PROJECT: BIO-MORPHOLOGICAL AND GENETIC CHARACTERIZATION OF THE BRASSICA WORKING GROUP COLLECTION

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Final Report

INTRODUCTION

During the meeting of the Brassica WG held in Olomouc (2007) it was decided to focus activities on the priority actions for the development of AEGIS (A European Genebank Integrated System). Recently, during the ECPGR Vegetable Network meeting (2009) and the ECPGR Brassica working group meeting (2010), held respectively in Catania and in Linguaglossa, all the members proposed to point the attention on *B. rapa,* which could be defined a multicrop species for its several uses, and on wild *Brassica* species (n=9) diffused in the Mediterranean basin (Branca and Iapichino, 1997; Branca *et al*., 2012). For these species few are the accessions held in the European collections and it was suggested to point our attention on Balkan, Mediterranean, Eastern and Western European areas.

The general idea has been to collect and/or set up an European core collection of about 20 accessions of wild *Brassica* species (n=9) to characterize and evaluate them for bio-morphological and DNA traits. These activities are strictly related to other activities carried out by the Institute of Sustainable Agriculture of Cordoba (CSIC-Spain) concerning evaluation for antioxidant compounds. The activities will be mainly co-funded by other specific projects.

Description of the work

We sown 26 accessions of wild Brassica species collected from several European gene banks by the Centre for Genetic Resources of Wageningen (CGN). Seed samples were sown and plantlets were transplanted at the experimental farm of Catania University (37°N, 20 m a.s.l.) in a cold greenhouse. For each accession were transplanted 10 plants. After two months from transplant all individuals were characterized utilizing IPGRI Brassica descriptors. Leaf samples of accessions of Wild Brassica species (n=9) were collected after one year from transplanting per accession and were utilized for glucosinolate and DNA analysis. Leaf samples were conserved at -80°C and freeze-dried. A sample of the leaf freeze-dried per accession were sent to the Institute of Sustainable Agriculture of Cordoba (CSIC-Spain) for glucosinolate analysis whereas the same samples were analyzed by SSRr markers by DISPA. At the end of the project all data acquired were inserted in the Bras-EDB.

Activities covered in the report

1) Bio-morphological characterization by the selected IBPGR and UPOV descriptors indicated by Brassica Working Group;
2) DNA analysis by five polymorphic SSR;
3) Provision of information and discussion about the obtained data to facilitate the preparation of a scientific publication on the results of this project, in collaboration with CGN, DISPA, VIR and Bioversity (to be completed after expiration of this agreement).

MATERIALS AND METHODS

Plants characterization

We characterized 26 accessions provided by four European genebanks (Tab.1). These were sown in trays in April 2009 and transplanted in June in cold greenhouse at the experimental farm of the Catania University. Sowing was carried out in alveolar containers but the seed samples and their quality did not permit to obtain the same amount of plants for each accession which varied from seven to twenty-one. The plantlets were transplanted at 3-4th leaf stage, in rows 100 cm apart and 50 cm apart along the row (2 plants per m^{-2}); for each accessions we transplanted 7 plants and for some of them 14 plants. The experimental design was randomized blocks. Characterization was made after one year after transplanting. Several IBPGR descriptors were utilised for morphological characterization (Tab. 2).

DNA extraction and SSR assays

For DNA extraction and SSR assays 25 accessions were utilized. Leaf tissues were collected upon 6th-8th leaves stage. The young leaf tissues were taken to the laboratory in an icebox and were used directly to isolate DNA by crushing in the extraction buffer. DNA was extracted from young leaves of seven a single seedling or single seeds as described in Tonguc and Griffiths (2004) utilizing the kit GenElute^{TM} Plant Genomic DNA Miniprep (Sigma Aldrich Inc.). The concentration and purity of each DNA sample was measured using a spectrophotometer Shimadzu at wave-lengths of 260 and 280 nm, quantified by visual comparison to λ DNA standards on ethidium bromide-stained agarose gels.

PCR amplification

PCR-based amplification of the purified DNA was carried out in a 20 µl reaction mixture. The reaction mixture contained 200 ng template DNA, 200 µM of each dNTP, 3.75 mM $MgCl_2$, 1X *Taq* DNA polymerase buffer and 2 mM Primer (Shah *et al.*, 2000). Five SSRs were selected for characterization: BoTHL1 (Genomic location: A02 from bp 12939222 to 12939238 strand -); BoAP1 (Genomic location: A02 from 11232063 to 11232079 strand -); PBCGSSRBO39 (Genomic location: A04 from 10029610);

BoPLD1(Genomic location: A05 from 19720558 to 19720576 strand -); BoABI1(Genomic location: A01 from 8639437 to 8639456 stand +) (Tab. 3). The primers flanking SSRs sequences were obtained from paper of Tonguç and Griffiths (2004) for BoTHL1, BoAP1, BoPLD1 and BoABI1. For PBCGSSRBO39 the primers flanking SSRs sequences were retrieved from the paper of Burgess B. *et al*. (2006).

Amplification of the DNA was done using a Perkin Elmer 9700 thermocycler (ABI, Foster City, CA, USA) with the following parameters: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 50°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. The reaction was stored at 4°C, until it was loaded onto the gel.

Agarose gel electrophoresis

PCR products were loaded into 4% agarose gels (UNILAB Life Science) and the electrophoresis run was performed at 100Vfor 5–6 h in 1 X TBE buffer (Sambrook *et al*. 1989).

Capillary electrophoresis

For each marker, 2 µl of the PCR product were mixed with 12 µl of formamide and 1 µl of Genescan 500 ROX size standard (Applied Biosystems, USA). The mixture was denaturated at 96°C for three minutes and placed on ice until further analysis. Capillary electrophoresis was performed using POP7 gel and ABI PRISM 3130 Genetic Analyser (Applied Biosystems, USA). Fragments' sizes were determined by the GeneMapper 3.7 software (Applied Biosystems, USA).

Data analysis

For the characterization bio-morphologic plants, the data matrix obtained was processed using SPSS 8.0 program by applying the hierarchical cluster analysis (Chatfield and Collins, 1980) with the complete linkage (furthest neighbour) method (Massie *et al*., 1996).

For the molecular characterization, the diversity within the wild species of *Brassica* was assessed using F_{ST} statistic with sample size bias correction (Latter, 1972; Nei, 1987). A Neighbour-Joining tree (Fig. 2) was constructed from the SSR data using the software package POPTREE2 and the $\mathbf{F_{ST}}$ statistic (Fig 3).

RESULTS AND DISCUSSION

Characterization bio-morphologic plants

After one year, only 20 accessions reached the reproductive stage. The characters showing higher variability were plant height, plant diameter, leaf blade shape and length, petiole length and width and the number of leaves per main stem.

The two accessions of *Brassica cretica* (CRE1 and CRE2) were different for vegetative stem width, ratio length/diameter, leaf blade length and width, petiole length, width, enlargement and colour. *Brassica incana* (BRA 2918) was different for vegetative stem width, plant height and branching, and leaf antocyanin coloration, blade length and shape. *Brassica macrocarpa* (MAC 1 and MAC2) were different for plant branching, leaf lobes, and color, petiole length, number of leaves for plant main stem and flowering time. *Brassica montana* (MON1 and MON2) differed mainly for the ratio plant length/diameter, plant branching, plant height, leaf antocyanin colour, petiole length and colour and number of leaves for plant main stem. *Brassica rupestris* (RUP1, RUP2, RUP3) were different for the ratio length/diameter, plant shape, leaf shape, plant branching and number of leaves for plant main stem. *Brassica villosa* VIL3 is different from VIL1 and VIL2 for ratio plant length/diameter, plant height, plant shape, leaf blade length, plant branching and number of leaves for plant main stem (Tab. 4).

Group A (INS, HIL, BOU1, BOU2, MAC1, RUP2, INC1 and MON1) is mainly different from the other groups for the light green colour of the leaves and the white colour of the petiole; group B (CRE2, RUP3, FRU1, OLE and CRE1) is different mainly for a more green coloration of the petiole; group C (FRU2, VIL2, RUP1, VIL1, RAP and MON2) is different for larger width of the petiole and number of scars; Group D (INC2 and VIL3) is different for the larger vegetative stem width, higher relation length/diameter, plant height, leaf blade length and width, higher angle of petiole, with leaf lamina more straight and less branching of the plant; Group E (DES and BAR) is mainly different for lower height and relation length/diameter of the plant, lower plant diameter, anthocyanin coloration and blade width of the leaf, lower petiole length and width and lower number of leaves and scars. Group F (DRE and MAC2) mainly differed for longer diameter, more elliptic leaf blade shape and longer petiole length. Group G (BAL) was mainly different for lower vegetative stem width, leaf blade length, leaf blade blistering and for higher branching of the plant and average number of leaves per plant (Fig. 1).

Molecular characterization

The alleles observed for the SSR primer utilized by capillary electrophoresis were nine for BoTHL1, eight for BoAP1, fifteen for BCGSSRBO, seven for BoPLD1 and no one for BoABI1 (Tab. 5). The alleles showed highest frequence in this Brassica CWRs collection were 153 and 162 for BoTHL1, 157 for BoAP1, 308 for BCGSSRBO and 291 for BoPLD1 (Tab. 5). The species showing the highest diversity for the alleles expressed by the primers utilized were *B. montana*, *B. hilarionis*, *B. fruticulosa*, *B. cretica*, *B. balearica*, *B. insularis*, *B. incana* and *B. rupestris*, whereas the lowest diversity was observed for *B. barrelieri*, *B. desnottesii*, *B. drepanensis*, *B. balearica*, *B. villosa* and *B. rapa oleifera*.

Among the markers utilized BoTHL1 and BoAP1 showed both eight alleles, PBCGSSRBO39 showed 15 alleles and BoPLD1 showed seven alleles. Pairwise genetic similarity (F_{ST} distance with sample size bias correction) was calculated for all cultivars (Fig. 3) and a dendrogram of relationships was produced. Major genetic similarities were found between *B. montana* and *B. bourgeaui*, at the same time major genetic distances were found between all accession of *B. rapa* and *B. barrelieri*, *B. cretica*, *B. desnottesii* and *B. drepanensis* and between *B. villosa* and *B. desnottesii* and *B. drepanensis* (Fig. 3). The

phylogenetic analysis grouped the *B. villosa* accessions on three different branches. Moreover the three accessions of *B. villosa* showed a considerable distance each other.

This observation can be explained with the esistence of several subpopulations of *B. villosa* which show morphological variances.

CONCLUSIONS

This work allowed us to observe a considerable variance among *Brassica* wild species as well as diversity among the same species widespread in different distribution areas. It could be noted that the used morphological and genetic descriptors could not discriminate the species according to the existing taxonomy. The additional biochemical studies foreseen in the frame of the BWG project will provide new data in view to offer additional indication about the diversity of the studied CWR population. Among the characterized species, *B. macrocarpa* and *B. balearica* were markedly different from other *Brassica* species.

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Tables

Table 1. Accessions characterized

INSTCODE	ACCENUMB	GENUS AND SPECIES	WORKING CODE	ORIGIN
DEU 146	BRA 2850	*Brassica balearica*	BAL	
DEU 146	BRA 2990/K10127	*Brassica barrelieri*	BAR	PRT
DEU 146	BRA 2998	*Brassica bourgeaui*	BOU1	ESP
DEU 146	BRA 2848	*Brassica bourgeaui*	BOU2	ESP
DEU 146	K 6631	*Brassica cretica*	CRE 1	GRC
DEU 146	K 10120	*Brassica cretica*	CRE 2	TUR
DEU 146	BRA 2919	*Brassica desnottesii*	DES	
DEU 146	BRA 2923	*Brassica drepanensis*	DRE	ITA
DEU 146	BRA1810	*Brassica fruticulosa*	FRU 1	ESP
DEU146	BRA 1727	*Brassica fruticulosa*	FRU 2	ITA
DEU146	BRA 2918	*Brassica incana*	INC 2	ITA
DEU146	K 5997	*Brassica insularis*	INS	ITA
DEU146	BRA 2944	*Brassica macrocarpa*	MAC 2	
DEU146	BRA 1644	*Brassica montana*	MON 2	
DEU146	CR 2929	*Brassica rapa*	RAP	DEU
DEU146	BRA 2945/K7690	*Brassica rupestris*	RUP 1	ITA
DEU146	BRA1896	*Brassica villosa*	VIL 1	ITA
GBR006	HRIGRU 124838	*Brassica hilarionis*	HIL	
GBR006	HRIGRU 6691	*Brassica incana*	INC 1	ITA
NLD037	CGN06903	*Brassica oleracea*	OLE	FRA
NLD037	CGN18472	*Brassica montana*	MON 1	ITA
NLD037	CGN14116	*Brassica villosa*	VIL 2	Mediterranean
ITA 227	UNICT 2973	*Brassica macrocarpa*	MAC 1	ITA
ITA 227	UNICT 3824	*Brassica rupestris*	RUP 2	ITA
ITA 227	UNICT 3844	*Brassica rupestris*	RUP 3	ITA
ITA 227	UNICT 3944	*Brassica villosa*	VIL 3	ITA

Table 2. IBPGRI and UPOV descriptors utilized

IBPGR descriptors		
Plant		
STDI	4.2.55	Vegetative stem width cm (measure diameter of widest point on stem)
ALDI	4.2.56	length/ diameter (cm)
PIAL	4.2.3	Plant height cm (measure extremity of plant)
PIDI	4.2.4	Plant diameter cm **(**measure extremity of plant)
PIRA		Branching plant (0=absent, 3=intermediate, 7=high)
MEFP	4.2.11	Average number of leaves for plant main stem (3=few, 5=intermediate, 7=many)
Leaf		
FGCO	4.2.24	Leaf color (1= yellow green, 2=light green, 3=green, 4=dark green, 5=purple green, 6=purple, 7=other)
FGFO	4.2.16	Leaf blade shape (1= orbicular, 2=elliptic, 3=obovate, 4=spathulate, 5=ovate, 6=lanceolate, 7=oblong)
FGLU	4.2.12	Leaf blade length (measure largest leaf including petiole)
FGLA	4.2.13	Leaf blade width (widest point on largest leaf)
FGAG	4.2.15	Angle of petiole with horizontal :1=erect(>87°, 2=open(~67°), 3=semiprostrate(~45°),
FGAT	4.2.23	Leaf lamina attitude (3=convex, 5=straight, 7=concave, drooping)
FGMA	4.2.21	Leaf blade blistering (0=none, 3=low, 5=intermediate, 7=high)
FGLO		Leaf lobes (1= absent, 9= present)
MEDC	4.2.10	Average leaf scars (n.)
Petiole		
PELU		Petiole length (cm)
PELA		Petiole width (cm)
PEAL	4.2.27	Petiole enlargement (3=narrow, 5=intermediate, 7=enlarged)
PECO	4.2.33	Petiole and/or midwein colour (1=white, 2=light green, 3=green, 4=purple, 5=red, 6=other)
UPOV descriptors		
Plant		
PIFO	UPOV_3	Plant shape (Plant growth habit)
Leaf		
FGAN	UPOV_5	Leaf anthocyanin coloration (1= absent, 9=present)
FGCU	UPOV_14	Leaf blade density of curling (1=absent or very low, 3= low, 5= medium, 7=high)
FGSE	UPOV_15	Folding leaf section (3=weak, 5=medium, 7= strong)

Tab. 3 Primers identified and the sequences corresponding

Sign	**Markers**	**Primer forward**	**Primer revers**
P2	BoTHL1	GCCAAGGAGGAAATCGAAG	AAGTGTCAATAAGGCAACAAGG
P3	BoAP1	GGAGGAACGACCTTGATT	GCCAAAATATACTATGCGTCT
P5	PBCGSSRBo39	AACGCATCCATCCTCACTTC	TAAACCAGCTCGTTCGGTTC
P6	BoPLD1	GACCACCGACTCCGATCTC	AGACAAGCAAAATGCAAGGAA
P7	BoABI1	TATCAGGGTTTCCTGGGTTG	GTGAACAAGAAGAAAAGAGAGCC

Table 4. Morphological characterization based on both IBPGR and UPOV descriptors

Plant	IBPGR	BRA 2850	BRA 2990/K10127	BRA 2998	BRA 2848	K 6631	K 10120	BRA 2919	BRA 2923	BRA 1810	BRA 1727	BRA 2918	K5597	BRA 2944
STDI	4.2.55	1,37	1,80	3,14	5,02	4,47	1,78	0,99	1,84	1,47	4,16	3,59	3,05	3,51
ALDI	4.2.56	5,99	3,89	11,92	9,87	4,63	15,40	3,55	10,13	9,51	4,21	17,51	12,41	14,29
PIAL	4.2.3	24,78	15,00	77,23	91,43	49,13	57,33	11,00	57,33	32,20	64,50	129,80	66,50	79,67
PIDI	4,2,4	39,67	16,00	60,31	60,71	39,80	53,50	14,50	87,33	51,20	59,00	63,40	58,60	116,17
PIRA		7,00	7,00	2,38	1,00	1,73	1,50	3,00	4,33	7,00	3,00	0,00	0,00	5,67
MEFP	4.2.11	166,33	9,00	45,92	24,14	27,93	19,83	36,00	142,00	0,00	38,00	27,60	29,00	138,50
Leaf	**IBPGR**													
FGCO	4.2.24	4,56	1,00	2,15	2,86	4,07	3,00	4,00	2,67	0,00	2,77	2,00	2,00	3,00
FGFO	4.2.16	2,11	6,00	3,00	3,00	3,87	2,67	6,00	2,00	0,00	5,00	6,00	2,00	2,00
FGLU	4.2.12	8,44	15,20	27,62	39,29	12,60	29,67	5,50	29,67	0,00	11,57	41,80	30,83	25,00
FGLA	4.2.13	5,11	6,43	20,62	26,64	5,87	10,83	2,50	19,67	0,00	9,42	14,40	8,67	17,17
FGAG	4.2.15	3,22	4,75	4,23	3,14	4,73	3,83	3,00	4,00	0,00	4,44	4,60	5,00	3,50
FGAT	4.2.23	5,00	5,00	5,31	5,29	5,40	5,33	5,00	5,00	0,00	5,00	6,60	5,00	5,00
FGMA	4.2.21	2,56	3,00	3,00	2,57	3,53	3,00	7,00	6,33	0,00	1,00	3,40	3,00	4,00
FGLO		1,78	9,00	1,00	1,00	1,40	1,17	2,00	2,00	0,00	1,00	1,20	1,00	2,00
MEDC	4.2.10	39,67	10,00	44,08	49,29	41,67	20,67	15,00	29,33	7,40	33,50	24,20	41,20	32,67
Petiole	**IBPGR**													
PELU		9,06	0,50	0,00	0,00	7,53	2,00	3,50	20,00	0,00	8,44	0,00	0,00	23,17
PELA		0,31	0,25	0,00	0,00	0,29	0,09	0,18	0,93	0,00	0,75	0,00	0,00	0,86
PEAL	4.2.27	3,00	4,86	0,00	0,00	2,80	0,50	3,00	3,67	0,00	2,53	0,00	0,00	4,00
PECO	4.2.33	2,44	2,00	0,00	0,00	3,20	0,33	2,00	2,67	0,00	2,00	0,00	0,00	2,00
Plant	**UPOV**													
PIFO	UPOV_3	3,00	1,00	5,00	5,00	2,87	5,00	2,00	3,67	1,00	1,00	5,00	5,00	3,00
Leaf	**UPOV**													
FGAN	UPOV_5	7,22	1,00	4,69	3,29	7,93	7,67	1,00	3,67	1,00	1,82	4,20	1,00	7,67
FGCU	UPOV_14	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,00	1,00	1,00	1,00	1,00
FGSE	UPOV_15	4,78	3,00	3,00	3,00	3,00	3,00	7,00	5,00	3,00	3,00	3,00	3,00	4,33

Follows

Plant	IBPGR	BRA 1644	CR 2929	BRA 2945/K7690	BRA 1896	HRI : 08 : 0124838	HRI : 02 : 006691	CGN 06903	CGN 18472	CGN 14116	UNICT	UNICT	UNICT	UNICT
STDI	4.2.55	3,77	2,95	2,50	3,36	2,14	2,00	2,16	6,63	2,43	3,43	3,69	1,75	3,30
ALDI	4.2.56	13,54	16,30	10,01	17,54	13,41	18,53	7,13	3,54	5,57	8,54	8,64	29,40	29,57
PIAL	4.2.3	87,43	107,50	59,20	89,67	60,15	72,14	35,79	47,33	30,00	61,00	62,14	48,14	140,67
PIDI	4,2,4	82,86	80,50	68,80	96,67	52,38	67,29	61,71	53,93	83,50	67,75	81,14	48,71	80,00
PIRA		3,86	0,00	3,00	4,67	0,00	1,71	1,86	1,33	3,50	0,00	0,00	1,71	0,00
MEFP	4.2.11	94,00	55,00	61,60	45,00	29,69	25,14	37,57	28,07	83,00	35,25	25,43	27,57	19,33
Leaf	**IBPGR**													
FGCO	4.2.24	2,00	3,00	2,00	2,33	2,69	2,00	3,00	2,53	3,00	2,00	2,29	3,00	3,00
FGFO	4.2.16	2,29	6,00	6,00	2,00	4,62	2,43	5,50	2,33	3,00	2,00	2,00	2,00	2,00
FGLU	4.2.12	29,00	28,20	24,60	33,00	27,92	23,14	19,11	27,67	26,00	21,75	25,14	18,71	55,67
FGLA	4.2.13	17,14	12,50	14,40	22,67	14,62	14,14	10,00	14,20	18,00	13,5	16,14	11,14	25,33
FGAG	4.2.15	3,14	2,62	3,00	5,00	4,62	3,86	3,43	3,67	3,00	4,00	4,00	3,86	5,00
FGAT	4.2.23	5,00	5,10	5,00	7,00	5,00	5,29	5,00	4,73	6,00	5,00	5,00	5,00	5,00
FGMA	4.2.21	3,57	3,40	5,00	3,00	2,77	4,71	3,86	3,27	3,00	3,5	3,00	5,00	3,67
FGLO		1,86	1,00	2,00	2,00	1,00	2,00	1,86	1,20	1,00	1,00	2,00	2,00	2,00
MEDC	4.2.10	53,71	78,50	50,60	60,00	47,08	20,86	25,50	29,20	46,00	46,50	45,14	23,86	29,33
Petiole	**IBPGR**													
PELU		18,43	11,63	23,00	23,67	0,00	20,00	14,07	9,87	24,50	11,00	13,86	15,43	0,00
PELA		0,71	0,71	0,84	1,04	0,00	0,72	0,78	0,62	1,29	0,74	0,84	0,52	0,00
PEAL	4.2.27	3,43	0,00	3,00	4,33	0,00	3,86	3,21	2,53	5,00	3,00	3,00	3,00	0,00
PECO	4.2.33	1,29	3,89	2,00	1,00	0,00	1,29	2,50	1,33	1,00	2,50	2,43	3,14	0,00
Plant	**UPOV**													
PIFO	UPOV_3	5,00	1,00	3,00	3,00	5,00	5,00	1,71	3,13	1,00	1,00	1,00	5,00	5,00
Leaf	**UPOV**													
FGAN	UPOV_5	2,14	9,00	4,20	1,00	4,08	1,00	9,00	1,00	1,00	5,00	6,71	9,00	1,00
FGCU	UPOV_14	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
FGSE	UPOV_15	3,57	3,00	5,00	5,00	3,00	3,00	3,00	3,13	3,00	3,00	3,00	3,57	3,00

Table 5. Molecular characterization

Primers	Alleles	BRA 2850	BRA 2990/K10127	BRA 2998	BRA 2848	K6631	K 10120	BRA 2919	BRA 2923	BRA1810	BRA1727	BRA 2918	K 5997	BRA 2944
BoTHL	153	0	0	0	0	0	0	0	0	0	1	0	0	0
	155	0	0	0	0	0.34	1	0	0	0	0	0	0	0
	157-2	0	0	0	0	0	0	0	0	0	0	0	0	0
	158	0	0	0	0	0	0	0	0	0	0	0	0	1
	162	0	0	1	0.57	0.08	0	0	0	0	0	1	0.5	0
	165	0	0	0	0	0	0	0	1	0	0	0	0	0
	168	0	0	0	0.43	0.58	0	0	0	0	0	0	0.5	0
	171	0	0	0	0	0	0	0	0	0	0	0	0	0
	308	0	0	0	0	0	0	0	0	0	0	0	0	0
BoAP1	154	0	0	0	0	0	1	0	0	0	0.5	0	0	0
	155	0	0	0	0	0	0	0	0	0	0	0	0	0
	156	0	0	0	0	0.33	0	0	0	0	0	0	0.8	0
	157	0	1	0	0.75	0.67	0	1	0	1	0.5	0	0.2	0
	160	0	0	0	0	0	0	0	0	0	0	0	0	0
	163	0	0	0	0	0	0	0	0	0	0	0	0	0
	165	0	0	0	0.25	0	0	0	0	0	0	0	0	0
	171	0	0	0	0	0	0	0	0	0	0	1	0	0
PBCGSSRBo39	155	0	0	0	0	0	0	0	0	0	0	0	0	0.1
	157	0	0	0	0	0	0	0	0	0	0	0	0	0
	294	0	0	0	0	0	0	0	0	0	0	0	0	0
	298	0.5	0	0	0	0	0	0	0	0	0	0	0	0
	300	0	0	0	0	0	0	0	0	0	0	0	0	0.6
	302	0	0	0	0	0	0	0	0	0	0	0	0	0
	304	0	0	0.1	0.07	0.7	0	0	0	0	0	0	0.25	0
	308	0.5	1	0	0.57	0.3	0	1	0	1	1	0	0.63	0
	313	0	0	0.7	0.36	0	1	0	0	0	0	0	0	0
	313-2	0	0	0	0	0	0	0	0	0	0	0	0	0
	316	0	0	0.2	0	0	0	0	0	0	0	0	0	0.3
	322	0	0	0	0	0	0	0	0	0	0	0	0.12	0
	325	0	0	0	0	0	0	0	0	0	0	0.25	0	0
	328	0	0	0	0	0	0	0	0	0	0	0.75	0	0
	330	0	0	0	0	0	0	0	0	0	0	0	0	0
BoPLD1	282	0	0	0	0	0	0.88	0	0	0	0.5	0	0.4	0
	287	0	0	0	0	0	0	0	0	0	0	0	0	0
	288	0	0	0	0	0	0	0	0	0	0	0	0.6	0
	289	0	0	0	0	0	0	0	0	0	0	0	0	0
	291	0	0	1	0	1	0	0	0	0	0.5	0	0	0
	293	0	0	0	0	0	0	0	0	0	0	0	0	0
	301	0	0	0	0	0	0.12	0	0	0	0	0	0	0

Follow

Primers	Alleles	BRA 1644	CR 2929	BRA 2945/K7690	BRA1896	HRI:08:0124838	HRI:02:006691	CGN06903	CGN18472	CGN14116	S 542	S 401	BV 7
BoTHL	*153*	0	0	0	0	0	0	0	0	0	0	0	0
	155	0.21	0	0	0	0	0	0	0	0	0	0	0
	157-2	0	0	0.7	0	0	0	0	0	0	0	0.5	0
	158	0	0	0	0	0	0.07	0.5	0	0	0	0	0
	162	0.79	0	0	1	0.08	0.79	0.125	0.6	0	0.93	0	1
	165	0	0	0.3	0	0.92	0	0	0	0	0	0	0
	168	0	0	0	0	0	0.14	0.375	0.4	0	0.07	0	0
	171	0	0	0	0	0	0	0	0	0	0	0.5	0
	308	0	0	0	0	0	0	0	0	0	0	0	0
BoAP1	*154*	0	0	0	0	0	0	0	0	0	0	0	0
	155	0	0	0	0	0	0	0.17	0	0	0.2	0.5	0
	156	0	0	0	1	0	0.4	0.5	0	0.5	0.2	0	0
	157	0.5	0	0	0	0.5	0.6	0.33	0.8	0.5	0.2	0.5	1
	160	0.5	0	0	0	0.5	0	0	0	0	0	0	0
	163	0	0	0	0	0	0	0	0.2	0	0	0	0
	165	0	0	0	0	0	0	0	0	0	0.1	0	0
	171	0	0	0	0	0	0	0	0	0	0	0	0
PBCGSSRBo39	*155*	0	0	0	0	0	0	0.25	0	0	0	0	0
	157	0	0	0	0	0	0.17	0	0	0	0	0	0
	294	0	0	0	0	0.375	0	0	0	0	0	0.29	0
	298	0	0	0	0	0.625	0	0	0	0	0.17	0.07	0
	300	0	0	0	0	0	0	0	0	0	0	0.14	0
	302	0	1	0	0	0	0	0	0	0	0	0	0
	304	0	0	0	0	0	0	0	0	0	0.33	0	0
	308	0.67	0	0	0	0	0.67	0.75	0.6	0	0.5	0	0
	313	0	0	0	0	0	0	0	0.1	0	0	0	0
	313-2	0	0	0	0	0	0	0	0	0	0	0	0.67
	316	0	0	0	0	0	0.16	0	0.1	0	0	0.29	0
	322	0	0	1	0	0	0	0	0.1	0	0	0.21	0
	325	0.33	0	0	0	0	0	0	0	0	0	0	0
	328	0	0	0	0	0	0	0	0	0	0	0	0
	330	0	0	0	0	0	0	0	0	0	0	0	0.33
BoPLD1	*282*	0	0	0	0	0	0	0	0	0	0.57	0	0
	287	0	0	0	0	0	0	0	0	0	0	0.1	0
	288	0	0	1	1	0	0.8	0.33	0.67	0	0.36	0.3	0
	289	0	0	0	0	0	0	0	0	0	0	0.1	0
	291	1	0	0	0	1.17	0.2	0.67	0.33	0	0	0.3	0
	293	0	0	0	0	0.83	0	0	0	0	0.07	0.2	0
	301	0	0	0	0	0	0	0	0	0	0	0	0

Figures

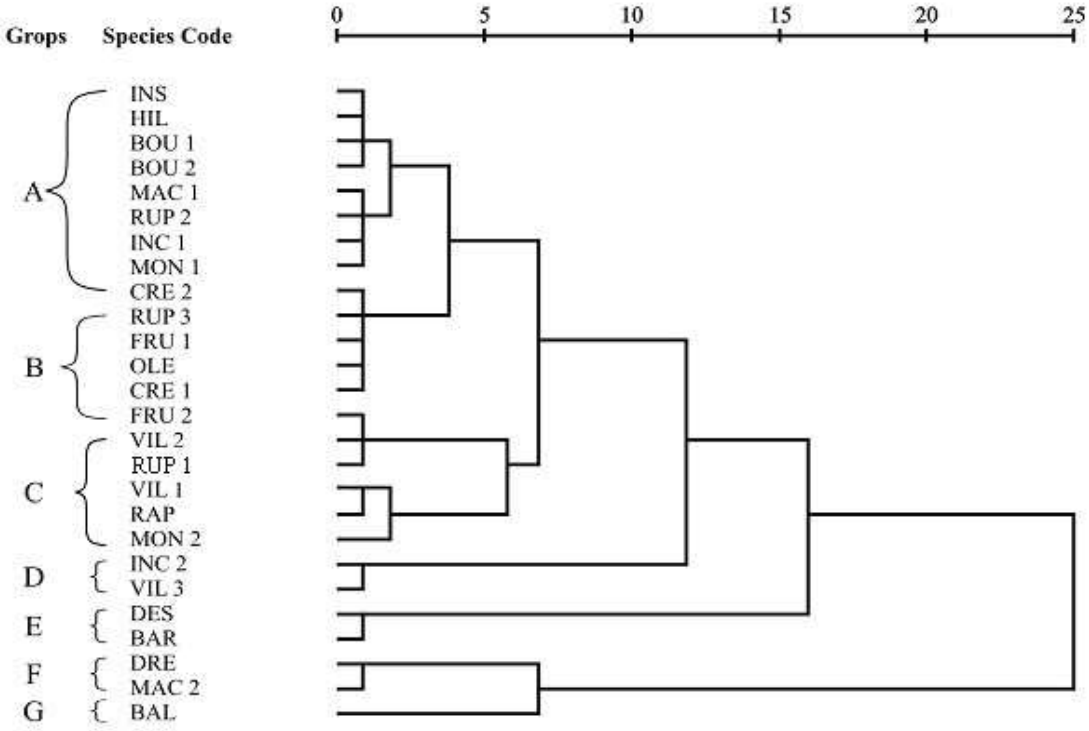

Fig. 1. Cluster analysis of the analyzed materials

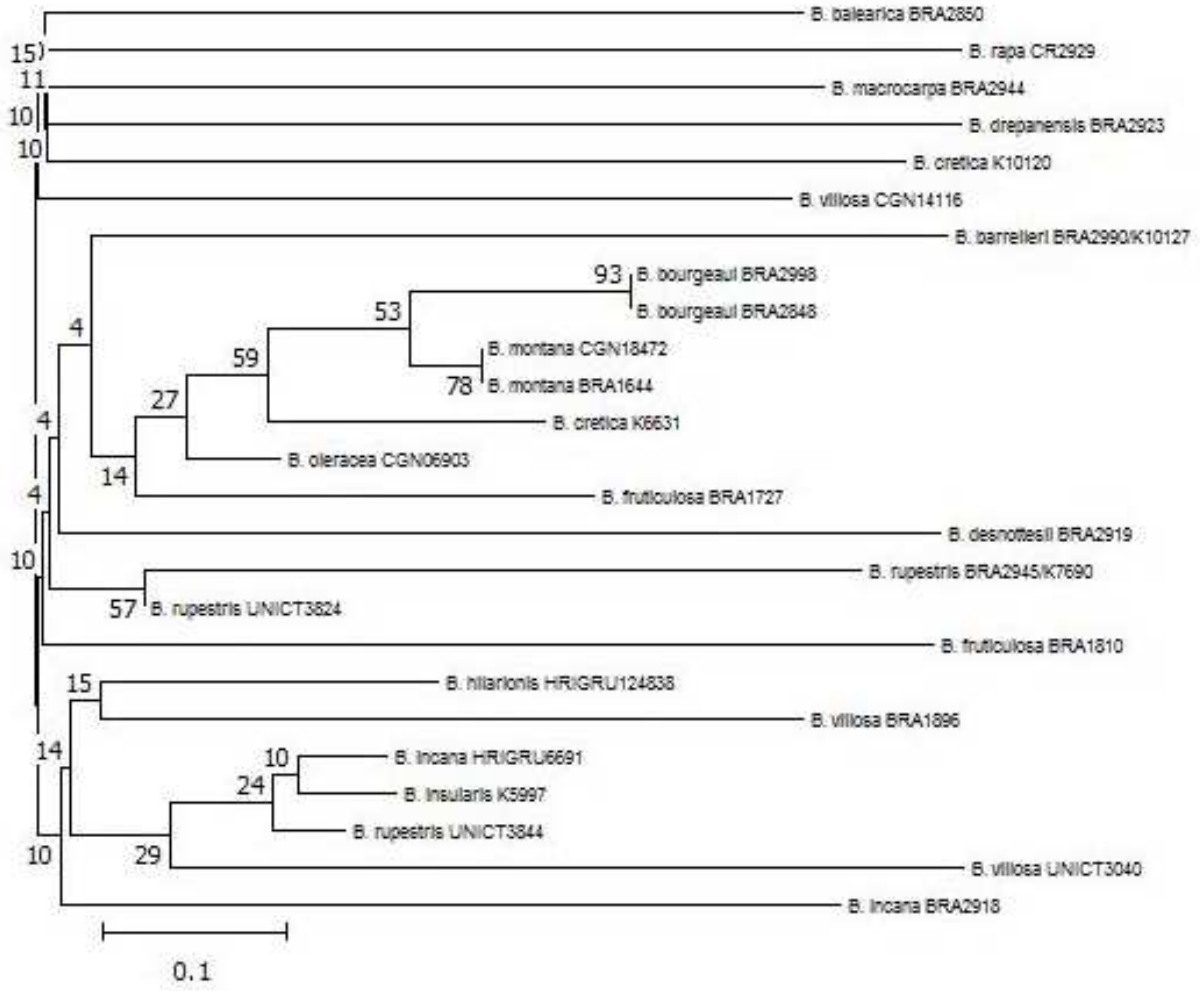

Fig. 2. Neighbor-joining (NJ) tree of pairwise genetic distance (F_{ST} distance with sample size bias correction). Numbers at nodes indicate % bootstrap support (out of 100 bootstrap replicates).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	0,00	0,91	0,72	0,71	0,71	0,89	0,91	0,92	0,91	0,71	0,63	0,65	0,85	0,63	0,84	0,65	0,62	0,56	0,92	0,86	0,45	0,61	0,85	0,92	0,83
2	0,91	0,00	0,75	0,73	0,74	0,97	1,00	1,00	1,00	0,73	0,71	0,66	0,94	0,67	0,93	0,64	0,60	0,57	1,00	0,94	0,48	0,64	0,91	1,00	0,91
3	0,72	0,75	0,00	0,00	0,28	0,75	0,75	0,82	0,75	0,50	0,43	0,33	0,70	0,43	0,74	0,09	0,03	0,29	0,82	0,76	0,22	0,32	0,60	0,77	0,71
4	0,71	0,73	0,00	0,00	0,26	0,74	0,73	0,81	0,73	0,48	0,42	0,31	0,68	0,42	0,73	0,07	0,00	0,28	0,81	0,75	0,21	0,30	0,58	0,76	0,69
5	0,71	0,74	0,28	0,26	0,00	0,75	0,74	0,80	0,74	0,49	0,47	0,41	0,73	0,37	0,73	0,26	0,21	0,23	0,80	0,74	0,22	0,39	0,67	0,78	0,68
6	0,89	0,97	0,75	0,74	0,75	0,00	0,97	0,97	0,97	0,74	0,71	0,74	0,91	0,68	0,89	0,72	0,69	0,66	0,97	0,91	0,51	0,65	0,90	0,97	0,89
7	0,91	1,00	0,75	0,73	0,74	0,97	0,00	1,00	1,00	0,73	0,71	0,66	0,94	0,67	0,93	0,64	0,60	0,57	1,00	0,94	0,48	0,64	0,91	1,00	0,91
8	0,92	1,00	0,82	0,81	0,80	0,97	1,00	0,00	1,00	0,83	0,71	0,77	0,94	0,74	0,93	0,76	0,74	0,69	1,00	0,94	0,54	0,73	0,93	1,00	0,92
9	0,91	1,00	0,75	0,73	0,74	0,97	1,00	1,00	0,00	0,73	0,71	0,66	0,94	0,67	0,93	0,64	0,60	0,57	1,00	0,94	0,48	0,64	0,91	1,00	0,91
10	0,71	0,73	0,50	0,48	0,49	0,74	0,73	0,83	0,73	0,00	0,54	0,45	0,77	0,45	0,76	0,38	0,34	0,32	0,83	0,78	0,31	0,41	0,73	0,83	0,73
11	0,63	0,71	0,43	0,42	0,47	0,71	0,71	0,71	0,71	0,54	0,00	0,40	0,64	0,44	0,67	0,36	0,33	0,38	0,74	0,67	0,15	0,32	0,57	0,71	0,64
12	0,65	0,66	0,33	0,31	0,41	0,74	0,66	0,77	0,66	0,45	0,40	0,00	0,63	0,10	0,68	0,15	0,10	0,09	0,77	0,64	0,17	0,10	0,54	0,54	0,64
13	0,85	0,94	0,70	0,68	0,73	0,91	0,94	0,94	0,94	0,77	0,64	0,63	0,00	0,63	0,86	0,63	0,60	0,61	0,94	0,88	0,48	0,56	0,83	0,92	0,85
14	0,63	0,67	0,43	0,42	0,37	0,68	0,67	0,74	0,67	0,45	0,44	0,10	0,63	0,00	0,67	0,31	0,27	0,09	0,74	0,61	0,22	0,11	0,61	0,51	0,61
15	0,84	0,93	0,74	0,73	0,73	0,89	0,93	0,93	0,93	0,76	0,67	0,68	0,86	0,67	0,00	0,69	0,66	0,55	0,93	0,87	0,44	0,65	0,86	0,93	0,84
16	0,65	0,64	0,09	0,07	0,26	0,72	0,64	0,76	0,64	0,38	0,36	0,15	0,63	0,31	0,69	0,00	0,00	0,16	0,76	0,68	0,15	0,20	0,52	0,69	0,65
17	0,62	0,60	0,03	0,00	0,21	0,69	0,60	0,74	0,60	0,34	0,33	0,10	0,60	0,27	0,66	0,00	0,00	0,12	0,74	0,65	0,12	0,16	0,48	0,66	0,62
18	0,56	0,57	0,29	0,28	0,23	0,66	0,57	0,69	0,57	0,32	0,38	0,09	0,61	0,09	0,55	0,16	0,12	0,00	0,69	0,58	0,11	0,22	0,57	0,56	0,56
19	0,92	1,00	0,82	0,81	0,80	0,97	1,00	1,00	1,00	0,83	0,74	0,77	0,94	0,74	0,93	0,76	0,74	0,69	0,00	0,94	0,54	0,73	0,93	1,00	0,92
20	0,86	0,94	0,76	0,75	0,74	0,91	0,94	0,94	0,94	0,78	0,67	0,64	0,88	0,61	0,87	0,68	0,65	0,58	0,94	0,00	0,34	0,64	0,88	0,92	0,86
21	0,45	0,48	0,22	0,21	0,22	0,51	0,48	0,54	0,48	0,31	0,15	0,17	0,48	0,22	0,44	0,15	0,12	0,11	0,54	0,34	0,00	0,17	0,40	0,50	0,42
22	0,61	0,64	0,32	0,30	0,39	0,65	0,64	0,73	0,64	0,41	0,32	0,10	0,56	0,11	0,65	0,20	0,16	0,22	0,73	0,64	0,17	0,00	0,47	0,56	0,61
23	0,85	0,91	0,60	0,58	0,67	0,90	0,91	0,93	0,91	0,73	0,57	0,54	0,83	0,61	0,86	0,52	0,48	0,57	0,93	0,88	0,40	0,47	0,00	0,91	0,83
24	0,92	1,00	0,77	0,76	0,78	0,97	1,00	1,00	1,00	0,83	0,71	0,54	0,92	0,51	0,93	0,69	0,66	0,56	1,00	0,92	0,50	0,56	0,91	0,00	0,91
25	0,83	0,91	0,71	0,69	0,68	0,89	0,91	0,92	0,91	0,73	0,64	0,64	0,85	0,61	0,84	0,65	0,62	0,56	0,92	0,86	0,42	0,61	0,83	0,91	0,00

Fig. 3. $\mathbf{F_{ST}}$ statistic pairwise values with sample size correction (Nei 1987). The accession codes are the following: 1= *B. balearica* BRA2850, 2= *B. barrelieri* BRA2990/K10127, 3= *B. bourgeaui* BRA2998, 4= *B. bourgeaui* BRA2848, 5= *B. cretica* K6631, 6= *B. cretica* K10120, 7= *B. desnottesii* BRA2919, 8= *B. drepanensis* BRA2923, 9= *B. fruticulosa* BRA1810, 10= *B. fruticulosa* BRA1727, 11= *B. hilarionis* HRIGRU124838, 12= *B. incana* HRIGRU6691, 13= *B. incana* BRA2918, 14= *B. insularis* K5997, 15= *B. macrocarpa* BRA2944, 16= *B. montana* CGN18472, 17= *B. montana* BRA1644, 18= *B. oleracea* CGN06903, 19= *B. rapa* CR2929, 20= *B. rupestris* BRA2945/K7690, 21= *B. rupestris* UNICT3824, 22= *B. rupestris* UNICT3844, 23= *B. villosa* BRA1896, 24=*B. villosa* UNICT3040, 25= *B. villosa* CGN14116.