UNO II (Biometra) thermo cycler was used for the PCR. 5 µl of preselective amplification product was visualised on a 1.5% agarose gel and the remaining 5 µl were diluted by 45 µl $TE_{0.1}$. The selective amplification reaction was prepared as a multiplex PCR in a 10 µl-reaction mixture [0.2 mM dNTP, 1 µM MseI primer, 3x 0.1 µM fluorescently-marked EcoRI primers, 0.5 U Taq polymerase (Qiagen, Germany), 1x buffer with 25 mM $MgCl_2$ and 1 µl diluted preselective amplification product] in the ABI PRISM 7700 thermocycler (Applied Biosystems, USA). Samples were prepared for electrophoresis using 4.8 µl of formamide, 0.5 µl of GS-500 ROX-labelled size standard and 1 µl of selective amplification product. The amplification products were separated by capillary electrophoresis in a Perkin Elmer Genetic Analyser ABI PRISM 310 and analysed using GeneScan™ and Genotyper™ software (Applied Biosystems, USA).

AFLP markers were generated with the Selective Amplification Start Up Kit for Regular Plant Genomes (Applied Biosystems, USA). Initially, 64 primer combinations involving 8 MseI and 8 EcoR1 primers grouped in 24 multiplexes (Table 2) were screened for polymorphisms using two different cabbage cultivars, 'Inter' (white form) and 'Mars' (red form). After the pre-screening, 30 primer combinations (arranged as multiplex groups 1 to 12) involving 8 EcoR1 and 6 MseI primers with the highest number of detectable polymorphic peaks within a size range of 100 to 500 bp were used for analyzing all accessions.

Using the Genescan™ and Genotyper™ software (Applied Biosystems), the peaks representing AFLP fragments were exported into Excel as a 1-0 matrix for additional data analysis.

DARwin software was employed to determine the level of dissimilarity between accessions, and a dendrogram was constructed based on a bootstrapped neighbour-joining algorithm using the Jaccard's similarity coefficient.

Table 1. List of *Brassica oleracea* var. *capitata* cultivars and landraces used in this study.

Evigez accessions number	Evigez accessions name	Form	Year of registration	Status	Breeeder
09H1800003	Zora	alba	1976	breeding variety	Genebank Department - RICP Prague Vegetable Section Olomouc Holice
09H1800004	Inter	alba	1966	breeding variety	SEMPRA, Plant Breeding Station Turnov
09H1800005	Kodanske trzni ranne	alba	1950	breeding variety	-
09H1800007	Mars	rubra	1982	breeding variety	Res. Instit of Vegetables Plant Breeding Station Dobra voda u Horic
09H1800009	Dobrovodske pozdni (Pourovo)	alba	1939	breeding variety	Res. Instit of Vegetables Plant Breeding Station Dobra voda u Horic
09H1800010	Pourovo cervene	rubra	1960	breeding variety	Res. Instit of Vegetables Plant Breeding Station Dobra voda u Horic
09H1800011	Holt	alba	1985	breeding variety	Res. Instit of Vegetables Plant Breeding Station Dobra voda u Horic
09H1800013	Polar	alba	1964	breeding variety	Res. Instit of Vegetables Plant Breeding Station Dobra voda u Horic
09H1800014	Krimicke	alba	1952	landrace	-
09H1800015	Vysocke	rubra	-	landrace	-
09H1800263	Pluto	alba	1990	breeding variety	Res. Instit of Vegetables Plant Breeding Station Dobra voda u Horic
09H1800268	Kalibos	rubra	1998	breeding variety	-
09H1800269	Rufus	rubra	1996	breeding variety	Moravoseed Mikulov
09H1800274	Klokotske	alba	1952	landrace	-
09H1800276	Taborske	rubra	1960	landrace	SEMPRA, Plant Breeding Station Slapy u Tabora
09H1800277	Turnovske	rubra	-	landrace	-
09H1800283	Bohmerwaldkohl- Sumavske	alba	-	landrace	-
09H1800287	Vysocke (Frydstejn)	rubra	-	landrace	-
09H1800319	Vysocke krajove (Jenisovice)	rubra	-	landrace	-
09H1800332	Dita	alba	1996	breeding variety	Moravoseed Mikulov

Evigez- Czech Plant Genetic Resources Documentation System (http://genbank.vurv.cz/genetic/resources/).

The total and mean numbers of distinct AFLP profiles generated by each selective primer combination were obtained. The discriminatory power ($D_j = 1\text{-}C_j$) and confusion probability (C_j) of the jth assay (Tessier et al. 1999) were calculated according to $C_j = \sum^{I}_{i=1} p_i (N_{pi} - 1)/N\text{-}1$, where p_i represented the frequency of the ith pattern, N the sample size and I the total number of patterns generated by the jth assay. The confusion probability reflects the probability that two randomly chosen accessions share an identical pattern. The effective number of patterns per assay was given by $P = 1/1\text{-}D_L$ (Belaj et al. 2003), where $D_L = \lim(D_j) = 1\text{-}\sum^{I}_{i=1} p_i^2$, as sample size tends toward infinity.

Table 2. Selective primer combinations used for prescreening in *Brassica oleracea* var. *capitata* (accessions 'Inter' and 'Mars').

MSel	**EcoR1fluorescent dye-labeled**		
	FAM	NED	JOE
CAA	ACT ACA	AAC ACC AGC	AAG AGG ACG
CAC	ACT ACA	AAC ACC AGC	AAG AGG ACG
CAG	ACT ACA	AAC ACC AGC	AAG AGG ACG
CAT	ACT ACA	AAC ACC AGC	AAG AGG ACG
CTA	ACT ACA	AAC ACC AGC	AAG AGG ACG
CTC	ACT ACA	AAC ACC AGC	AAG AGG ACG
CTG	ACT ACA	AAC ACC AGC	AAG AGG ACG
CTT	ACT ACA	AAC ACC AGC	AAG AGG ACG

RESULTS

The 30 primer-AFLP combinations used in this study produced 1084 fragments in total, of which 286 fragments were species-specific for *B. oleracea* var. *capitata* (detected in all samples of cabbage) and 231 fragments were present only in *B. pekinensis*. Most of the species-specific markers (17 fragments) in *B. oleracea* var. *capitata* were produced from primer combinations CAC/AAC (from multiplex 3), CTT/AGC (from multiplex 8) and CAC/AGC (from multiplex 9). In *B. pekinensis*, most of the species-specific markers were produced from primer combinations CAG/AGC (from multiplex 10) (16 fragments), CAG/AAG (from multiplex 4) (15 fragments) and CTT/ACA (from multiplex 2) (14 fragments). For discrimination between *B. oleracea* var. *capitata* and *B. pekinensis*, the most informative primer combinations were multiplexes 12, 9, 2 and 3 (Table 3). Accessions of *B. oleracea* var. *capitata* and *B. pekinensis* were strictly differentiated.

Across 20 *B. oleracea* var. *capitata* (cabbage) accessions, we detected 806 different fragments, 365 of which were polymorphic (45%). The average number of polymorphic fragments per combination was 12. The most informative primer combinations were CAT/AGG (from multiplex 1), with 70% polymorphic fragments and CAG/AAC (from multiplex 4) and CTC/AAG (from multiplex 5), with 63.3% polymorphic fragments for both of them (Table 4).

Table 3. Summary of detected specific AFLP fragments in *Brassica oleracea* var. *capitata (B. o.)* and *Brassica pekinensis (B. p.)* gene bank accessions.

multiplex	primer combinations		No. of specific frag.		No. of total detected frag.	% of specific frag.		average % of specific frag. per multiplex		average % of specific frag. per multiplex
	MseI	EcoR1	*B. o.*	*B. p.*	*B. o.+ B. p.*	*B. o.*	*B. p.*	*B. o.*	*B. p.*	*B. o.+B. p.*
	CAT	ACA$_{FAM}$	7	4	26	26,9	15,4			
1	CAT	AGG$_{JOE}$	2	4	24	8,3	16,7	24,3	14,9	39,2
	CAT	ACC$_{NED}$	9	3	24	37,5	12,5			
	CTT	ACA$_{FAM}$	8	14	40	20,0	35,0			
2	CTT	AGG$_{JOE}$	6	6	26	23,1	23,1	27,4	26,6	54,0
	CTT	ACC$_{NED}$	9	5	23	39,1	21,7			
	CAC	ACT$_{FAM}$	6	13	39	15,4	33,3			
3	CAC	AAG$_{JOE}$	11	10	40	27,5	25,0	27,5	25,6	53,1
	CAC	AAC$_{NED}$	17	8	43	39,5	18,6			
	CAG	ACT$_{FAM}$	7	7	25	28,0	28,0			
4	CAG	AAG$_{JOE}$	14	15	55	25,5	27,3	24,3	25,7	50,0
	CAG	AAC$_{NED}$	8	9	41	19,5	22,0			
	CTC	ACT$_{FAM}$	10	4	27	37,0	14,8			
5	CTC	AAG$_{JOE}$	7	6	36	19,4	16,7	27,9	18,1	46,0
	CTC	AAC$_{NED}$	13	11	48	27,1	22,9			
	CTG	ACT$_{FAM}$	8	5	28	28,6	17,9			
6	CTG	AAG$_{JOE}$	13	6	33	39,4	18,2	31,5	18,9	50,4
	CTG	AAC$_{NED}$	9	7	34	26,5	20,6			
7	CAT	ACG$_{JOE}$	2	6	19	10,5	31,6			
	CAT	AGC$_{NED}$	15	7	67	22,4	10,4	16,5	21,0	37,5
8	CTT	ACG$_{JOE}$	5	12	30	16,7	40,0			
	CTT	AGC$_{NED}$	17	9	61	27,9	14,8	22,3	27,4	49,7
9	CAC	ACG$_{JOE}$	13	4	31	41,9	12,9			
	CAC	AGC$_{NED}$	17	9	49	34,7	18,4	38,3	15,6	53,9
10	CAG	ACG$_{JOE}$	10	5	31	32,3	16,1			
	CAG	AGC$_{NED}$	11	16	53	20,8	30,2	26,5	23,2	49,7
11	CTC	ACG$_{JOE}$	5	6	37	13,5	16,2			
	CTC	AGC$_{NED}$	12	7	47	25,5	14,9	19,5	15,6	35,1
12	CTG	ACG$_{JOE}$	5	5	17	29,4	29,4			
	CTG	AGC$_{NED}$	10	8	30	33,3	26,7	31,4	28,0	59,4

frag. = AFLP fragments; FAM, JOE, NED = blue, green, yellow fluorescent dyes.

Accessions were clustered into two main groups, with this division correlating the white and red forms of cabbage. Only one of the white cabbages, ‘Bohmerwaldkohl’ was clustered with the red cabbage accessions. Special subgroups were observed within the groups, reflecting the region of plant breeding and pedigree relationships. In cluster A, cultivar ‘Holt’ was bred from cultivar ‘Polar’ (bootstrap value 100); in cluster B1, cultivar ‘Mars’ was bred from cultivar ‘Pourovo cervene’ (bootstrap value 91); and in cluster B2, cultivar ‘Kalibos’ was bred from landrace ‘Vysocke’ (bootstrap value 60) (Figure 1).

Table 4. Summary of obtained AFLP fragments in *Brassica oleracea* var. *capitata* and *Brassica pekinensis* (22 accessions). Results within *Brassica oleracea* var. *capitata* (20 accessions) are noted separately.

multiplex	primer combinations		*B. oleracea* var. *capitata* + *B. pekinensis*			*B. oleracea* var. *capitata*		
	MseI	EcoR1	No. of frag.	No. of polymorphic frag.	Percentage of polymorphic frag.	No. of frag.	No. of polymorphic frag.	Percentage of polymorphic frag.
	CAT	ACA_{FAM}	26	22	84,6	22	10	45,5
1	CAT	AGG_{JOE}	24	20	83,3	20	14	70,0
	CAT	ACC_{NED}	24	22	91,7	20	9	45,0
	CTT	ACA_{FAM}	40	35	87,5	25	11	44,0
2	CTT	AGG_{JOE}	26	20	76,9	18	6	33,3
	CTT	ACC_{NED}	23	21	91,3	16	5	31,3
	CAC	ACT_{FAM}	39	37	94,9	22	13	59,1
3	CAC	AAG_{JOE}	40	37	92,5	30	12	40,0
	CAC	AAC_{NED}	43	39	90,7	30	9	30,0
	CAG	ACT_{FAM}	25	23	92,0	17	7	41,2
4	CAG	AAG_{JOE}	55	51	92,7	38	19	50,0
	CAG	AAC_{NED}	41	39	95,1	30	19	63,3
	CTC	ACT_{FAM}	27	25	92,6	21	9	42,9
5	CTC	AAG_{JOE}	36	32	88,9	30	19	63,3
	CTC	AAC_{NED}	48	41	85,4	33	15	45,5
	CTG	ACT_{FAM}	28	25	89,3	23	12	52,2
6	CTG	AAG_{JOE}	33	31	93,9	26	11	42,3
	CTG	AAC_{NED}	34	25	73,5	25	6	24,0
7	CAT	ACG_{JOE}	19	15	78,9	12	6	50,0
	CAT	AGC_{NED}	67	51	76,1	57	25	43,9
8	CTT	ACG_{JOE}	30	25	83,3	17	7	41,2
	CTT	AGC_{NED}	61	56	91,8	52	29	55,8
9	CAC	ACG_{JOE}	31	27	87,1	27	10	37,0
	CAC	AGC_{NED}	49	41	83,7	38	12	31,6
10	CAG	ACG_{JOE}	31	24	77,4	26	8	30,8
	CAG	AGC_{NED}	53	49	92,5	33	18	54,5
11	CTC	ACG_{JOE}	37	31	83,8	28	16	57,1
	CTC	AGC_{NED}	47	38	80,9	37	16	43,2
12	CTG	ACG_{JOE}	17	15	88,2	12	5	41,7
	CTG	AGC_{NED}	30	26	86,7	21	7	33,3
Total no. of peaks			1084	943	87,0	806	365	45,3
Mean no. of peaks per combination			36,1	31,4		26,9	12,2	

frag. = AFLP fragments; FAM, JOE, NED = blue, green, yellow fluorescent dyes.

Finally, we found that all 30 used primer pairs showed high discrimination power for the differentiation of cabbage accessions. Ten of the primer pairs were most informative within cabbage because they showed a discriminatory power value of 1 (Table 5). Each of these 10 primer combinations was able to differentiate 20 cabbage accessions, some of them closely related. The complex of these 10 primer combinations is very effective for assessing genetic diversity within cabbage and was found to be very efficient for gene bank accession fingerprinting. For the multiplex-arranged primer combinations for AFLP, we suggest the use of multiplex 3, 4, 5 or 11.

In addition, we detected three markers that would be candidate markers for white cabbage accessions. These markers were not present in tested red cabbage accessions, whereas they were present in most

of the white cabbage accessions. Marker CTG/AAG_{192} was detected in 8 white cabbages accessions, and markers CAG/AAC_{487} and CAT/AGC_{160} were detected in 7 white cabbage accessions.

DISCUSSION

Characterisation of diversity in the genetic resources of gene banks is important for managing these gene banks. Among the generally used molecular techniques, AFLP is a powerful technique for cultivar identification and fingerprinting (Powell et al. 1996; Lombard et al. 2000; Garcia et al. 2004). In *Brassica* species, the most-studied cultivated plants were *Brassica rapa* (Zhao et al. 2005; Liu and Meng, 2006; Takuno et al. 2007; Warwick et al. 2008) or *Brassica napus* (Lombard et al. 2000; Čurn et al. 2002; Liu and Meng, 2006) and some varieties of *Brassica oleracea* (Gu et al. 2008- *B. o.* var. *botrytis*), compared with the rare AFLP studies of genetic diversity performed on *Brassica oleracea* var. *capitata* (Van Hintum et al. 2007). Compared to other studies, we used extensive sets of selective primers. We pre-screened 64 primer combinations and selected 30 primer combinations with the highest number of detectable polymorphic peaks; 45% of the detected fragments within *B. oleracea* var. *capitata* were polymorphic. The rate of polymorphism in AFLP fragments described for *Brassica* is from 23% (*B. carinata*- Warwick et al. 2006) to 86.6% (*B. rapa*- Takuno et al. 2007). The value of polymorphism is dependent on the diversity of the chosen plant material to a certain degree because the high level of polymorphism described by Takuno et al. (2007) was obtained from plants of morphologically distinct *Brassica rapa* subspecies. Despite this fact, the value of polymorphic fragments in our collection of cabbage is high.

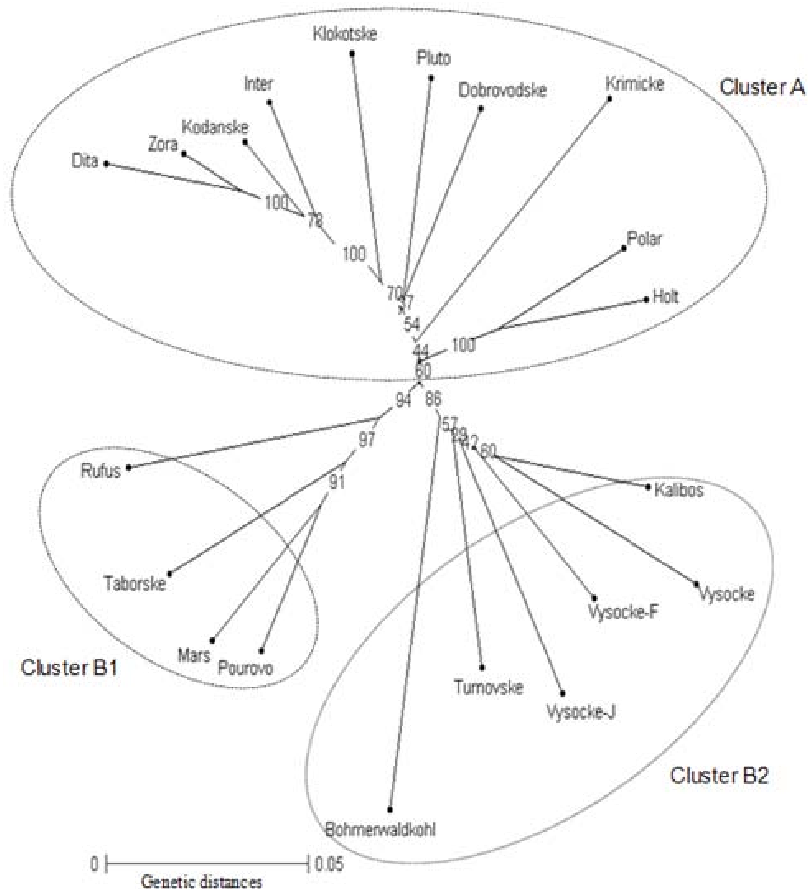

Fig. 1 Dendrogram showing relationships among 20 Czech cabbage gene bank accessions, based on a bootstrapped neighbour-joining algorithm using the Jaccard's similarity coefficient. The values on the branches represent the results of 1000 bootstrap replications.

Furthermore, 10 selective primer pairs having a discrimination power value of 1 could be effectively used for fingerprinting cabbage accessions. Some of the primers that we selected for cabbage fingerprinting have also been used in other AFLP *Brassica* studies. The most commonly used selective primer combination, CAC/AAG (Lombard et al. 2000, Srivastava et al. 2001; Lionneton et al. 2002; Liu and Meng, 2006; Takuno et al. 2007), showed a discrimination power of 0.9684. The second most commonly used primers, CTC/ACT (Lombard et al. 2000; Srivastava et al. 2001; Negi et al. 2004; Takuno et al. 2007), showed a discrimination power of 0.9842. Primers CTC/AAG (Lombard et al.

Table 5. The effective number of patterns and discriminatory power obtained for assay unit for *Brassica oleracea* var. capitata AFLP.

multiplex	primer combinations		Ns = 20						
	MseI	EcoR1	I	I/Ns	P	%P	Pu	Pu/Ns	Dj
1	CAT	ACA_{FAM}	18	0.9	15,4	85.5			0.9842
	CAT	AGG_{JOE}	19	1.0	18,2	95.7	15,1	0.757	0.9947
	CAT	ACC_{NED}	16	0.8	11,8	73.5			0.9632
2	CTT	ACA_{FAM}	17	0.9	15,4	90.5			0.9842
	CTT	AGG_{JOE}	12	0.6	8,7	72.4	9	0.448	0.9275
	CTT	ACC_{NED}	6	0.3	2,8	46.8			0.6743
3	CAC	ACT_{FAM}	20	1.0	20,0	100.0			1.0000
	CAC	AAG_{JOE}	17	0.9	12,5	73.5	17,5	0.875	0.9684
	CAC	AAC_{NED}	20	1.0	20,0	100.0			1.0000
4	CAG	ACT_{FAM}	16	0.8	13,3	83.3			0.9737
	CAG	AAG_{JOE}	20	1.0	20,0	100.0	17,8	0.888	1.0000
	CAG	AAC_{NED}	20	1.0	20,0	100.0			1.0000
5	CTC	ACT_{FAM}	17	0.9	15,4	90.5			0.9842
	CTC	AAG_{JOE}	20	1.0	20,0	100.0	18,5	0.923	1.0000
	CTC	AAC_{NED}	20	1.0	20.0	100.0			1.0000
6	CTG	ACT_{FAM}	15	0.8	10,5	70.2			0.9526
	CTG	AAG_{JOE}	18	0.9	16,7	92.6	12,2	0.612	0.9895
	CTG	AAC_{NED}	13	0.7	9,5	73.3			0.9421
7	CAT	ACG_{JOE}	11	0.6	6,7	60.6			0.8947
	CAT	AGC_{NED}	19	1.0	18,5	97.3	12,6	0.630	0.9952
8	CTT	ACG_{JOE}	17	0.9	15,4	90.5			0.9842
	CTT	AGC_{NED}	20	1.0	20,0	100.0	17,7	0.885	1.0000
9	CAC	ACG_{JOE}	16	0.8	14,5	90.5			0.9794
	CAC	AGC_{NED}	19	1.0	18,2	95.7	16,4	0.818	0.9947
10	CAG	ACG_{JOE}	18	0.9	15,4	85.5			0.9842
	CAG	AGC_{NED}	20	1.0	20,0	100.0	17,7	0.885	1.0000
11	CTC	ACG_{JOE}	20	1.0	20.0	100.0			1.0000
	CTC	AGC_{NED}	20	1.0	20.0	100.0	20.0	1.000	1.0000
12	CTG	ACG_{JOE}	9	0.5	5,8	64.4			0.8632
	CTG	AGC_{NED}	12	0.6	9,5	79.4	7,7	0.383	0.9421

N_s = number of samples, I = number of distinct patterns, P = effective number of patterns per assay unit, % P = percentage of a effective number of patterns from the number of patterns, P_u = average effective number of patterns per multiplex, D_j = discriminatory power. $_{FAM,\ JOE,\ NED}$ = blue, green, yellow fluorescent dyes.

2000; Srivastava et al. 2001; Lionneton et al. 2002; Takuno et al. 2007) and CTC/AAC (Srivastava et al. 2001; Lionneton et al. 2002; Negi et al. 2004; Sabharwal et al. 2004) showed discrimination power values of 1 among cabbage accessions used in our study.

The 20 cabbage accessions were divided according to their origin and form. Only the white accession 'Bohmerwaldkohl' was clustered with the red cabbages, but the group in which it was clustered are old landraces that originated in mountain regions with similar conditions, which may explain this genetic similarity.

AFLP was successfully used for the assessment of genetic diversity within cabbage cultivars and landraces, and we suggest effective primer combinations for cabbage gene bank accession fingerprinting. Furthermore, candidate AFLP markers for white cabbage were identified.

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