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Interspecific relationship and genetic diversity in wild beets in section Corollinae genus Beta: Isozyme and RAPD analyses

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Abstract

Interspecific relationship among wild beets in section Corollinae was analyzed. Isozyme allele composition supports the concept of three basic species consisting of two diploid species, *Beta lomatogona* and *Beta macrorhiza* and the tetraploid species, *Beta corolliflora*. The polyploid and apomictic species referred to as *Beta trigyna* and *Beta intermedia* were found as hybrids. Based on isozyme and RAPD results, *B. corolliflora* was also found more related to *B. macrorhiza* than to *B. lomatogona*. From the phylogenetic trees constructed from isozyme data, *B. corolliflora* and *B. macrorhiza* seem to be the end members of the evolutionary line. Analysis of genetic diversity showed that *B. corolliflora* was more polymorphic and heterozygous than *B. lomatogona* and *B. macrorhiza*. Differences between *B. macrorhiza* accessions from Turkey and Dagestan were found.

Introduction

The genus Beta of the family Chenopodiaceae consists of four sections namely: Beta (Vulgares), Procumbentes (Patellares), Corollinae and Nanae. Section Beta contains both wild and cultivated beets including sugar beet, *Beta vulgaris* L. The other three sections comprise only wild beets.

In section Corollinae, there are about five known species. These are the diploid *Beta lomatogona* Fisch. et Mey. and *Beta macrorhiza* Stev. and the polyploid species *Beta corolliflora* Zoss. ex Buttler, *Beta intermedia* Bunge and *Beta trigyna* Wald. et Kit. The Corollinae species are mainly distributed in the highlands of Asia Minor encompassing Turkey, Iran, Armenia, Georgia and Dagestan. They can also be encountered in Central to Eastern Europe (Crimea, Bulgaria, Romania and Hungary).

The basic chromosome number of Corollinae species is n = 9. They are characterized by a very hard seed coat and slow growth. Corollinae species are known as sources for resistance to several diseases affecting sugar beet (Van Geyt et al., 1990). In addition, they show high tolerance to drought, cold, frost and salinity.

Apomictic reproduction is one important characteristic of this section (Barocka, 1966; Jassem, 1990; Reamon-Büttner, 1994). The species are known to cross pollinate each other giving rise to new different morphotypes that are then later fixed by apomixis. This leads to difficulty in the taxonomy of the Corollinae species, which is further aggravated by the lack of morphological markers to distinguish the species. Until now the taxonomy in this section is not clear.

In an extensive study of the Corollinae species using morphological characters and geographic distribution patterns, Buttler (1977) suggested that only the sexual species, namely *B. lomatogona*, *B. macrorhiza* and *B. corolliflora* should be considered as the basic species and the rest, as hybrids or apomictic forms. *B. trigyna* has been presumed to be a hybrid form between *B. corolliflora* and *B. lomatogona* (Zossimovitch, 1940). *B. intermedia* has been likewise described by Scheibe (1934) as an intermediary form of *B. trigyna* and *B. lomatogona*. Barocka (1966) earlier proposed the subdivision of section Corollinae into Lomatogonae and Trigynae based on differences in

floral morphology between *B. lomatogona* and *B. trigyna*. Such subdivision has been supported by two studies using molecular techniques (Fritzsche et al., 1987; Santoni & Berville, 1992). Moreover, Buttler (1977) suggested that aside from the *B. macrorhiza* gene pool in Turkey, there could be a second one in the Eastern Caucasus. To acquire authentic material, two plant explorations were conducted in the Caucasus in 1990 and 1991 (Frese & Burenin, 1991).

This study was, therefore, conducted to help clarify the taxonomy of section Corollinae and to verify assumptions made earlier about Corollinae species using molecular techniques. In addition, improved understanding of the structure of genetic diversity can contribute to a more rational collection and preservation of Corollinae germplasm in genetic resources holdings as well as to its utilization in sugar beet breeding programs.

Materials and methods

Plant material

The different accessions of Corollinae species used in this study are shown in Table 1. Except for the two *B. corolliflora* accessions (labeled USA and Bulgaria) which were obtained from the Institute of Plant Breeding and Acclimatization, Bydgoszcz, Poland, most accessions were provided by the German-Dutch Beta collection held by the Institute of Crop Science in Braunschweig, Germany. The *B. corolliflora* (USA) accession was originally from the Coons collection (Beltsville, Maryland). Chromosome number was determined using the technique described in Löptien (1984).

To overcome poor germination due to the very hard seed coat, seeds were treated with concentrated sulphuric acid for 1–2 h in a beaker, transferred to a sieve and then washed in running water to remove debris. Under the stereomicroscope, while holding the seeds with forceps, the apical lid was removed using a scalpel. The seeds were planted singly in sterile soil; germination started in about a week, which would otherwise take months.

The mode of reproduction, i.e. sexual or apomictic, was determined through isozyme analysis. Isozyme loci which were in heterozygous condition were selected as markers and segregation of alleles was observed after self- or open-pollination. Progenies that displayed the maternal zymogram and chromosome number were considered of apomictic origin.

Isozyme analysis

The enzymes were extracted from the leaves of about 2–3-month-old plants. This was carried out through an extraction equipment using 250 μl of a 5% sucrose solution with 0.1% mercaptoethanol for every 2–3 leaves. The extract was centrifuged at 14 000 rpm at 15 min at 4 °C.

The enzymes isocitrate dehydrogenase (ICD), malate dehydrogenase (MDH), peroxidase (PRX), leucine aminopeptidase (LAP), glutamate oxaloacetate (GOT), superoxide dismutase (SOD) phosphoglucomutase (PGM) and acid phosphatase (ACP) were used. The electrophoretic conditions and staining procedures were adapted from Wagner (1990) and Wagner & Wricke (1991). The isozymes observed migrated anodally except PRX which migrated to the cathode.

Isozyme loci are written in italics i.e. *Lap1*. Then this is followed by the allele number such as in *Lap1*-1. Number 1 designates the fastest locus and the fastest allele. When the alleles are separated by commas, it means that the accession or the species is polymorphic in these given alleles. In *B. trigyna* and *B. intermedia*, the isozyme phenotypes or allele combinations are given e.g. *Lap1*-135, *Lap1*-14, *Lap1*-15. The phenotype *Lap1*-135, shows the combination of allele numbers 1, 3 and 5.

RAPD analysis

DNA was extracted adapting the CTAB method of Saghai-Maroof et al. (1984) from representatives of several accessions totalling from 2–18 plants for each species: *B. corolliflora* (18), *B. macrorhiza* (2), *B. lomatogona* (11), *B. trigyna* (11) and *B. intermedia* (5). A double strength CTAB extraction buffer was used in the isolation of DNA from fresh young leaves. The arbitrary decamer primers A-05, A-18, and A-19 were purchased from Operon Technologies (Almeda, California). DNA amplification was done according to the method described by Uphoff & Wricke (1992).

Genetic diversity and phylogenetic trees

For the study of genetic diversity and construction of phylogenetic trees, the computer programs Biosys-1

Table 1. The different Corollinae accessions used for isozyme and RAPD analyses including alleles observed in three isozyme loci per accession

No.	BGRC/IDBB No.	Species	Country of origin and district	Chromosome no. (2n)	No. of plants	Isoenzyme locus/ observed alleles		
						Lap1	*Prx1*	*Got3*
1	017812 2523	*B. corolliflora*	Turkey/Kars	36	43	1, 2, 3, 4, 5	1, 2, 3	1, 2, 3, 4
2	017822 2533	*B. corolliflora*	Turkey/Kars	36	74	3, 4, 5	1, 2, 3	1, 2, 3, 4
3	018254 2790	*B. corolliflora*	Turkey/Erzurum	36	104	3, 4, 5	1, 2, 3	1, 2, 3, 4
4	018255 2791	*B. corolliflora*	Turkey/Erzurum	36	75	3, 4, 5	1, 2, 3	1, 2, 3, 4
5	035314 3221	*B. corolliflora*	Transcaucasian	36	5	3, 4	1, 2	2, 4
6	018252 2788	*B. corolliflora*	Turkey	36	2	3, 4, 5	1, 2	2, 3
7	018156 2373	*B. corolliflora*	Turkey	36	4	–	–	2, 4
8	018246 2326	*B. corolliflora*	Turkey	36	24	2, 3, 4, 5	1, 3	1, 2
9	Bulgaria	*B. corolliflora*	Bulgaria	36	36	1, 2, 3, 4, 5	1, 2	2, 4
10	USA	*B. corolliflora*		36	24	1, 2, 3, 4, 5	1, 2	2, 4
11	061245 8539	*B. macrorhiza*	Dagestan	18	9	3	1	2
12	061247 8541	*B. macrorhiza*	Dagestan	18	4	3	1	2
13	061248 8542	*B. macrorhiza*	Dagestan	18	14	3	1	2
14	061249 8543	*B. macrorhiza*	Dagestan	18	2	3	1	2
15	061250 8544	*B. macrorhiza*	Dagestan	18	3	3	1	2
16	061251 8545	*B. macrorhiza*	Dagestan	18	5	3	1	2
17	061252 8546	*B. macrorhiza*	Dagestan	18	21	3	1	2
18	061254 8548	*B. macrorhiza*	Dagestan	18	14	3	1	2
19	058253 7315	*B. macrorhiza*	Dagestan	18	2	3	1	2
20	058255 7317	*B. macrorhiza*	Dagestan	18	48	3	1	2
21	049830 3688	*B. macrorhiza*	Turkey	18	7	4, 5	2, 3	2
22	018256 2792	*B. macrorhiza*	Turkey	18	29	4, 5, 6	3	1, 2
23	018257 2793	*B. macrorhiza*	Turkey	18	1	4, 5	3	2
24	018259 2795	*B. macrorhiza*	Turkey	18	4	4, 5	3	1, 2
25	054215 6120	*B. macrorhiza*	Turkey	18	5	4	3	1, 2
26	018242 2322	*B. macrorhiza*	Turkey/Erzurum	18	2	4, 5	3	2
27	017829 2998	*B. lomatogona*	Turkey/Erzurum	18, 27	40	1	1, 2	2
28	017830 2999	*B. lomatogona*	Turkey/Erzurum	18	55	1	2, 3	1, 2, 4
29	017831 3000	*B. lomatogona*	Turkey/Cankiri	18	40	1	2	2
30	017832 3001	*B. lomatogona*	Turkey/Erzurum	18, 27	52	1, 3	1, 2, 3	2
31	054213 6121	*B. lomatogona*	Turkey	18	37	1	2, 3	2
32	017971 2945	*B. lomatogona*	Turkey/Eskisehir	18	7	1	2, 3	2
33	058251 7313	*B. lomatogona*	Armenia	–	19	1	1, 2	2
34	058258 8155	*B. lomatogona*	Iran/Ardabil	–	15	1	1, 2	2
35	035294 3201	*B. lomatogona*	Turkey	18, 27	4	1	–	–
36	035306 3213	*B. trigyna**		54	1	135	12	22
37	035308 3215	*B. trigyna**		54	6	135	–	22
38	035344 3250	*B. trigyna**	Turkey	54	3	135	12	22
39	035313 3220	*B. trigyna**	Turkey	54	34	135	–	22
40	035309 3216	*B. trigyna**		45	9	135	12	12
41	035343 3249	*B. trigyna**	Turkey	45	1	135	–	22
42	010084 3083	*B. trigyna**		45	23	135	12	12
43	018152 2369	*B. intermedia**	Turkey/Erzincan	36	17	14	12	12
44	017919 2483	*B. intermedia**	Turkey/Eskisehir	45	2	13	12	22
45	049849§3707	from *B. trigyna* to species mixture (?)	Turkey	36, 45	8	1, 3, 5		1, 2, 4

Table 1. Continued

No.	BGRC/IDBB No.	Species	Country of origin and district	Chromosome no. (2n)	No. of plants	Isoenzyme locus/ observed alleles		
						Lap1	*Prx1*	*Got3*
46	035304§3211	from *B. trigyna* to *B. intermedia* (?)*	Turkey	45	10	16	12	22
47	035311§3218	from *B. trigyna* to *B. corolliflora* (?)	Turkey	36	59	1, 2, 3, 4, 5	1, 2, 3	1, 2, 4
48	017811§2522	from *B. corolliflora* to *B. trigyna* (?)*	Turkey	45	24	135	12	12
49	049696§3620	from *B. lomatogona* and hybrids (?)	Turkey	18, 27, 36	45	1, 2, 3, 4, 5	1, 2	2
50	010081§3080	from *B. lomatogona* and hybrids (?)	Turkey	18, 36	18	1, 3, 5	1, 2	22

* For these species, the isozyme phenotypes are given.
§Discussed in text.

(copyright 1989 David L. Swofford, Illinois Natural History Survey) and Phylip 2.1 (copyright 1993 Joseph Felsenstein and the University of Washington) were utilized. The allele frequency in each locus was determined and served as the input data for both programs. For the tetraploid *B. corolliflora*, it was assumed that a plant possessed four gene doses and the banding intensity reflected allele dosage. Genetic variability measures used were the number of polymorphic loci and average heterozygosity. Genetic distance was based on Nei (1972). The phylogenetic trees were constructed with the UPGMA method (Sneath & Sokal, 1973) and Neighbor Joining (NJ) method (Saitou & Nei, 1987). The UPGMA method constructs rooted trees which means that the ancestor is identified.

Results

Isozyme screening of different Corollinae species and accessions

Table 2 and Fig. 1 show the isozyme loci and alleles found in the Corollinae species. A total of six alleles were observed at *Lap1*, four alleles at *Got3*, and three alleles at both *Prx1* and *Sod3*. *Lap1* and *Prx1* behaved as monomers while *Got3* and *Sod3* as dimers.

The two PGM loci were polymorphic. *Pgm1* consisted of three bands in heterozygous condition and two bands when homozygous. In *B. corolliflora* which produced a few seeds when selfed, the two bands of the homozygous phenotype did not segregate in the progeny; the middle band was always present. This locus has been interpreted as polymorphic for two alleles and a monomer. Five alleles were observed at *Pgm2* which also behaved as a monomer.

ICD showed in homozygous condition a three-banded phenotype. Three alleles were noted at *Icd1* and it is interpreted as a dimer. The products formed between two alleles could be seen in the combination *Icd1*-13. *Mdh1* is a dimer and two alleles were observed in the Corollinae species. *Mdh2* comigrated with *Mdh1*.

B. lomatogona

In this study, *B. lomatogona* refers to a diploid and a sexual species with chromosome number $2n = 2x = 18$. As shown in Table 2, the investigated accessions of *B. lomatogona* differed from the other basic species, *B. macrorhiza* and *B. corolliflora* by the type of alleles it contained. *B. lomatogona* exhibited only the alleles *Lap1*-1, *Pgm1*-1 and *Icd1*-3. It did not contain *Pgm2*-1. Although the presence of the three *Sod3* alleles was noted in *B. lomatogona*, *Sod3*-1 was seldom. It was only found in one accession and with a low frequency of 0.069. Most of the plants screened carried the *Sod3*-2 allele. *Got3*-3 was not observed in the accessions studied. *Got3*-1 and *Got3*-4 were found in just one accession with frequencies 0.076 and 0.015. Obviously, *Got3*-2 was prevalent in *B. lomatogona*. *Prx1*-1 was not present and the average frequency of *Prx1*-3 in three accessions was 0.03.

Table 2. Isozyme alleles observed in Corollinae species

Species	Chromosome number	Isozyme loci							
		Sod3	*Prx1*	*Lap1*	*Got3*	*Pgm1*	*Pgm2*	*Icd1*	*Mdh1*
B. macrorhiza	2n = 18	1, 2	1, 2, 3	3, 4, 5, 6	1, 2	2	1	1	1
B. corolliflora	2n = 4x = 36	1, 2, 3	1, 2, 3	1, 2, 3, 4, 5	1, 2, 3, 4	1, 2	1, 2, 3, 4, 5	1, 2	1, 2
B. lomatogona	2n = 18	1, 2, 3	2, 3	1	1, 2, 4	1	2, 3, 4, 5	3	1, 2
*B. intermedia**	2n = 4x, 5x = 36, 45	12	12	13, 14	22	12	13	13	12
*B. trigyna**	2n = 5x, 6x = 45, 54	12	12	135	12, 22	12	12, 13, 15	13	12
	Total no. of alleles	3	3	6	4	2	5	3	2

* For these species, the isozyme phenotypes are given.

B. corolliflora

B. corolliflora is an autotetraploid (Reamon-Büttner & Wricke, 1993) with chromosome number 2n = 4x = 36. Several morphotypes of these species were observed. *B. corolliflora* exhibited sexual reproduction with apomictic tendencies such as spontaneous dihaploidy. A dihaploid plant was found in the progeny of a *B. corolliflora* plant which produced a few seeds when selfed. It did not vary in allele content from the other progeny except that it appeared weak and had narrower lamina.

This species was found highly polymorphic. It had the most number of alleles compared to *B. lomatogona* and *B. macrorhiza*. For instance, *B. corolliflora* exhibited three alleles in *Sod3* and *Prx1*; four alleles in *Got3*; five alleles in *Lap1* and *Pgm2*.

B. corolliflora had more common alleles with *B. macrorhiza* than with *B. lomatogona*. This pertains especially to those alleles not found in *B. lomatogona*. These included *Prx1*-1, *Pgm1*-2 and *Icd1*-1. *Lap1*-1 which is a characteristic allele of *B. lomatogona* and not present in *B. macrorhiza* occurred at a low average frequency of 0.04 in four *B. corolliflora* accessions. In the accessions studied, *B. corolliflora* and *B. macrorhiza* shared at least three LAP alleles. *Got3*-3 and *Icd1*-2 were only observed in *B. corolliflora*.

B. macrorhiza

B. macrorhiza is diploid with chromosome number 2n = 2x = 18. It showed sexual reproduction and characteristically fleshy, oblong ovate leaves. As already mentioned, the investigated accessions of *B. macrorhiza* shared more alleles with *B. corolliflora* than with *B. lomatogona*. Compared to *B. corolliflora*, however, *B. macrorhiza* contained fewer alleles and was also less polymorphic (Table 2). For example, only one kind of an allele such as *Mdh1*-1, *Icd1*-1 and *Pgm1*-2 was present in *B. macrorhiza*. The alleles found at *Lap1*, *Pgm1*, *Pgm2* and *Icd1*, provided a distinct difference between *B. macrorhiza* and *B. lomatogona*. For instance, at *Pgm1* where there were two alleles namely: *Pgm1*-1 and -2, *B. macrorhiza* had the allele *Pgm1*-2 while *B. lomatogona*, *Pgm1*-1. Another example was at *Pgm2*, where there were five alleles; *B. macrorhiza* exhibited *Pgm2*-1 while *B. lomatogona*, *Pgm2*-2 to -5.

B. intermedia

Two *B. intermedia* accessions were studied; one was tetraploid and the other one was pentaploid. No variation in isozyme pattern within the accession was found. It seemed to be fixed. When plants from the tetraploid accession were either selfed or open-pollinated, no segregation of alleles was observed in the progeny. They all exhibited the maternal zymogram, indicating apomictic reproduction.

The two accessions showed the same allele components except at *Lap1* wherein the pentaploid accession had the phenotype *Lap1*-13 and the tetraploid, *Lap1*-14. If *B. intermedia* is a hybrid form, then this would somehow be reflected in the allele combinations it has. *B. intermedia* exhibited the phenotypes *Lap1*-13, -14, *Prx1*-12, *Pgm1*-12, *Pgm2*-13 and *Icd1*-13. Taking into consideration the alleles present in the basic species especially *B. lomatogona* and *B. corolliflora*, the allele combinations in *B. intermedia* would already suggest hybridity. But the occurrence of the phenotype *Icd1*-13 gave the strongest indication for the hybrid nature of *B. intermedia*. *Icd1*-13 never occurred within any of the basic species accessions. The allele *Icd1*-1 was only observed in *B. corolliflora* and *B. macrorhiza* while *Icd1*-3, in *B. lomatogona*.

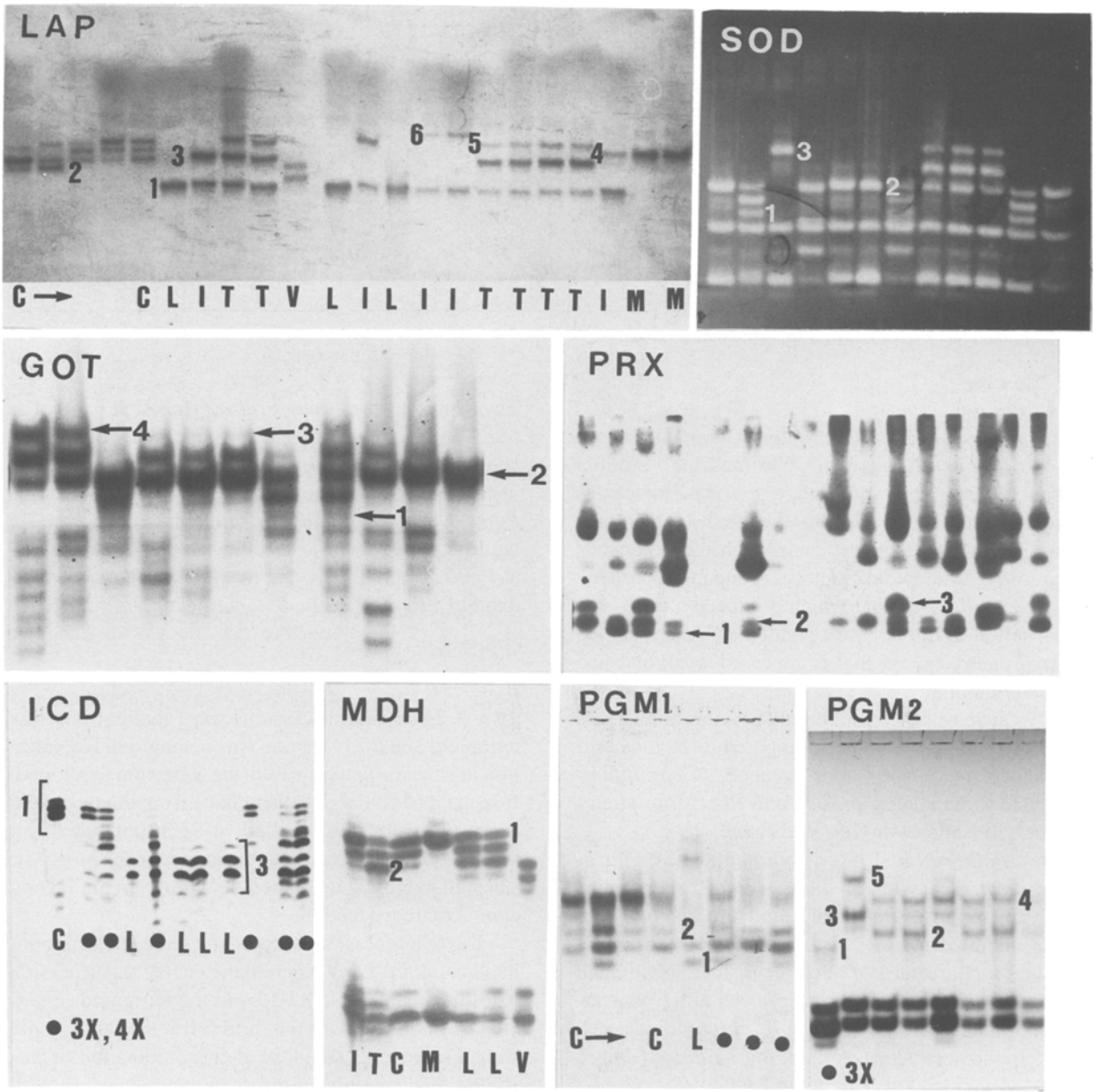

Fig. 1. The enzyme systems and alleles (indicated by numbers) observed in the Corollinae species. The SOD and PGM2 zymograms were from *B. lomatogona*, PRX and GOT from *B. corolliflora*, LAP, PGM1, ICD and MDH from different Corollinae species and *Beta vulgaris*: (C) *B. corolliflora*, (L) *B. lomatogona*, (M) *B. macrorhiza*, (I) *B. intermedia*, (T) *B. trigyna* and (V), *B. vulgaris*. At ICD, 3x and 4x were triploids and tetraploid plants from *B. lomatogona* populations.

B. trigyna

The *B. trigyna* accessions studied were either pentaploid or hexaploid. Plants belonging to both ploidy levels showed large leaves that were papery in texture. Similarly in *B. intermedia*, isozyme patterns in *B. trigyna* did not vary within the accession. Most interesting was that all the plants exhibited the phenotype *Lap1*-135.

B. trigyna is considered as a hybrid form probably from *B. lomatogona* and *B. corolliflora* (Zossimovitch, 1940). The alleles found in five isozyme loci namely: *Lap1*, *Pgm1*, *Pgm2*, *Prx1* and *Icd1* support hybridity. Like in *B. intermedia*, the most convincing combina-

tion could be seen at *Icd1*. *B. lomatogona* contained the *Icd1*-3 allele only, while *B. corolliflora*, *Icd1*-1 and -2. *Icd1-2* was seldom in the *B. corolliflora* accessions screened. Therefore, *B. lomatogona* could have been the likely donor of *Icd1*-3 in *B. trigyna* while *B. corolliflora* contributed *Icd1*-1.

Other Corollinae accessions

Some accessions of Corollinae species screened for isozyme analysis could be other forms of hybrids or inadvertently misclassified. For example, BGRC 035311 was classified as *B. trigyna*. The 59 plants studied had chromosome number $2n = 4x = 36$ and exhibited polymorphism in at least five isozyme loci. Based on plant morphology and isozyme alleles found, this accession could be more appropriately classified as *B. corolliflora*.

Another accession BGRC 017811 that was classified as *B. corolliflora* resembled *B. trigyna* more than *B. corolliflora*. No variation in isozyme pattern was found. The plants exhibited a characteristic *B. trigyna* isozyme phenotype *Lap1*-135 and with chromosome number $2n = 5x = 45$. This accession could likewise be classified as *B. trigyna*.

BGRC 035304 was classified as *B. trigyna*. The plants were pentaploid and similar to *B. trigyna* in gross plant morphology. They also contained similar alleles found in *B. trigyna* except that instead of the *Lap1*-135 phenotype, they had the phenotype *Lap1*-16. The allele *Lap1*-6 was only observed in *B. macrorhiza* in the present study. Considering, however, the many similarities in *B. macrorhiza* and *B. corolliflora*, *Lap1*-6 could also be present in *B. corolliflora*. If *B. trigyna* would only comprise plants with the phenotype *Lap1*-135, then this accession would appropriately fall under *B. intermedia* which could also be a hybrid form of *B. lomatogona* and *B. corolliflora*. A significant difference between *B. trigyna* and *B. intermedia* could be that the former possessed the phenotype *Lap1*-135 while *B. intermedia*, *Lap1*-13 and -14. Taking into account the other LAP alleles in *B. corolliflora*, allele combinations such as *Lap1*-12 and -15 could not be discounted.

BGRC 049849 was classified as *B. trigyna*. Five plants were tetraploid and three plants were pentaploid. The plant morphology of the pentaploids was in the direction of *B. trigyna*/*B. intermedia*. Isozyme analysis showed that the three pentaploids, on one hand, had the *Lap1*-13, which was the typical isozyme phenotype for *B. intermedia*. The tetraploids, on the other hand, showed polymorphism involving the alleles *Lap1*-3, -4 and -5 and exhibited plant morphology like *B. corolliflora*. This accession, therefore, could not be considered as *B. trigyna* but a mixture of species. It was demonstrated in this accession that isozyme alleles could be used to identify a certain Corollinae species.

Two accessions, namely, BGRC 049696 and 010081 were classified under *B. lomatogona*. Chromosome counts gave a mixture of diploids, triploids and tetraploids. The diploids all looked like *B. lomatogona* in plant morphology and also showed the alleles expected of a diploid *B. lomatogona*. These, however, differed from the polyploids in their allele composition. The polyploids had the following allele combinations: *Lap1*-13, -14, -15 and -135 as well as gross plant morphology observed in *B. intermedia* and *B. trigyna*. In addition to the LAP alleles, they exhibited *Prx1*-1, *Pgm1*-2, *Pgm2*-1 and *Icd1*-1, alleles that were typical for *B. corolliflora*. It could be observed in these two accessions that only the diploids fit in as a basic species while the polyploids as hybrids fall into the category of *B. intermedia* or *B. trigyna*.

Genetic diversity and phylogenetic relationship in the basic species B. corolliflora, B. macrorhiza and B. lomatogona

The accessions which were studied for genetic diversity are shown in Table 3. The hybrid species *B. intermedia* and *B. trigyna* were not included since due to apomictic breeding systems, no polymorphism was found.

The *B. corolliflora* accessions differed in the type and frequency of alleles they contain. At *Prx1*, the alleles *Prx1*-1 and -2 were most frequent but *Prx1*-2 was not observed in the accession BGRC 018246 (C1). *Prx1*-3 was frequent in C2 (Erzurum), C3 (Erzurum) and C5 (Kars). While *Got3*-2 with frequency ranging from 0.53 to 0.86 was the most common allele in the *B. corolliflora* accessions, *Got3*-3 was rare with a maximum frequency of 0.09. *Got3*-3 was present in the Erzurum and Kars accessions. *Lap1*-1 was not usually found in *B. corolliflora*. *Lap1*-2 appeared most in C4 (Kars), *Lap1*-4 in C8, *Lap1*-5 in C2 (Erzurum) and C3 (Erzurum). *Lap1*-3 was frequent in all accessions. *Sod3*-1 was most frequent while *Sod3*-3 with a maximum frequency of 0.13 was seldom. *Sod3*-3 was detected in C2 (Erzurum), C3 (Erzurum) and C4 (Kars).

Table 3. The accessions of *B. lomatogona*, *B. macrorhiza* and *B. corolliflora* included in the study of genetic diversity

Population	BGRC No.	Species	Origin	Location	Longitude	Latitude	Sea level
C1	BGRC 018246	*B. corolliflora*	Turkey				
C2	BGRC 018254	*B. corolliflora*	Turkey/ Erzurum	Domlu/Tortum, 3 km N Karayollari Guze	04120E	04000N	2170
C3	BGRC 018255	*B. corolliflora*	Turkey/ Erzurum	Domlu/Tortum, 3 km N Karayollari Guze	04120E	04000N	2170
C4	BGRC 017812	*B. corolliflora*	Turkey/Kars	Kars/Arpacay, 5 km S Melik Koyu	04306E	04040N	1700
C5	BGRC 017822	*B. corolliflora*	Turkey/Kars	Ladikars/Kotek, 1 km S Pasli Koyu	04300E	0400N	1850
C6	Bulgaria	*B. corolliflora*	Bulgaria				
C7	USA	*B. corolliflora*	–				
C8	BGRC 035311	*B. corolliflora**	Turkey				
M1	BGRC 018256	*B. macrorhiza*	Turkey				
M2	BGRC 061282	*B. macrorhiza*	Dagestan				
M3	BGRC 058255	*B. macrorhiza*	Dagestan				
L1	BGRC 017830	*B. lomatogona*	Turkey/ Erzurum	Karaurgan/ Horasan, 7 km S Yukari/Asagi	04200E	0400N	1990
L2	BGRC 017829	*B. lomatogona*	Turkey/ Erzurum	Horasan/ Pasinler, 9 km	04200E	0400N	1220
L3	BGRC 017831	*B. lomatogona*	Turkey/ Cankiri	Sabanozu/ Cankiri, 2 km W Kreuz. Sedi.	03320E	04020N	930
L4	BGRC 017832	*B. lomatogona*	Turkey/ Erzurum	Horasan/ Pasinler, 9 km W	04200 E	04000N	1600
L5	BGRC 054213	*B. lomatogona*					

* Received under the name *B. trigyna*.

There was not much difference among the *B. lomatogona* accessions except at *Sod3*. *Sod3*-3 was found in *B. lomatogona* collected in Erzurum but was absent in L3 (Cankiri). *Got3*-1 and *Got3*-4 were only observed in L1 (Erzurum) but with low frequencies of 0.076 and 0.015, respectively. *Prx1*-3 was also rare in *B. lomatogona*. The most frequent alleles in all accessions were *Sod3*-2 (0.50–1.0), *Got3*-2 (0.91–1.0) and *Prx1*-2 (0.93–1.0). *Pgm1*-1 and *Lap1*-1 were characteristic of *B. lomatogona*.

The Turkey and Dagestan accessions of *B. macrorhiza* differed from each other. Although only three accessions were included for the analysis of genetic diversity, a total of 16 accessions were screened. The *B. macrorhiza* (Dagestan) accessions were characterized by the alleles *Prx1*-1 and *Lap1*-3, while *B. macrorhiza* (Turkey) contained *Prx1*-2 and -3 and *Lap1*-4, -5 and -6.

Tables 4 and 5 show the genetic variability in *B. corolliflora*, *B. lomatogona* and *B. macrorhiza*. The *B. macrorhiza* accessions and one *B. corolliflora* accession could not be included for the genetic variability at *Sod3* and *Pgm1* in Table 4 since not enough data were obtained. Based on % polymorphic loci, *B. corolliflora* was highly polymorphic compared to *B. macrorhiza* or *B. lomatogona*. *B. corolliflora* showed also the high-

Table 4. Genetic variability in five isozyme loci* in *B. corolliflora* and *B. lomatogona* populations

Population	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity	
				Observed	Expected** (Hardy-Weinberg)
C2	72.6	3.0	100.0	0.725	0.543
C3	61.2	3.0	100.0	0.779	0.538
C4	24.8	3.4	100.0	0.574	0.452
C5	48.8	3.0	100.0	0.683	0.401
C6	24.4	2.6	100.0	0.797	0.471
C7	21.2	2.6	100.0	0.746	0.466
C8	39.2	3.0	100.0	0.805	0.520
L1	44.2	1.8	40.0	0.077	0.096
L2	23.8	1.2	20.0	0.200	0.102
L3	30.0	1.0	0.0	0.000	0.000
L4	31.0	1.6	40.0	0.113	0.123
L5	37.0	1.4	0.0	0.016	0.016

* *Prx1*, *Got3*, *Lap1*, *Sod3* and *Pgm1*.
** Nei (1978).

Table 5. Genetic variability in three isozyme loci* in *B. corolliflora*, *B. macrorhiza* and *B. lomatogona* populations

Population	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity	
				Observed	Expected** (Hardy-Weinberg)
C1	24.0	2.7	100.0	0.485	0.367
C2	91.3	3.3	100.0	0.688	0.565
C3	72.0	4.0	100.0	0.744	0.531
C4	27.3	4.0	100.0	0.547	0.546
C5	49.3	3.7	100.0	0.788	0.447
C6	24.7	3.0	100.0	0.870	0.577
C7	20.3	3.0	100.0	0.844	0.566
C8	48.0	3.7	100.0	0.838	0.587
M1	28.7	2.0	66.7	0.213	0.172
M2	21.0	1.0	0.0	0.000	0.000
M3	48.0	1.0	0.0	0.000	0.000
L1	43.7	2.0	33.3	0.040	0.070
L2	25.0	1.0	0.0	0.000	0.000
L3	30.0	1.0	0.0	0.000	0.000
L4	28.3	1.3	33.3	0.016	0.047
L5	37.0	1.3	0.0	0.009	0.009

* *Prx1*, *Got3* and *Lap1*.
** Nei (1978).

est mean number of alleles per locus. In terms of mean heterozygosity, *B. corolliflora* likewise exhibited higher values than the two other species. For *Prx1*, *Got3* and *Lap1*, *B. corolliflora* had an average of 0.73, *B. lomatogona*, 0.01 and *B. macrorhiza*, 0.07.

The genotype frequencies in *Sod3* in three geographically closely located populations of *B. corolliflora* are exhibited in Table 6. Since the genotype frequencies in the three populations were very similar, the values were summed up and placed under the

Table 5. Genotypic frequency at *Sod3* in three *B. corolliflora* populations at Hardy-Weinberg equilibrium and chromosome segregation

Genotypes	Population			Observed		Expected		$(E-O)^2/E$
	C2 (Erzurum)	C3 (Erzurum)	C5 (Kars)	(O)		(E)		
Sod-3-								
1111	7	3	4	14		16.70		
2222				0	14	0.92	17.72	0.74
3333				0		0		0
1112	17	16	7	40		32.30		1.83
1122	10	9	3	22		23.45		0.08
1222	2	4		6		7.57		0.32
1113	2	3	7	12		8.61		
1133				0	13	1.67	10.42	0.63
1333			1	1		0.14		
2223		1		1		0.97		
2233				0	1	0.40	1.44	0.13
2333				0		0.07		
1123				0		12.52		
1223	7	4	1	12	17	6.05	20.20	0.50
1233	1	3	1	5		1.63		
Total	46	43	24	113		113		$\chi^2 = 4.23$ ns

column "observed". These summed values were then used for the estimation of the expected values under Hardy-Weinberg conditions. The expected frequencies after chromosome segregation did not vary from that of the observed values.

Figure 2 shows the constructed phylogenetic tree based on the UPGMA method. There are four distinct groups: (1) *B. macrorhiza* (M1, Turkey), (2) *B. lomatogona* group, (3) *B. corolliflora* group, and (4) *B. macrorhiza* (M2 and M3, Dagestan) and *B. corolliflora* (C1). The plants in C1 looked intermediate between *B. corolliflora* and *B. macrorhiza*. This tree shows that *B. corolliflora* is more related to *B. macrorhiza* than to *B. lomatogona*. *B. corolliflora* and *B. macrorhiza* are also the end members of the divergence line. There is also a distinct difference between the Dagestan and Turkey *B. macrorhiza* accessions. Similar results were found in the tree constructed from the Neighbor-Joining method (Fig. 3).

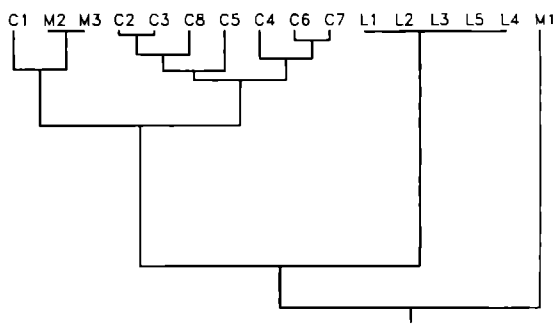

Fig. 2. The tree constructed after the Neighbor-Joining Method and genetic distance of Nei (1972): (C) *B. corolliflora*, (M) *B. macrorhiza*, and (L) *B. lomatogona*.

RAPD analysis

In the screening of Corollinae species, the primers A-05, A-18 and A-19 were used. After amplification with primer A-05 three fragments were scored (Fig. 4a). The Corollinae species exhibited Fragment 1 but *B. lomatogona* was polymorphic. Fragment 2 seemed to be species specific. It was found in *B. lomatogona* although polymorphic but not in *B. macrorhiza* and *B. corolliflora*. This fragment was also found in *B. intermedia* and *B. trigyna*. Fragment 3 was observed only in *B. corolliflora*. A total of 10 fragments were scored for primer A-18 (Fig. 4b). These fragments were all found in *B. corolliflora*. Fragment 6 was detected in *B. corolliflora* and *B. macrorhiza* but not in *B. lomatogona*. It

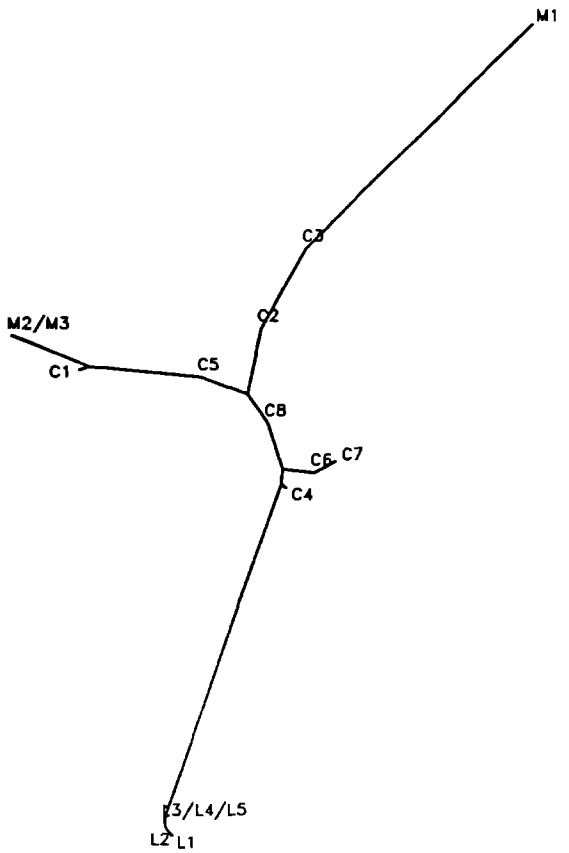

Fig. 3. The tree constructed after the Neighbor-Joining Method and genetic distance of Nei (1972): (C) *B. corolliflora*, (M) *B. macrorhiza*, and (L) *B. lomatogona*.

was also present in *B. intermedia* and *B. trigyna*. In the material investigated with primer A-19, one fragment could be used to separate the species. This fragment (Fig. 4c) was found in *B. corolliflora* and *B. macrorhiza* but not in *B. lomatogona*. In *B. trigyna* and *B. intermedia*, it was detected in some plants. Based on the three RAPD primers, it was observed that *B. corolliflora* is more related to *B. macrorhiza* than to *B. lomatogona*. The hybridity in *B. intermedia* and *B. trigyna* could not be clearly determined. However, RAPD results do not contradict the assumptions that hybrid species probably stem from *B. corolliflora* and *B. lomatogona*.

Discussion

Isozyme analysis showed that the Corollinae species could be classified into three basic species: *B. lomatogona* (2x), *B. macrorhiza* (2x) and *B. corolliflora* (4x), and that *B. intermedia* and *B. trigyna* are hybrids. These results support the taxonomy of section Corollinae advanced by Buttler (1977) who introduced the concept of three basic species in Corollinae namely: *B. lomatogona*, *B. macrorhiza* and *B. corolliflora*. This concept was also supported by Özgör (1992). If the three basic species represent a continuous variation of isozyme alleles, *B. lomatogona* occupies one end and *B. macrorhiza*, the other end. *B. corolliflora* is in between but much more related to *B. macrorhiza* than to *B. lomatogona*.

Hybridity in *B. trigyna* and *B. intermedia* was reflected in their allele composition found in different isozyme loci. *B. trigyna* could be a hybrid form of *B. lomatogona* and *B. corolliflora* as earlier proposed by Zossimovitch (1940). Based on the alleles found, *B. intermedia* could be more likely also a hybrid form of *B. corolliflora* and *B. lomatogona* and not *B. trigyna* and *B. lomatogona* as described by Scheibe (1934). Results also showed that there is confusion in the classification of Corollinae species. Buttler (1977) already traced the case of *B. trigyna* and *B. corolliflora*. According to this author, *B. corolliflora* was once considered as identical to *B. trigyna* until the separation of these two species by Zossimovitch (1940). Other reasons given by Buttler were the underevaluation of geographical distribution as well as reproduction systems as relevant characters in systematic classification.

B. corolliflora was more polymorphic and heterozygous than *B. macrorhiza* and *B. lomatogona*. The remarkably high level of heterozygosity in *B. corolliflora* is in accordance with tetrasomic inheritance which was observed in this species (Reamon-Büttner & Wricke, 1993) and confirming further its autotetraploid origin. Similarly in naturally occurring autotetraploids belonging to the family Saxifragaceae, it was observed that they showed a much increased level of heterozygosity as compared to the diploid cytotypes (Soltis & Riesenberg, 1986, Wolf et al., 1987). The genotypic frequencies in *Sod3* found likewise in three geographic closely related wild populations of *B. corolliflora* support further the autotetraploid origin of *B. corolliflora*.

The close relationship between *B. corolliflora* and *B. macrorhiza* was again clearly depicted in the phylogenetic trees. This finding is in agreement with that of Buttler (1977) who found that *B. corolliflora* and *B. macrorhiza* correspond in many features. These include flower structure; special form and position of the tepals; similar variability; orientation of the tepals in the fruit and in the degree of polycarpy; and similar

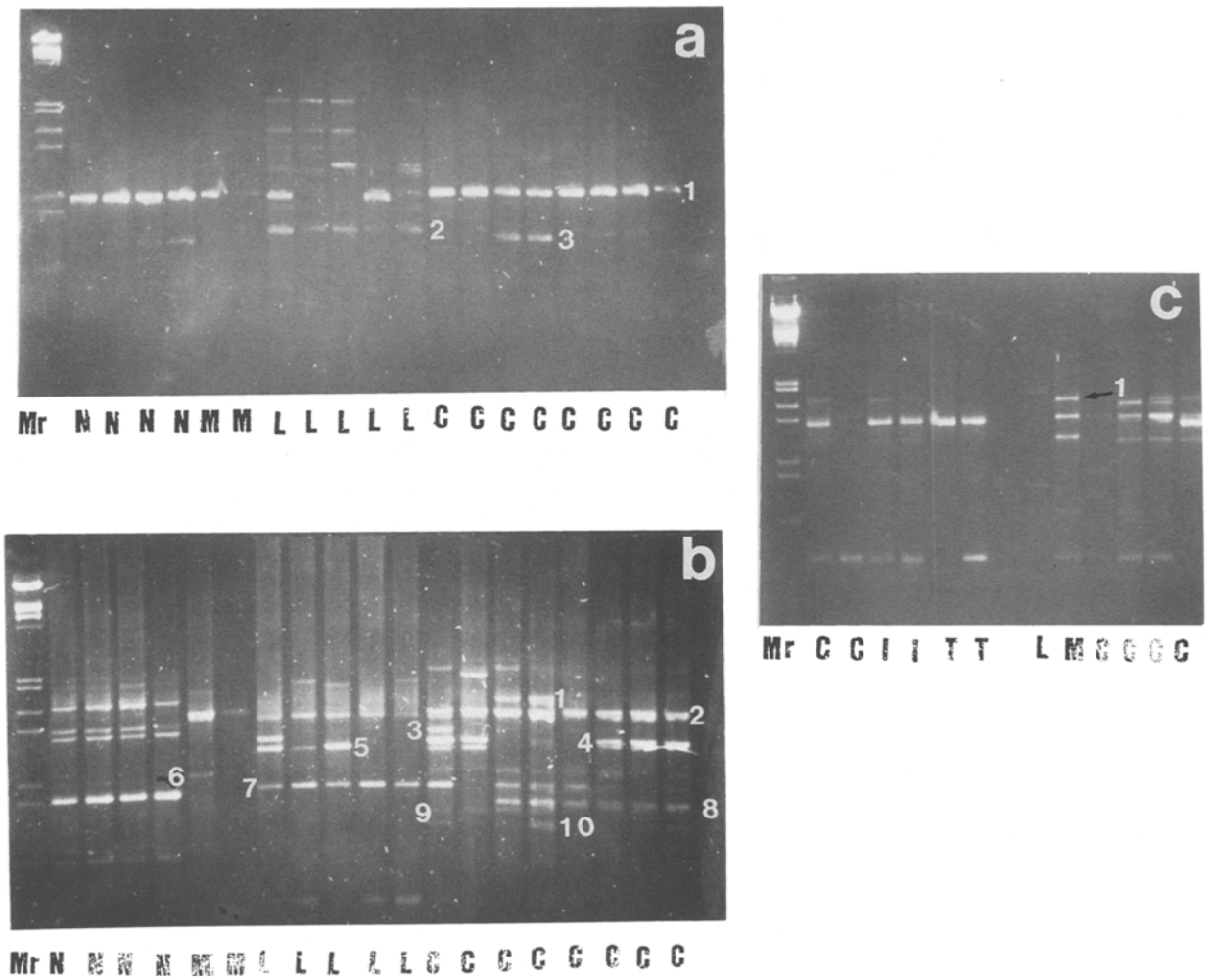

Fig. 4. Amplification with primers A-05 (a) and A-18 (b) and A-19 (c) in different accessions of *B. corolliflora* (C), *B. macrorhiza* (M), *B. lomatogona* (L) and *B. nana* (N). (Mr) size marker.

distribution area with corresponding areal diagnosis. With these criteria, *B. lomatogona* deviates from *B. corolliflora*. Similar relationship was found among *B. corolliflora*, *B. macrorhiza* and *B. lomatogona* based on DNA fingerprinting (Jung et al., 1993) and rDNA markers (Santoni & Berville, 1992).

Buttler (1977) claimed that *B. macrorhiza* originating from the Caucasus and the *B. macrorhiza* (*B. foliosa*) originating in Turkey belong to one species, although a very variable one. Due to lack of authentic material from the Caucasus, he could not confirm the possibility of a sub-species separation of the two areal populations. The data on at least two isozyme loci *Prx1* and *Lap1* from authentic *B. macrorhiza* accessions from the Caucasus confirm the existence of two different gene pools in these species. These two *B. macrorhiza* gene pools were also depicted in the phylogenetic trees.

In the UPGMA tree, *B. corolliflora* and *B. macrorhiza* (Dagestan) are at the end of the evolutionary line. This is also in agreement with earlier conclusion of Buttler (1975; 1977) that *B. corolliflora* and *B. macrorhiza* are the end members of the evolutionary line in section Corollinae. Buttler (1997) considering the differences between *B. corolliflora* and *B. macrorhiza* proposed that evolution would be possible in both directions except that of chromosome number which is from diploid to tetraploid. This implies that *B. corolliflora* is more recent than *B. macrorhiza*.

B. macrorhiza (Turkey) appears to be the most ancestral accession in the UPGMA tree. According to Buttler (1977) the progenitor of section Corollinae has probably the morphology within the range of the recent species *B. corolliflora* and *B. macrorhiza*. The region of East Anatolia and Armenia is the assumed evolutionary centre of section Corollinae, where the distribution areas of all three basic species overlap. A more detailed study of the East Anatolia genepool of *B. macrorhiza* to which M1, BGRC 018256 belongs, can perhaps provide valuable information on the evolution of section Corollinae.

If *B. corolliflora* is an autotetraploid, what could have been its progenitor? No reports of diploid *B. corolliflora* populations have been found until now. One *B. corolliflora* accession (C1, BGRC 018246, Turkey) clustered with the *B. macrorhiza* accessions collected in Dagestan. The plants in this accession looked intermediate between *B. corolliflora* and *B. macrorhiza*. In view of this, a question is posed as to whether *B. macrorhiza* is the ancestor of *B. corolliflora*. Considering the alleles observed in different *B. corolliflora* accessions, they are generally found in *B. macrorhiza*.

The tendency to develop unreduced male gametes has been observed in *B. macrorhiza* (Jassem et al., 1985) while that of unreduced female gametes has not yet been reported. Perhaps the combination of two unreduced gametes could be a possible mechanism for the development of an autoteraploid from *B. macrorhiza*. Asker (1979) reported on such a mechanism in the Bothriochloa-Dicanthium Complex (Panicoideae).

From *B. macrorhiza* accessions from Turkey, Buttler (1977) found two triploid plants which could have been resulted from the combination of unreduced and reduced gametes. The triploid plants developed weakly and did not survive. Assuming that *B. macrorhiza* is the ancestor of *B. corolliflora*, a possible mechanism would also be the fertilization of a triploid plant (the 2x gamete) with unreduced diploid pollen. A spontaneous dihaploid *B. corolliflora* has been observed also in the present study and in another one (Jassem, 1990). Perhaps this phenomenon might also have played a role in the evolution of the *B. corolliflora*/*B. macrorhiza*. Many wild populations of these two species have been discovered but have not yet been analyzed.

The results obtained with RAPD analysis of Corollinae support further the relationship that *B. corolliflora* has more similarities with *B. macrorhiza* than with *B. lomatogona*. However, the RAPD results with *B. macrorhiza* are preliminary until more plants are screened. From the isozyme data, it could be observed that *B. trigyna* and *B. intermedia* are hybrid forms, most likely from *B. corolliflora* and *B. lomatogona*. With the primers used, hybridity could not be determined with certainty although the results do not contradict the findings on isozymes. Actually, the RAPD markers behaved as expected if the genomes of *B. corolliflora* and *B. lomatogona* were combined.

Based on floral morphology Barocka (1966) proposed a subdivision of the Corollinae section into Trigynae and Lomatogonae. This subdivision was considered by Buttler (1977) as not justifiable arguing among others that difference in the two flower types is not enough to create sub-sections since even with this criterion at the species level *B. lomatogona* separates from *B. macrorhiza*/*B. corolliflora*. Two other studies, however, using molecular techniques support Barocka's proposal. Fritzsche et al. (1987) found differences in the restriction patterns of chloroplast DNA between *B. trigyna* and *B. lomatogona*, which they used to support the subdivisions Trigynae and Lomatogonae. Unfortunately, other Corollinae species were not included in their study. With RAPD markers, different conclusions could be made if only *B. trigyna* and *B. lomatogona* would be compared and also without taking into consideration the data on isozyme analysis. Santoni & Berville (1992) using nuclear rDNA markers found that the BamHI site was absent in *B. lomatogona* and *B. intermedia* while it was present in *B. trigyna*, *B. corolliflora* and *B. macrorhiza*. With this observation, which was based on a single plant per species or seed material, they also proposed the subdivisions Lomatogonae and Trigynae. In the RAPD analysis, no such subdivisions have been observed.

As observed in the study of Corollinae species, it would be wise to consider at least the plant morphology and chromosome number of plant material if used for further investigation. Isozyme screening would be a helpful method in differentiating the species and in determining genetic diversity for genetic resources conservation of wild beets. A better approach to taxonomy in this section, however, would be multidisciplinary where morphological, biochemical and molecular data are taken into consideration.

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