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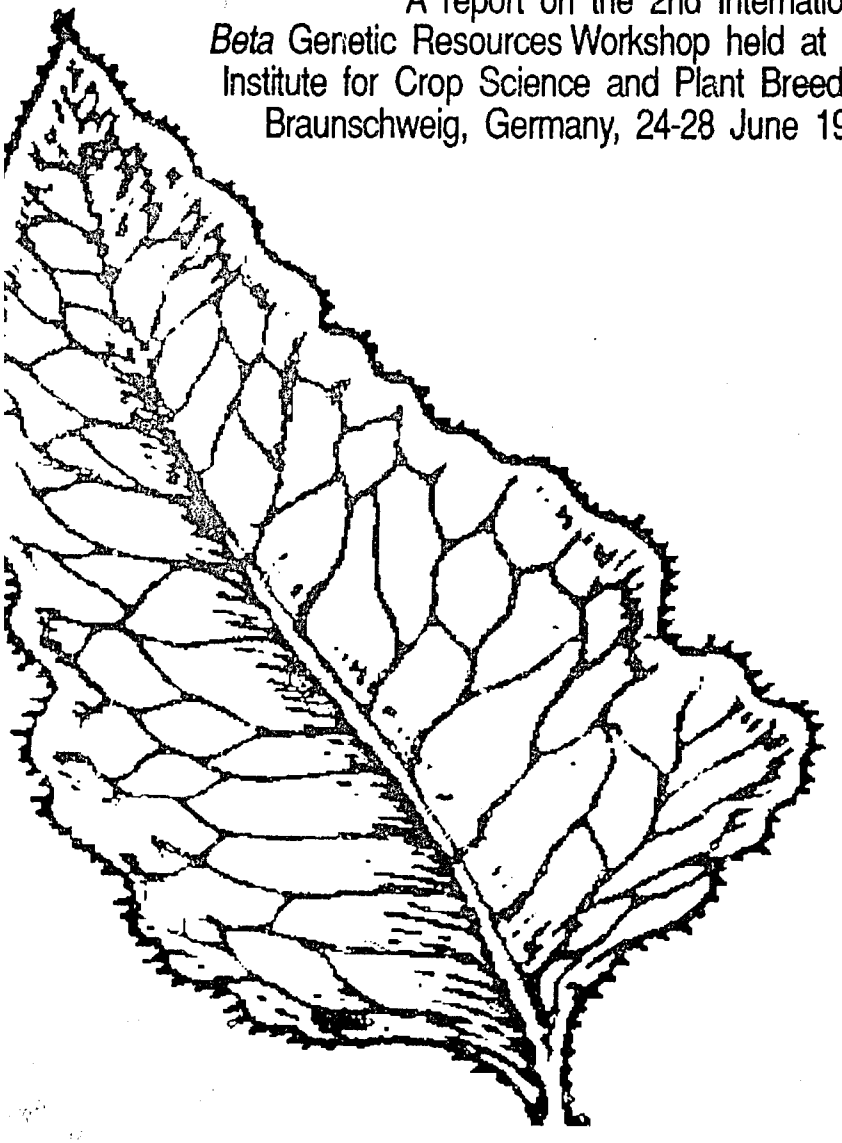
INTERNATIONAL CROP NETWORK SERIES. 7

International *Beta* genetic resources network

A report on the 2nd International *Beta* Genetic Resources Workshop held at the Institute for Crop Science and Plant Breeding Braunschweig, Germany, 24-28 June 1991



IBPGR

INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES

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IBPGR
ROME 1992

The International Board for Plant Genetic Resources (IBPGR) is an autonomous international scientific organization under the aegis of the Consultative Group on International Agricultural Research (CGIAR). IBPGR was established by the CGIAR in 1974. The basic function of IBPGR is to promote and coordinate an international network of genetic resources centres to foster the collecting, conservation, documentation, evaluation and use of plant germplasm and thereby contribute to raising the standard of living and welfare of people throughout the world. Financial support for the core programme is provided by the Governments of Australia, Austria, Belgium, Canada, China, Denmark, France, Germany, India, Italy, Japan, the Netherlands, Norway, Spain, Sweden, Switzerland, the UK and the USA, the United Nations Development Programme and the World Bank

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Working session

Business report of the *Beta* Coordinating Committee

Activity report

The *Beta* Coordinating Committee (BCC) meetings have lead to the following activities:

Participants who had not sent their passport and seed stock data to the IDBB have been asked to provide this information.

Dr. Ponz-Ascaso of the Madrid (Spain) genebank, was contacted to ascertain whether any *Beta* collecting mission is planned in Spain. Information received from various Spanish colleagues indicates that there is no national *Beta* genetic resources project. All the material collected, as yet, originates from multi-crop expeditions. With the exception of the collection maintained by Prof. Nuez, it is difficult to assess where newly collected material ends up and whether such material is properly maintained.

It seems that interesting inland types of wild beets and landraces still exist in Spain. In addition, no wild beets have been collected along the sea shore of Galicia (NW Spain). Since vast parts of the Iberian peninsula have never been systematically explored, additional collecting trips could render valuable *Beta vulgaris* germplasm.

Wild beet populations and landraces also occur in Bulgaria and Romania. Dr. Lozanov (Sadovo, Bulgaria) is interested in implementing a joint *Beta* collecting mission. Dr. Stanescu (Bucharest) has already started to make a survey of the distribution of wild beets and landraces in Romania. No attempt has been made to establish contacts with colleagues in Yugoslavia and Albania.

Mr. M. Nasser Arjmand (Karaj, Iran) provided evidence for the existence of *B. lomatogona* and *B. vulgaris* ssp. *maritima* in Iran. It would also be interesting to explore adjacent areas in Armenia, Azerbaidjan, Syria and Iraq but, considering the political situation in this region, such plans for joint plant exploration are unlikely to materialize in the short term.

Additional information on the distribution of *Beta* germplasm and plans for *Beta* collecting missions in China, India, Pakistan, Afghanistan, and Egypt were reported during the presentation of regional genetic resources activities.

Some cooperation between genebanks was achieved in the field of germplasm rejuvenation e.g.(request-support): USA-Turkey, Greece-East Germany, the Netherlands-Turkey, the Netherlands-Iran.

Some information on seed dormancy has been collated but research on this subject is still pending. Because of reorganization activities, the University of Hohenheim (FRG) could not assume such research work.

A meeting with commercial plant breeders at Braunschweig (December 1989) and a first discussion on a pre-breeding project were organized. A short description of a project proposal was submitted to the Commission of the European Countries (CEC) in March 1990 to check whether research on genetic resources of sugar beets would fit into the objectives of the CAMAR programme. The full project description was submitted in

October 1990. This was rejected by the CEC in March 1991 but was resubmitted in April 1991, on the advice of the Dutch SCAR member.

The IBPGR booklet "Descriptors for *Beta*" was published in 1991.

The objectives of the second world *Beta* network meeting were discussed in October 1990 and the agenda approved. Organization of the meeting started in November 1990.

An application for financial support of network participants from EC-member states was submitted to the CEC, in February 1991. The request was rejected in March 1991.

Several breeding companies were asked to provide financial support for the second meeting. Their response has been very positive.

Financial account

The CGN, in 1988, received 58,529 HFL from IBPGR. This was 90% of a total budget of 33,500 US$ allocated for funding the *Beta* workshop held in February, 1989 at Wageningen. In total, 53,833 HFL were spent as itemized below:

Prepaid flight tickets:	37947.00 HFL
International Agricultural Centre:	199.15 HFL
Daily allowances and train tickets:	3852.00 HFL
Workshop dinner:	1891.50 HFL
CGN secretariat:	1265.00 HFL
Reception DLO:	- 320.75 HFL
	53833.90 HFL
Balance	+ 4695.35 HFL

In August 1989, the CGN received, from IBPGR, the remaining 10% of the workshop budget (=7328,00 HFL) for funding the first and second *Beta* Coordinating Committee meetings. Together with the rest of the budget of the 1989 workshop, this added up to 12029.35 HFL. In total, 11032.54 HFL were spent for both BCC meetings, the first at Wageningen (June 1989) and the second at the Plant Genetic Resources Research Institute at Menemen, Izmir (October 1990). The balance on 17-04-1991 was 996.81 HFL.

Progress report of the International Database for *Beta* (IDBB)

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Introduction

Since the seventies, wild *Beta* species, primitive types, landraces, advanced cultivars and breeding lines have systematically been collected by various national and international organizations with the primary aim of safeguarding the genetic resources of sugar beet breeding. Currently, the world *Beta* genetic resources seed stock consists of 8306 accessions. Regular seed regeneration, long-term cold storage and routine monitoring of the seed quality should guarantee that this germplasm remains available for future generations of researchers, breeders and farmers, even if wild populations are destroyed or obsolete cultivars are abandoned by breeders. However, the funds required for the implementation of crop genetic resources programmes are often limited and generally do not allow the work to be performed adequately.

It is now widely recognized that the available financial means could be used more effectively if it were possible to rationalize genetic resources activities at international and national levels. During the early years of genetic resources programmes much emphasis was given to rescuing endangered germplasm and too little attention was paid to coordination of activities. Lack of sufficient information exchange between genebanks and research units worldwide has resulted in duplication of activities and in unnecessary duplication of germplasm. In some crops, duplicates account for more than 40% of total stock of accessions. Through task sharing between genebanks and the rationalization of existing collections, genetic resources activities could be streamlined and use of funds be focussed on essential work. However, cooperation at international level requires a management structure. IBPGR, therefore, in 1989, launched the concept of crop networks (crop specific working groups) to stimulate joint efforts in the field of conservation and utilization. One of the first of such working groups, the *Beta* network, was established during a workshop held at Wageningen, in February 1989. The participants agreed to collaborate in collecting, documenting, conserving, regenerating and evaluating germplasm. The establishment of the International Database for *Beta* (IDBB) was one of the first joint actions of the working group.

A central database which collates, analyses and disseminates information is an essential requirement for an operational and viable network. During the meeting participants agreed upon a number of collaborative activities to improve the potential of the IDBB as a central management instrument. It was decided:

- to complete the inventory of passport data of all collections;
- to send seed stock data to the IDBB;
- to send plans for the next two years' national programmes for regeneration of accessions ;
- to list accessions needing urgent regeneration, and subsequently to prepare collaborative action; and
- to analyze the information of the IDBB for further selective germplasm collection.

The results of the various initiatives taken by the IDBB to match these aims are described below.

Inventory of collections

Between 1989 and April 1991 the IDBB received passport data information on 1431 new accessions (Fig.1). A complete survey of the two most important IDBB tables is presented in Appendix 1. Large datasets were provided by China, the USA, the USSR and Turkey. The dataset provided by the Italian genebank at Bari will be entered into the system soon. To date, the IDBB has compiled passport information on 8306 accessions. A comparison of the quantity of data stored in 1989 and 1991 showed that almost no progress was achieved in seed stock data exchange. The percentage of seed data still remains at a level of about 38% of the total number accessions for which passport data has been compiled (Appendix 1).

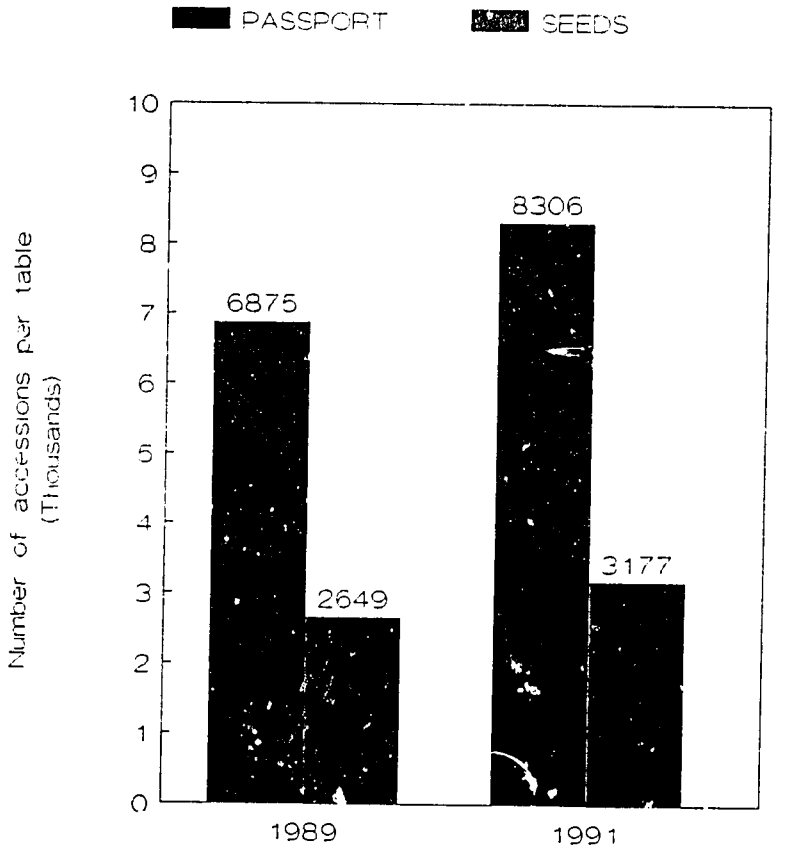

Figure 1: Comparison of table content in 1989 and 1991 (IDBB)

Compilation of the inventories of the Indian and Bulgarian *Beta* collections, as well as the working collection held at the Institute of Sugar Beet Research at Klein Wanzleben (East Germany), is in progress. The Japanese genebank was asked to

provide the *Beta* passport data of their recent plant exploration in Morocco. They have promised to do so.

Updating the information

Each time the IDBB received new datasets a search for duplicate accessions was performed. In general, probable duplicates (PRD) and samples not within genebank responsibility (NOG) were identified based on the variety name or on a combination of variety name and ploidy level (for NOG only). Varieties which were frequently exchanged and used by plant breeders of various countries are: red beet varieties named "Egyptian", "Crosby", "Detroit"; fodder beets named "Barres", "Eckendorfer", and sugar beet varieties from the breeding companies KWS, Hilleshög and Maribo. Open pollinated varieties belonging to such a group were classified as probable duplicates (PRD) since they probably stem from one base population. The grouping of accessions as most original sample (MOS) and PRD samples is described in figure 2. The hierarchical structure allows first the selection of information concerning all MOS category accessions, e.g. IDBBnrs. 3363, 3723, etc. (Fig.2). Subsequently, a search on IDBBnr. 3363 in the field MON-IDBBNR will render the passport information on all the related PRD samples of a particular accession (e.g. IDBBnrs. 7030, 1900, 7901, 6255, 4855 and 7848).

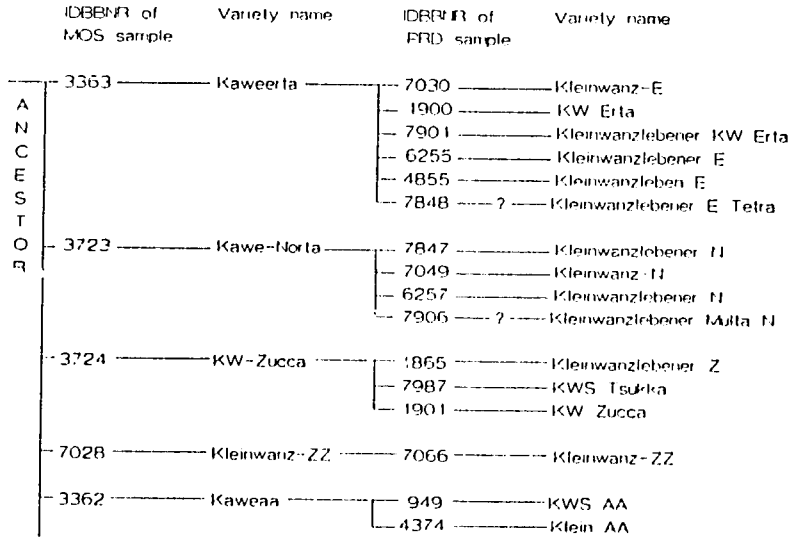

Figure 2: Classification of probable duplicates

During 1990/91 many geographic coordinates of collection sites were up-dated using the Times atlas and other suitable maps. The number of accessions with latitude/longitude data increased from 1671 to 3163. In sections *Beta, Nanae* and *Procumbentes,* incorrect coordinates of collecting sites could easily be detected since any deviation of the plotted site from the coast-line or the natural distribution area is suspicious. For inland wild types much more detailed digitized maps would be necessary to check the integrity of the coordinates. Mistakes in the latitude/longitude data generally took the form of typing errors, confusion of the hemisphere, or coordinates incorrectly read from topographic maps. Up-dating of the coordinates of

collection sites was very time consuming. Often it was not possible to trace the site on maps, in which cases it was decided to indicate, as accurately as possible, the area in which the sample was found and to describe the precision of the coordinates in the field "Remarks" using the code "MIA". For example, MIA:PRECISION LAT/LONGI POS.1-3 means that only the degrees of latitude and longitude of the collecting site are correct and the minutes a rough estimate. Since accurate geographic coordinates of the collecting site are needed for geographic analysis of any plant character or information, efforts to increase and improve the set of latitude/longitude data are certainly well invested.

Because many collectors recorded habitat data it was decided to add this information to the database. A start was made in March 1991. Examples of such data are shown on a printout in Appendix 2. If such data had been recorded systematically and in a standardized form they could, for instance, have been used to quantify the ecological amplitude of a particular species. The data stored in "Remarks" also form a source of information on the occurrence of pests and diseases at collecting sites and additional collector's notes which do not match the rigid definitions of the other database fields.

Analysis of the information

Twenty genebanks have sent their passport and part of their seed stock data to the IDBB. The total number of accessions per genebank ranges between 15 and 1950; the number of MOS ranges between 13 and 1773 (Table 1). The number of duplicates of all kinds, as can be counted by the field MON-IDBBNR, has increased from 1427 in 1989 to 2315 in 1991 (Appendix 1). Since the new datasets contained relatively more PRD or NOG than MOS, the percentage of the sample category MOS has decreased between 1989 and 1991 from 74.4% to 67.8% (Fig.3). The percentage of security duplicate samples (categories SDS and SDA) has not significantly changed, but the number of recorded security duplicates still remains below 8% of the total number of registered accessions. The actual degree of safety duplication may be higher since some of the genebanks, such as Beltsville (USA), Izmir (Turkey) and Leningrad (USSR), take care of their own security storage. The information on security duplication at national level is not yet available to the IDBB.

Composition of the world holding

The major part of the holding consists of *Beta vulgaris* germplasm (Fig. 4 and, in more detail, in Appendix 3). In comparison with the percentages of 1989, that of unclassified *B. vulgaris* germplasm and of cultivated germplasm has decreased, while the percentage of wild *B. vulgaris* germplasm has increased. The percentage of section *Corollinae* material also slightly increased (12.0% to 13.1%). Increases in wild germplasm result from collecting missions conducted by the USDA/ARS, Plant Genetic Resources Research Institute (Izmir), CGN/FAL and VIR, in 1989/90.

Further analysis showed that the holding of MOS of cultivated beets is composed of 61.6% unclassified accessions, 0.1% "weedy types", 8.5% "landraces", 12.9% "breeders' lines" and 26.8% "advanced cultivars" (Fig.5). Landraces originate from three major geographic regions, namely W/NW Europe (19.5%), E/SE Europe (32.1%) and the Mediterranean basin (46.5%). From Asia only very few accessions of landraces (1.9%) are available in collections (Fig.6).

Table 1: Number of accessions per *Beta* holding and sample category

Donor	MOS	SDS	SDA	PRD	NOG	NOC	Total
001CHN (China)	44			6			50
AARI (Turkey)	264			1			265
AISBRC (Ireland)	13			2			15
BARCPI (United States)	1773			159	16	2	1950
BIRDPB (United Kingdom)	768			244	12	1	1025
BLOBAI (Poland)	38			8	50		96
DYOSAP (France)	8			37			45
GGB (Greece)	679			61		1	741
INRALR (France)	14						14
MERRVP (Belgium)	41			31	54		126
NEDBEG (Netherlands)	1084	328	223	138	31	330	2134
NGB (Sweden)	19			8	2		29
NVRS (United Kingdom)	36			42		1	79
NYONRA (Switzerland)	56			10			66
OLORBI (Czechoslovakia)	102			85	1		188
PRAGGR (Czechoslovakia)	67			32	78		177
TAPRCA (Hungary)	70			21	19		110
VIR (USSR)	365			376	94		835
ZARAEE (Spain)				1	110		111
ZIGUK (Germany, East)	189			60	1		250
Total by sample category	5632	328	223	1300	468	335	8306

Abbreviations:

Beta holdings:
AARI= Plant Genetic Resources Research Institute, Izmir; BARCPI= Beltsville Agricultural Research Centre, Beltsville, Maryland; BIRDPB= University of Birmingham, Department of Plant Biology, Birmingham; GGB= Greek Gene Bank, Thessaloniki; MERRVP= Rijksproefstation voor Plantenveredeling, Merelbeke; NEDBEG= Dutch-German cooperation on beet genetic resources, Wageningen; NGB= Nordic Genebank, Alnarp; NVRS= Station Federale de Recherces Agronomique de Changins, Nyon; OLORBI= Research and Breeding Institute for Vegetable Crops, Holice; PRAGGR= Research Institute of Plant Production, Genebank, Praha; TAPRCA= Research Centre for Agrobotany, I.P.P.Q., Tapiozele; VIR= All-Union Vavilov Institute for Plant Industry, Leningrad; ZARAEE= Estacion Experimental 'Aula Dei', Zaragoza; ZIGUK= Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben.

Sample category:
MOS= most original accession, in the active collection; SDS= security duplicate sample, not in the active collection; PRD= probable duplicate, in the active collection; NOC= no longer in the collection, NOG= not within genebank responsibility.

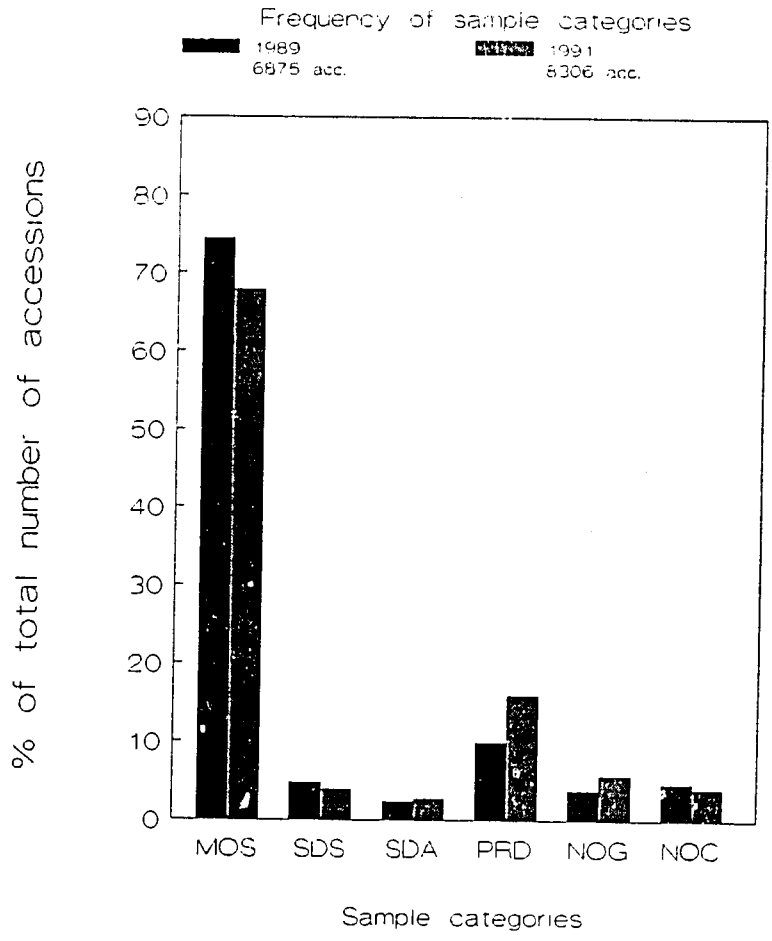

Figure 3: Frequency of sample categories in 1989 and 1991 (IDBB)

Identification of geographic gaps

The application of the program "Mapview" allowed visualization of geographic gaps in the world *Beta* collection. The information used to generate figures seven to nine was restricted to the MOS category and is presented separately for section *Beta*, sections *Corollinae* and *Nanae*, and section *Procumbentes*. More detailed information on the origin of particular species is presented in Appendix 4.

Figure 7 clearly shows coastlines where no wild *B.vulgaris* germplasm has been collected. Major geographic gaps are: northern and north-eastern Spain, large parts of the Italian coast, Yugoslavia and Albania, north-east Greece, the eastern and southern Black Sea coasts, Syria and Lebanon, large parts of the North African coast and Morocco. So far, almost no wild *B. vulgaris* germplasm has been collected east of Israel. Collecting missions in Morocco in 1990 (Japanese genebank), and in northern Turkey in 1988/89 (PGRRI, Izmir) did yield new material but, since the IDBB lacks this information, both regions occur as "gaps" on the figures.

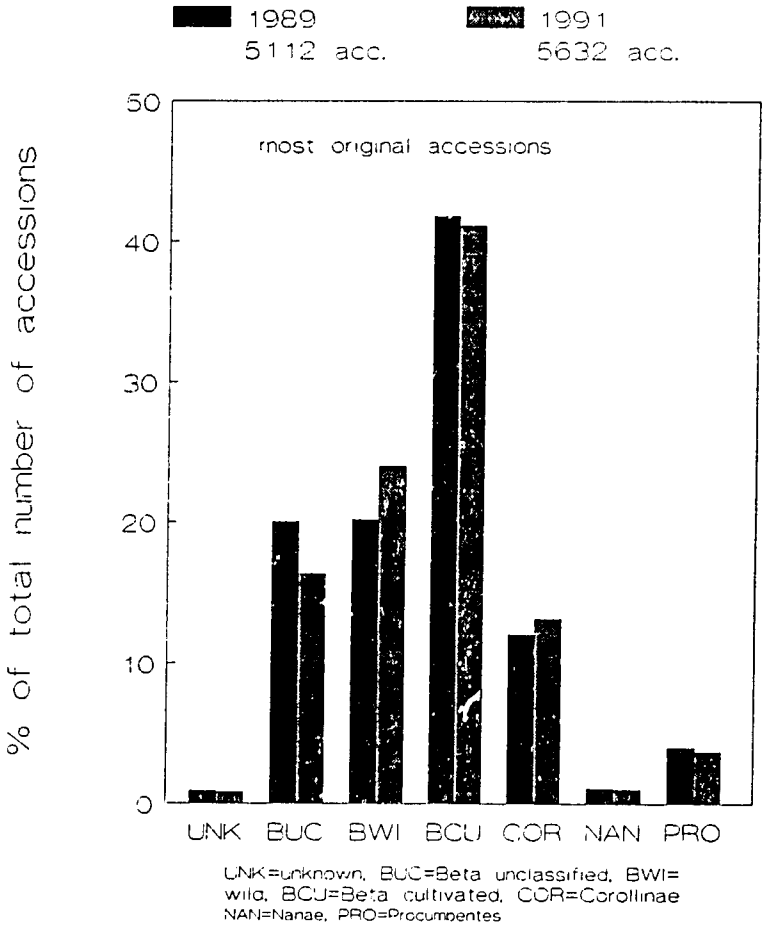

Figure 4: Composition of world holding by major germplasm groups (IDBB)

Collecting sites of *B.nana* are shown on the left of Fig. 8. Many samples of the species were collected in more or less the same area so that points were plotted several times on the same spot. The bulk of *Corollinae* species was sampled in western Anatolia. In central and eastern Turkey the points are more scattered and generally represent the collection made by the taxonomist K.Buttler between 1970 and 1976, as well as material stored at Izmir and Birmingham. Few accessions, mostly with very inaccurate coordinates, were collected in southern Russia (a single one named *B.trigyna* in the Altai mountains and a *B.lomatogona* sample in Iran). *Corollinae* material from the northern Black Sea region, Bulgaria, Romania and Hungary does not exist in any of the national holdings.

Figure 9 depicts the origin of the collection of section *Procumbentes*. A major part of the material was sampled on Gomera, Gran Canaria and Teneriffe. The exploration of the Japanese genebank in Morocco may have yielded additional material from the north-western African coast. No material is available from the Capverdian Islands where *Procumbentes* species, presumably, also occur.

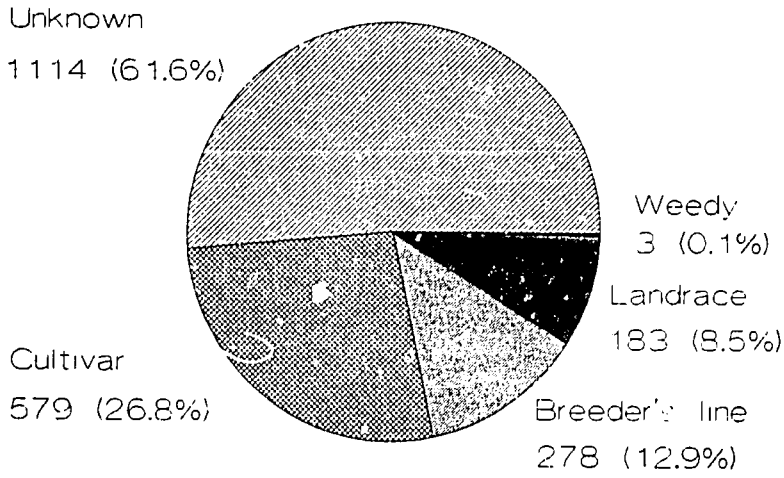

Figure 5: Composition of the world holding of most original samples of cultivated beets (IDBB)

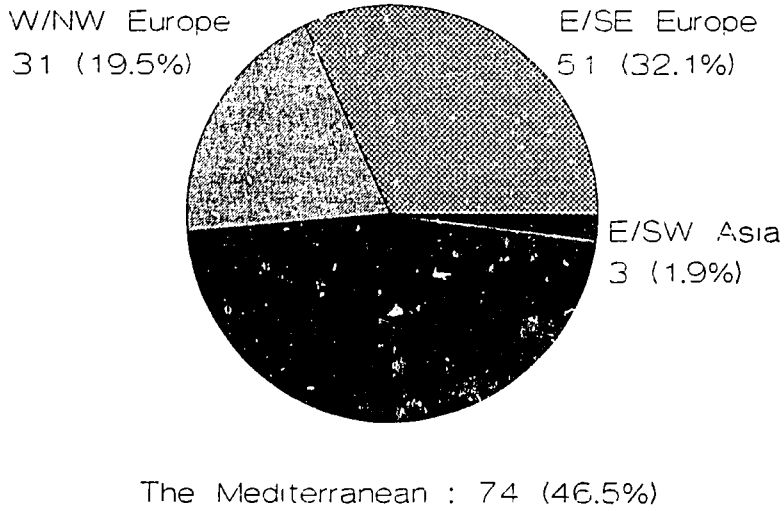

Figure 6: Geographic origin of most original landrace accessions (IDBB)

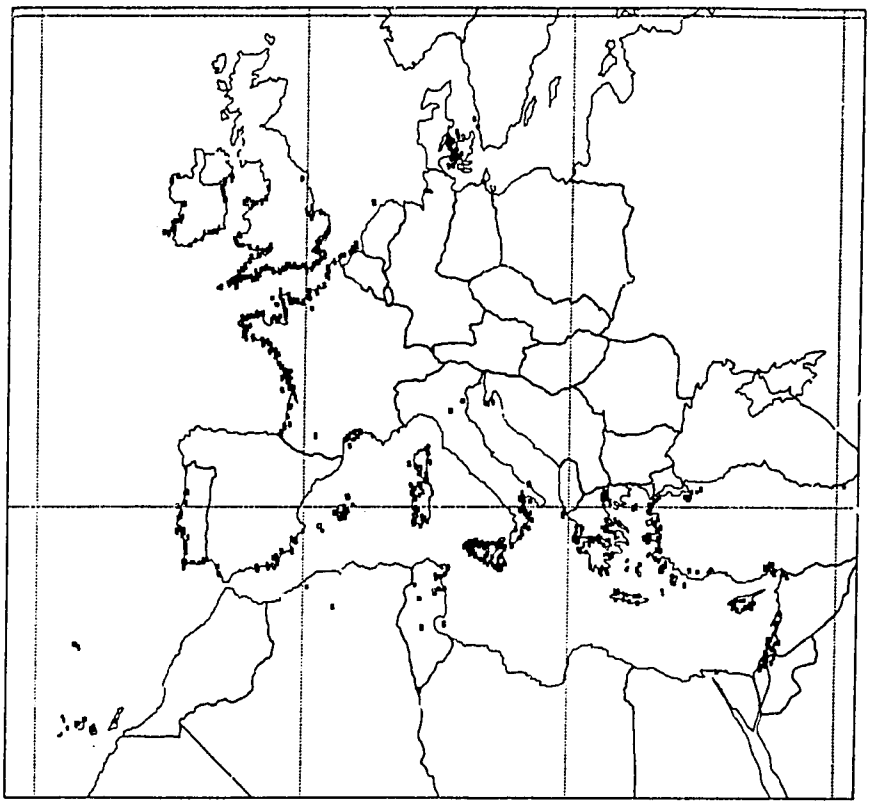

Figure 7: Collection sites of section *Beta* (most original accessions) in Europe, North Africa and the Middle East

Service to the users

The establishment of the IDBB has considerably improved the access to information on the type of germplasm stored at each of the national genebanks. This information system helps researchers and commercial plant breeders to trace specific material in the world *Beta* holding and to acquire seed samples. For that purpose the user is supplied, by the IDBB, with survey tables and/or other kinds of comprehensive information, which indicate the range of material stored worldwide. This provides a first insight into the type of material and helps users to specify their requests and to order the accessions from the storage location. Many of the requests addressed to the IDBB were already fairly detailed, in which cases a selection of material was generated and sent as a printout to the user. Where larger data volumes are requested the information is provided as a fixed format ASCII file on floppy disc. For example, a copy of the full content of the database was sent to the genebank at Gatersleben (East Germany).

Requests for information on *Beta* germplasm were addressed to the IDBB by genebanks, research institutes and the breeding industry. There were two major categories of requests: firstly, information on sugar beet breeding lines and varieties, and secondly, requests for very specific material. The latter information generally was used by research institutes to re-collect or to acquire specific accessions for evaluation and research projects.

This user service could be improved if the "Seeds" table of the IDBB were complete and sufficiently up-to-date. The IDBB could immediately restrict the passport information to accession numbers which are available at the national genebank. At present, the user will frequently find that some accession numbers listed by the IDBB are not available at the national genebank due to temporary depletion of the seed stock. The number of outside requests for information from the IDBB, since spring 1989, is still limited (about 30). This excludes the IDBB's own rather frequent use of the database. The number of requests will presumably increase when information on evaluation data can also be distributed by the central database.

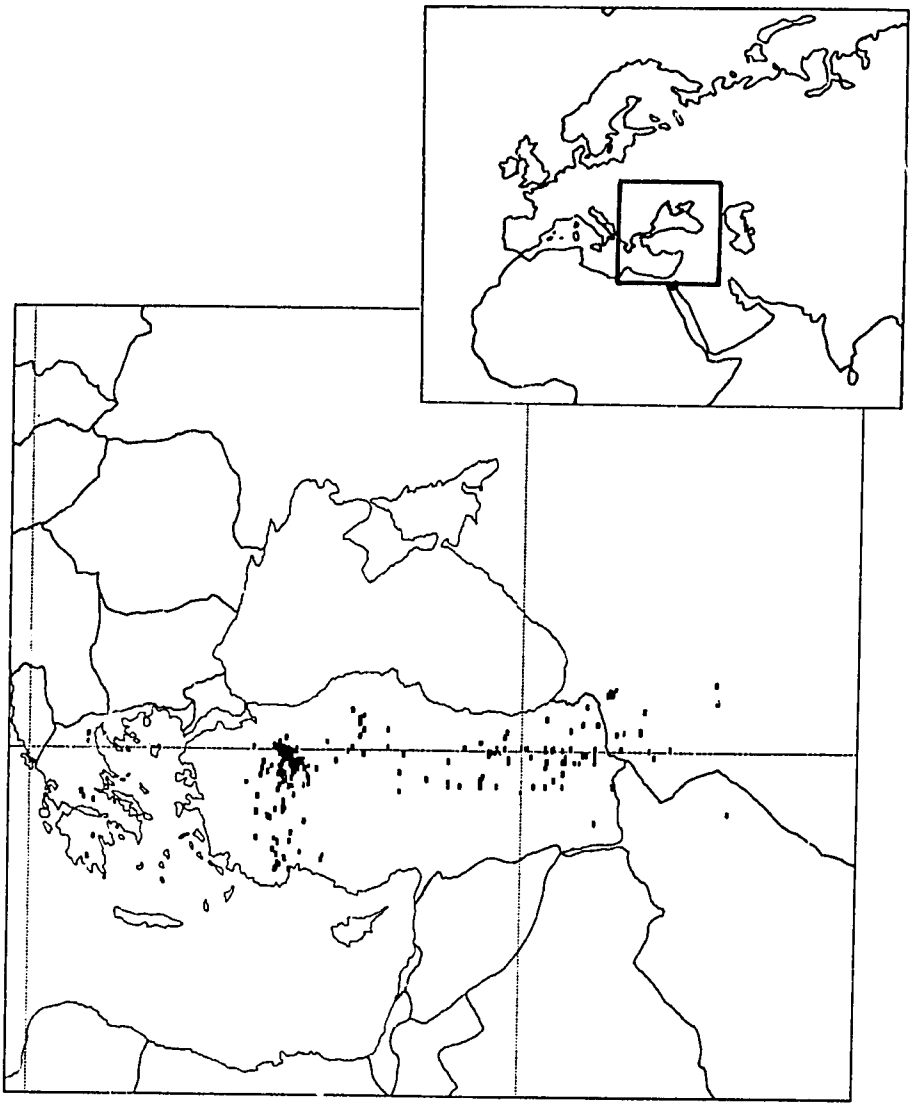

Figure 8: Collection sites of sections *Corollonae* and *Nanae* (most original accessions) in Greece, Turkey and Iran

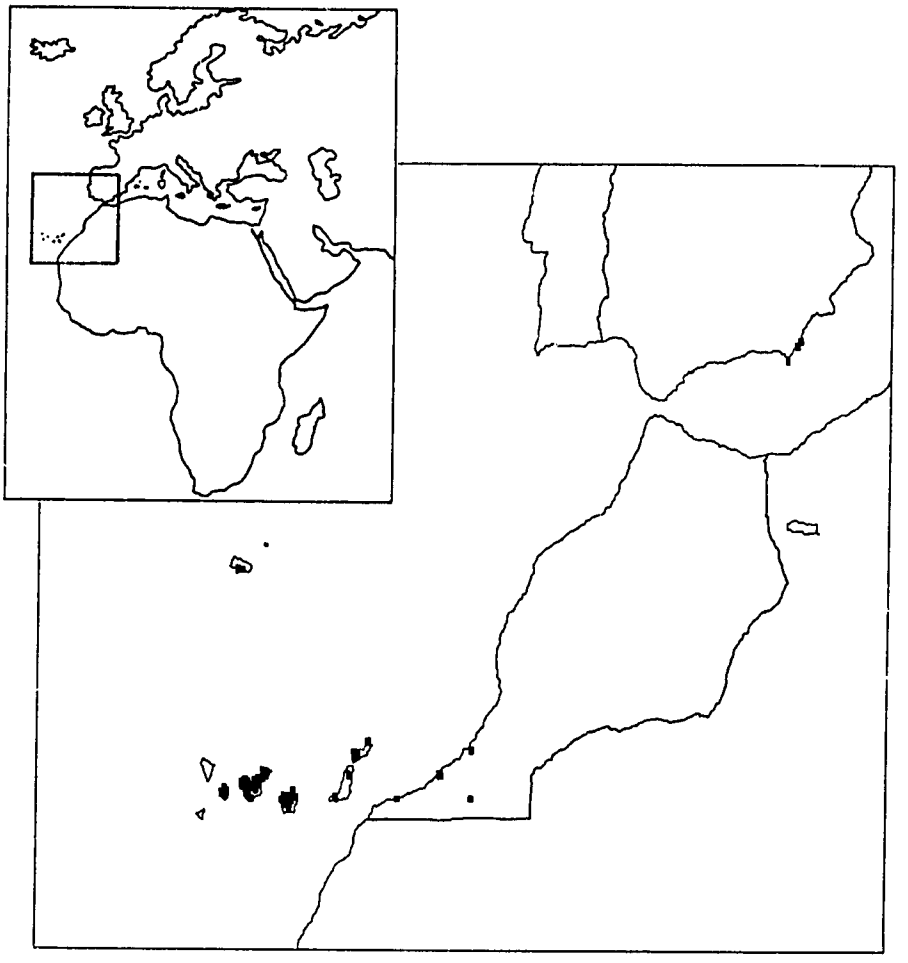

Figure 9: Collection sites of section Procumbentes (most original accessions) in Morocco, Canary Islands and Spain

Conclusions

Exchange of passport and seed stock data

This has become a routine process. Over the past two years, experience has shown that data on material collected during expeditions is usually provided by the institutes responsible after completion of the travel report. Information on new accessions acquired from elsewhere and added to the national collection was not systematically sent to the IDBB. In future, the accession numbers of each national collection should be compared with the accession numbers registered by the IDBB to detect discrepancies between them.

Though the IDBB improves the integrity of its data by continuous correction of the dataset(s), none of the connected national genebanks asked the IDBB to provide the improved set of passport data. The IDBB would, of course, be willing and interested to service counterparts in that field.

Repeated requests for the provision of seed stock data did not yield any positive response. This endangers, or at least considerably delays, the joint action which should be most suited to starting international collaboration. Intermediate solutions, such as coordination of bilateral cooperations through the central database, have rendered some results and now need to be enforced. Full cooperation however can only be achieved if the IDBB receives complete and sufficiently up-to-date information on the seed stocks. At present, the development of a priority list for regeneration would be rather meaningless and would not warrant the efforts the IDBB would have to spend on its distribution. This part of the network activity should be carefully reviewed and the reasons for slow dataflow from the national to the international level be identified and discussed frankly.

The lack of computerized seed stock data at the national level is presumably the major factor which hampers the dataflow from the national unit to the IDBB. Whether or not this is the case should be discussed and, if so, how the network could actively overcome this obstacle towards an operational joint regeneration programme. Members of the network should strive for conditions at the national level that allow curators or database managers to generate and dispatch an ASCII file with seed stock data within one hour. Then, with some goodwill, it should not be too difficult to run a joint regeneration programme.

Analysis of information

The relative amount of stored wild beet material has not changed considerably since 1989 and agrees fairly well with the size of the distribution area of each section and the abundance of populations within such areas. It seems that collection of wild *Beta* species has almost been accomplished. The remaining geographic gaps in the world *Beta* holding can be filled in the coming years by those plant explorations already projected and some additional ones. Many of the open pollinated, obsolete sugar beet, fodder beet, garden beet and Swiss chard varieties and a high number of breeding lines are available in collections. The number of landraces, however, is limited to 183 accessions (8.5% of all cultivated germplasm of the MOS category). In addition, only a few of them originate from Arabian and Asian countries. The biological significance of these ciphers however is not very clear.

The IDBB can count, summarize and visualize information; it cannot provide hard biological evidence for an economic need to collect more material. It seems advisable to put more emphasis on the collection of landraces but we really do not know whether unique germplasm will be gathered or whether only redundant material will be added to the existing seed stock.

The biological significance of the probable duplicates is unknown. Is it worth while to maintain PRD samples in collections as separate accessions or could many of them be seed-increased and maintained as bulk samples? Bulking of probable duplicates would reduce the world *Beta* holding by about 16% and could therefore help to save funds for maintenance and evaluation. How should another source of duplication, that of densely sampled areas, be treated? Are there good reasons to maintain, for instance, 61 samples of *B. vulgaris* from Corfu or each of the numerous section *Procumbentes* samples from the Canary Islands as separate accessions? It seems that the *Beta* collection can be rationalized but the genetic criteria required for a decisive finding are lacking. Intensified research on the structure of genetic diversity of beets could become a possible theme for research projects initiated by the network members. The necessary expertise to investigate this subject is certainly available within the network.

Appendix 1: Number of records/field in the tables PASSPORT AND SEEDS

PASSPORT	
Field	Number
IDBBNR	8306
R_DATE	8306
D_ADDR	8306
D_COUNTRY	8306
D_PNR	8064
S_NAME	8257
SS_NAME	5767
V_NAME	4013
O_ADDR	5362
O_TYPE	5064
COLLNR	3389
O_DATE	4747
O_COUNTRY	6857
DISTRICT	3639
LOCATION	3482
PLANT_NR	992
LONGI	3163
LAT	3163
ALT	2728
SAM_STAT	5791
SAM_CAT	8306
MON_IDBBNR	2315
REMARK	5048

PASSPORT	(REMARK)
Code	Number
OBN	2669
ONR	1070
PLO	229
RSN	1086
RVN	82
SDO	174
ANC	329
ASP	60
CHI	158
EPO	246
HAB	133
MIA	887
SLO	108
SPH	69

Codes used in REMARK:

OBN=original botanic name	ASP=aspect
ONR=other number	CHI=coded habiat information
PLO=ploidy level	EPO=effective population size
RSN=rest species name	HAB=habitat description
RVN=rest variety nam	SLO=slope in degrees
SDO=secondary donor	SPH=soil pH value
ANC=ancestry	MIA=more information available

SEEDS	
IDBBNR	3177
U_DATE	3177
AVAILAB	3118
S_WEIGHT	2185
S_NUMBER	744
E_YEAR	2128
LM_YEAR	996
PM-YEAR	424
GERM_YEAR	772
GERM_METH	579
GERM_PERC	7[illegible]3

Appendix 2: Habitat information (example)

IDBB	DON.NR	DISTRICT	SPE	LAF	SIT	SOT	STO	SAL	DRA	SCO	SNU	ASP	SLO	SPH	EPO	PLANT
8203	TR51212	CANAKKALE	vul	2	2	3	4	1	3	6	3	W	50	08.1	0015	10
8204	TR51214	CANAKKALE	vul	2	2	3	3	2	3	6	3	N+W	70	08.0	0020	20
8205	TR51215	CANAKKALE	vul	3	0	3	2	1	2	6	3		00	08.1	>150	>50
8206	TR51216	CANAKKALE	vul	3	0	3	2	1	2	6	2		00		>100	50
8207	TR51217	CANAKKALE	vul	5	3	3	2	0	3	2	2	W	05	07.9	0050	30
8208	TR51218	CANAKKALE	vul	4	0	3	2	1	3	2	2		00	08.0	0005	5
8209	TR51219	CANAKKALE	vul	3	0	2	2	1	3	2	2		05	08.0	0040	30
8210	TR51220	CANAKKALE	vul	3	0	2	1	2	3	2	2		05	07.9	>500	>100
8211	TR51221	CANAKKALE	vul	3	0	1	1	2	3	6	2		00	07.9	0010	9
8212	TR51222	CANAKKALE	vul	2	0	2	2	1	2	6	2			07.7	0075	50
8213	TR51223	CANAKKALE	vul	3	0	3	1	1	3	6	3		00	08.0	>200	75
8214	TR51224	CANAKKALE	vul	3	0	3	2	1	3	2	3		00		0020	15
8215	TR51226	BURSA	vul	7	2	3	1	1	3	2	3	W	20	08.0	0040	40
8216	TR51227	BURSA	vul	7	2	3	3	2	3	2	3	N	30	07.9	0010	10
8217	TR51230	BURSA	vul	7	2	1	3	2	3	2	2	N	45		0040	25
8218	TR51231	BURSA	vul										00	07.5		1
8219	TR51233	BURSA	vul	4	0	3	2		3	2	3		00	07.7	2001	>200
8220	TR51234	BURSA	vul	2	0	3	3	2	3	2	3	N	05	08.4	0050	30
8221	TR51235	BILECIK	cor	8	3	3	2	0	3	2	2	S	00	07.5	0150	50
8222	TR51237	BILECIK	cor	8	3	3	2	0	3	6	2	E	10	07.6	0100	50
8223	TR51238	BILECIK	cor	8	3	3	1	0	3	2	3	S	05	07.9	0003	3
8224	TR51239	BILECIK	int	8	5	3	2	0	3	6	2	W	20	08.4	0010	10

Abbreviations: SPE= species; vul=vulgaris, cor=corolliflora, int=intermedia; LAF= landform; SIT=collection site; SOT=soil type; STO=stoniness; SAL=salinity; DRA=drainage; SCO=soil color; SNU=soil nutrient content; ASP=aspect; SLO=slope in degrees; SPH=soil pH; EPO=effective population size; PLANT=number of plants sampled. For decoding see IBPGR Descriptors for Beets.

Appendix 3: Number and botanic names of most original accessions by genebank

Botanic name	AARI	BARCPI	BIRDPB	GGB	MERRVP	NEDBEG	PRAGGR	TAPRCA	NYONRA	ZIGUK	001CHN	VIR	Others	Total
Beta sp.unknown	8	6	10			19	1					3		47
B. vulgaris	67	487	63	222		46	12			16			2	915
ssp.*maritima*	20	432	137	417		142	6			17		6	15	1192
var.*maritima*						1				9				10
var.*macrocarpa*		9	8	12		33				4			1	67
var.*atriplicifolia*		7	3			2						1	2	15
var.*trojana*	16		1			2								19
ssp.*adanensis*	8		14			2								24
ssp.*orientalis*		1	2			1				1		3	2	10
ssp.*patula*		4	5			3	1						1	14
ssp.*cicla*	36	25	19			27				17		11	1	136
var.*cicla*			1				23		9	18			2	53
var.*flavescens*		6	6			1			46	6				65
ssp.*vulgaris*		3	79	1		41			1	8		15	2	150
var.*conditiva*		6	36			23	63	24		42		75	31	300
var.*crassa*			30		41	52	11	23		37		109	25	328
var.*altissima*		664	186			151	38	23		5	44	141	31	283
B. corolliflora	8	8	6			91	1			2			2	118
B. macrorhiza	1	12	7			32	1						2	55
B. lomatogona	59	24	26			104	3			3			3	222
B. intermedia	39	8	5			206						1		259
B. trigyna	2	17	26			32	6			1			2	86
B. nana	1	1	27		29									58
B. procumbens	16	45			6	2			1				2	72
B. patellaris		28	36			33	1			2			1	101
B. webbiana		9	18			5							1	33
Total (per genebank)	264	1773	770	679	41	1084	169	70	56	189	44	365	128	5632

Appendix 4: Number and botanic names of most original accessions by origin country

Botanic name	Tur	Cyp	Isr	Dza	Grc	Ita	Esp	Fra	Gbr	Irl	Nld	Hun	Sun	Others	Unknown	Total
Beta sp. unknown	23						5		1		1		1	2	14	47
B. vulgaris	194	6	1	25	216	20	45	3		1		6	13	255	130	915
ssp. *maritima*	34	47	60	1	254	203	52	185	157	47	6		1	84	61	1192
var. *maritima*					1	1		1						1	6	10
var. *macrocarpa*	1	3	4	2	2		29							15	11	67
var. *atriplicifolia*							5							2	8	15
var. *trojana*	19															19
ssp. *adanensis*	15				9											24
ssp. *orientalis*						1								5	4	10
ssp. *patula*														14		14
ssp. *cicla*	45				3	13	26	2			3		4	26	14	136
var. *cicla*	1					11	1	7	1		1		1	24	6	53
var. *flavescens*						11		6			2			27	19	65
ssp. *vulgaris*	50			1	16			3			2		13	36	29	150
var. *conditiva*	6				1	12		11	28		28	27	66	84	37	300
var. *crassa*	2				1	4	2	16	5		47	28	36	136	51	328
var. *altissima*	19				4	1	1	153	87	1	23	25	82	386	501	1283
B. corolliflora	88												10	2	18	118
B. macrorhiza	26												12	4	13	55
B. lomatogona	176												8	3	35	222
B. intermedia	248													1	10	259
B. trigyna	29							1					10	2	44	86
B. nana					58											58
B. procumbens							44							1	27	72
B. patellaris							47							7	47	101
B. webbiana							17								16	33
Total (per country)	976	56	65	29	565	277	274	388	279	49	113	86	257	1117	1101	5632

Report on the second meeting of the World *Beta* Network

FAL Braunschwieg (FRG), 24-28 JUNE 1991

Introduction

The second meeting of the World Beta Network was convened at the Institute for Crop Science and Plant Breeding (FAL) at Braunschweig (FRG) from 24-28 June 1991. The meeting was jointly organized by the Centre for Genetic Resources (CGN) within CPRO-DLO[1] and the FAL. The objective of the workshop was:

- to review the past activities and achievements of the Network;
- to further improve the cooperation between genebanks, research institutes and the plant breeding industry; and
- to receive technical reports on *Beta* genetic resources research.

A list of participants is provided (pp. lxxviii - lxxix).

After welcoming the participants, the director of the Institute for Crop Science and Plant Breeding of the FAL, Prof. M. Dambroth, explained that the Dutch-German *Beta* genetic resources programme currently located at Wageningen, the Netherlands will, at the end of September 1991, be transferred to Braunschweig. He expressed his willingness to assume respensibility for the IDBB and to continue the relevant activities.

In his welcoming address on behalf of the IBPGR, Dr. P. Perret stressed the strong interest of the IBPGR in the *Beta* Network and noted that the experiences gained from the network's implementation would allow the further improvement of the IBPGR network concept. He outlined previous CGN support and expressed the hope that FAL would do the same.

Prof. N.O. Bosemark was unanimously elected as chairman and Dr. L. Frese as the technical secretary. On Thursday, 27th June the meeting was chaired by Dr. W. Lange.

The meeting consisted of two parts: a working session on collaborative activities was followed by technical paper and poster presentations. On the afternoon of Thursday the 27th of June, the participants visited the Institute for Crop Science and Plant Breeding.

Review of activities

Report of the Beta Coordinating Committee (BCC)

Dr. L. Frese described the major results of the two BCC meetings: In 1989/90, on the initiative of Dr. N.O. Bosemark, a pre-breeding concept was developed in collaboration with European research institutes and breeding companies. The project proposal for the "Establishment of a European network for the exploitation of wild

[1] Since 1991 the centre for Genetic Resources (CGN) has been part of the CPRO-DLO

Beta section *Beta*" was submitted to the Commission of the European Communities (CEC) within the framework of the CAMAR programme of Directorate General VI. The decision on approval of the project is still pending. During the second BCC meeting the second World Beta Network meeting was planned. The various actions taken to acquire financial support for the 1991 workshop from the CEC and sugar beet breeding companies were reported by Dr. L. Frese.

Progress report of the IDBB

Participants were provided with summary tables and graphs and Dr. L. Frese described the current content of the IDBB. It was noted that the inventory of the passport data to the world *Beta* holding is almost completed. Concern was expressed about the slow progress of seed stock data exchange. A discussion of geographical gaps, genetic erosion and the concept of landraces in *Beta* followed. Dr. L. Frese explained that genetic erosion could occur in *B. nana.*

Regional Beta genetic resources activities

The various activities in the USA, Asian, East European, Mediterranean and Northwest European regions were presented by Dr. D. Doney, Dr .H.M. Srivastava, Dr. L. Dalke, Ms. A. Tan and Dr. B.V. Ford-Lloyd (principal authors), respectively (see pages xxxii - lxxiii).

The meeting noted:

- interesting enhancement (pre-breeding) activities in the USA;
- the level of evaluation and breeding in Asia especially in Iran and India, as well as collecting activities in China;
- the need to involve Yugoslavia, Spain and Pakistan in the network;
- the great diversity of open pollinated varieties available in the eastern European countries, as well as constraints being placed on the genebanks for *Beta* genetic resources conservation due to the changing economic situations; and
- the diverse but more or less uncoordinated activities within the European Community.

Review of collecting activities

The achievements of, and future plans for, germplasm collection were discussed. It was noted that considerable progress had been made during the past few years.

Working Session

Improvement of the IDBB

Passport data

Additional small collections, originating from explorations, need to be added to the database (e.g. the Japanese mission in Morocco, 1990). There is also a need for regular updating from the contributors. The IDBB will contact the participants and request the relevant information.

Type of germplasm registered by the IDBB

It was stressed that national genebanks should only provide passport data on germplasm which (i) is available for distribution and (ii) is maintained in the long term. This data should not only be related to genetic resources *sensu stricto* but should include, as far as possible, genetic stocks. Information on temporary working collections should not be sent to the IDBB. National genebanks have to decide on the extent of breeding material to be maintained in genetic resources collections and hence the type of material to be registered by the IDBB.

Seed stock data

No substantial progress has been achieved. The lack of manpower needed to register and continuously up-date the seed stock information was recognized as a much greater constraint than the lack of an effective information system. It was noted that the databases available at the national level would suffice to manage seed stock data. Dr. R. Jansen argued that curators of collections may not realize an immediate benefit to themselves of a joint regeneration programme and hence will consider exchange of seed stock data as a low priority task. It was restated by the workshop that the data on availability is vital for the network activities. Participants committed themselves to make special efforts to send availability data as soon as possible (by the end of 1991). IDBB also agreed to send reminders periodically. Lack of information is also impeding progress in safety duplication. As agreed in the previous meeting, each accession should be stored in two long-term storage facilities.

Evaluation and characterization data

Various approaches for dealing with evaluation and characterization data were outlined by Dr. L. Frese. They ranged from the registration of all data irrespective of quality, to only information on the availability of evaluation data at the national databases. After some discussion, it was stressed that the IDBB should serve as a supportive unit helping users to get access to the detailed information stored in the national documentation systems. The objectives of a central database were described as providing answers to the following questions:

- What kind of data are available?
- Where is the information stored?
- How can a user get rapid access to the data?

Participants agreed to provide the IDBB with information on evaluation data (descriptors used, accessions numbers described, etc.) together with all information which may facilitate contacts between specialists. It was also agreed not to limit the information to a selected set of characters but to include all.

The purpose and usefulness of the database

The usefulness of the database for botanists and plant breeders searching for material is evident. The IDBB has been requested by breeding companies and researchers to provide information on specific material. It was mentioned that the database will serve and benefit the user community for many years. Participants remarked that the IDBB is also of benefit to curators of national genebanks in such ways as joint management of a network of dispersed collections, sharing of workload, better services to their national users and re-collection of material.

Collecting plans

Participants reported on planned explorations. Within the framework of a cooperative programme between the Indian Council Council of Agricultural Research (ICAR) and the Office of International Cooperation (OICD) of the United States Department of Agriculture (USDA) plans for collecting missions in India are being prepared. Depending on approval of the budget, the programme will start in 1992. Contacts by members of the sugar beet CAC, USA have also been established with scientists in Pakistan for cooperative collection efforts. Prof. Sun Yi-Chu informed the meeting that collecting of beet landraces will be continued in China. Mr. Nasser Arjmand (Iran) said that exploration will be continued and that the missions could be implemented as joint expeditions. Egypt (Dr. El-Gharbawy) has developed, in cooperation with the OICD of USDA, a 4-year collection/enhancement programme. It is hoped that funding of expeditions will be approved soon. It is intended to first collect in the Nile delta and later along the Red Sea coast. According to Ms. A. Tan, the PGRRI at Izmir would set priority on sampling *B. macrorhiza*. In Romania (Prof. Z. Stanescu) a *Beta* genetic resources programme has just been established. The intention is to survey, in 1991, the distribution of wild beets in Romania, before determining collection activities. Dr. L. Frese informed the meeting of the close collaboration between the CGN/FAL and the VIR (exploration of the Caucasus area) as well as the PGRRI. He noted that the German-Dutch Board for Crop Genetic Resources could perhaps fund expeditions in Bulgaria, Romania, Italy and Spain, from 1993 onwards. Japan will be asked to provide information on future plans. Contacts with Yugoslavia will be established through IBPGR.

Joint seed increase programme

Members restated the necessity of sharing regeneration efforts and outlined the soundness of the concept elaborated during the first meeting. Progress will depend on receipt of seed stock data.

Germplasm evaluation and utilization

The meeting entirely supported the approach, as explained by Dr. L. Frese, of the evaluation project submitted to the EC and restated the stringent need to bring novel genetic variation into the crop. They also agreed that the two key objectives of the project would be:

- a large scale screening for new sources of pest and disease resistance,
- the creation of base populations with many useful characters including a reasonable yield.

It was outlined that all other objectives concerning ecogeographic aspects, extension of the IDBB, etc. were natural by-products which would result from the achievement of the key objectives. The meeting was convinced that the global approach adopted was the right one and emphasized that it should become international. Considering that the project would benefit the sugar industry, the industry should be approached through the scientific council of the International Institute for Sugar Beet Research at Brussels (IIRB) and any other channels, as necessary.

The participants gave full mandate to the new *Beta* Coordinating Committee to pursue these objectives and to use any external expertise available. There was general agreement that the BCC should, as soon as possible, reformulate the project proposal. A concise description of the project should be available before the October meeting of the Scientific Council of the IIRB.

The possibility of starting similar activities on a smaller scale through established programmes and goodwill was emphasized. This would not substitute for a global approach.

Research

Due to very intensive discussion during the presentation of scientific papers and posters, there was no time left to treat the issue of research in more detail. Information received from the Directorate General XII (DGXII) of the CEC indicates that it might be possible to get funds for a joint research project on genetic diversity in the genus *Beta* from the European Comunity. Research projects within the framework III (Biotech programme) of DGXII would have to focus on new approaches seeking to understand evolutionary relationships within the genus and on genetic screening. Use of advanced and innovative research methods is a essential requirement for project approval. DGXII will invite applications in November 1991. Because of his close contact with DGXII, Dr. B. Ford-Lloyd could take action in this matter. It was stressed that such research would not immediately benefit genebanks in their daily work. However, any action promoting the *Beta* Network is very useful since it helps to maintain the organization.

Programme of, and funding for, the next Network meetings

Participants emphasized that each Network meeting should combine a working session with lectures on scientific subjects. It was pointed out that the 1991 Network meeting had convened experts from all research disciplines and from all major sugar beet growing areas. Extensions of this truly international group would be highly desirable. Dr. W. Lange pointed out that the Network should not become competitive with the IIRB or ASSBT and should restrict activities to genetic resources subjects. Dr. D. Doney suggested that the third Network meeting be convened at Fargo (USA), in July 1993. This offer aroused considerable interest though concern about possible financial implications was expressed. It was suggested that the new BCC should assess the feasibility of a meeting in the USA. Pre-breeding was suggested as a theme for lectures presented during the third World *Beta* Netwok meeting.

A further suggestion was to convene the fourth Network meeting in Turkey and to link the meeting with a training course on *Beta* taxonomy. The PGRRI at Izmir (Turkey) would be the most appropriate location for training and field visits. Such a training course should not be restricted to the endemic species but should cover the whole range of diversity of the genus. Clarification of the taxonomic status of species such as *B. penta, B. foliosa, B. palonga* and *B. atriplicifolia* was suggested by Prof. R. Melzer. Dr. P. Perret supported this idea and suggested the development of a standard reference set for wild taxa. The BCC was asked to take the necessary initiatives.

Dr. H.M. Srivastava raised the question of funding of the Network meetings. Dr. P. Perret stressed that IBPGR will not increase its financial support but would try,

wherever possible, to support the Network. The meeting expressed its thanks to IBPGR for its catalysing role, as well as for its financial support. Because of the achievements of the network, participants expressed their willingness to try to continue on an entirely self-sustaining basis. Nevertheless, prompt support may be requested from IBPGR, to solve bottlenecks. It was hoped that such requests would be considered favourably.

Election of the Beta Coordinating Committee

Two members, namely, Dr. N.O. Bosemark and Ms. A. Tan resigned. It was agreed that the person representing the IDBB had to be a permanent member of the BCC. It was also decided to extend the BCC from three to four members, to allow for better task sharing and stronger support for Dr. L. Frese, the Network secretary. The following members were elected: D. Doney, H.M. Srivastava and B.V. Ford-Lloyd.

Session on biosystematic and taxonomic research

Ecotype formation and clinal variation in *Beta* section *Beta* were discussed by Dr. J. Smartt. Considering the distribution area, the typical habitat of the wild types of this section, the distribution mechanisms and potential geneflow, he concluded that genetic diversity in section *Beta* should be described in terms of clines rather than ecotypes. He stressed that clinal variation for morphological characters would not exclude the existence of cryptic ecotypes which can only be identified, for instance, by isozyme analysis. Prof. H. van Dijk described geographic variation patterns of flowering behaviour of *B. vulgaris* ssp. *maritima* populations collected in France while Dr. D. Doney described, by means of morphological characters, patterns of variation in a collection of North Atlantic and Mediterranean *B. vulgaris* ssp. *maritima* germplasm. Intervals of 25 - 50 km between populations suffice to induce significant genetic differentiation. An extraordinary amount of genetic variation can be found in the North Atlantic *Beta*. Natural selection from south to north appears to favour annual to biennial, erect to prostrate, increasing leaf thickness and increasing cold hardiness. The results of isozyme investigations in a broad geographic sample of wild populations of section *Beta* were presented by J. Letschert. He provided interesting information on the geographic distribution of alleles, the restriction of particular alleles to specific taxa and indicated the existence of a North Atlantic and a Mediterranean genepool. Dr. B. Ford-Lloyd stressed that the breeding genepool of cultivated beets still contains a large amount of genetic variation. The prominent use of wild beets could introduce novel genetic variation into the cultivated species. He then introduced random amplified polymorphic DNA (RAPD) as a relatively cheap and powerful tool for the analysis of genetic variation. He suggested that RAPD could be used to gain information on the genetic diversity between and within species. This information could be applied to rationalize the conservation of the genetic resources and the evaluation of germplasm. C. Jung presented results of RFLP investigations on species of all four sections of the genus. Apart from interesting variation in wild *Beta* section *Beta*, banding patterns of *B. nana* (section *Nanae*) and of species in section *Procumbentes* were reported to be strikingly different from those of section *Beta*. Further evolutionary studies are in progress. Dr. J. Cuguen presented investigations of the evolutionary relationship between populations growing in a small area of northern France and called attention to cytoplasmic genetic diversity. Though seed transport by sea movements and pollen transport by wind should favour exchange of genetic material, the effective geneflow between neighbouring populations seems to be low. This was a striking result which conflicts with the generally accepted assumption that genetic material can be easily exchanged between populations along the coast-line.

Prof. B. Jassem presented a comprehensive paper on species relationships and evolution. This contribution clearly demonstrated the current status of our knowledge and the need for more investigations into the evolution of the genus.

During the poster session, Prof. R. Melzer presented a survey of the breeding and research activities at Klein Wanzleben (East Germany). Dr. B. Ford-Lloyd described genetic stability in tissue cultures of beets. Prof. Sun Yi-Chu presented very interesting results of *Beta* explorations in China, indicating the existence and distribution of landraces in his country. Examples of the application of mapping systems to the analysis of the content of databases were given by Dr. T. Hazekamp. Dr. H.M. Srivastava described the special problems related to breeding of sugar beet varieties for sub-tropical climates.

Acknowledgements

Participants warmly thanked the members of the previous BCC for their dedication and Prof. Bosemark and Dr. W. Lange for their excellent chairmanship.

Appreciation was also expressed, to Dr. L. Frese for organizing this stimulating workshop, for the work performed by CGN and to Prof. M. Dambroth and his staff for their kind hospitality.

The participants requested the organizer of the meeting to convey their gratitude to the following breeding companies for their financial contributions to the cost of the meeting: Saatzucht Dieckmann-Heimburg (Germany), van der Have (the Netherlands), Hilleshög (Sweden), ICI-SES (Belgium), Kleinwanzlebener Saatzucht AG (Germany) and Saatzucht Strube (Germany).

Beta genetic resources: North American activities

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Introduction

Approximately 550,000 ha of sugar beets are grown in North America. Of that, about 24,000 ha are grown in Canada. U.S. production is centred in five regions: Great Lakes (69,000 ha), Red River Valley (208,000 ha), Great Plains (92,000 ha), Mountain States (78,000 ha), California (68,000 ha). There is also a small beet growing area in Texas (9,000 ha). Each region experiences somewhat different disease problems and climate. For this reason, the U.S. Department of Agriculture (USDA) has established four sugar beet (*Beta*) research centres: East Lansing, Michigan; Fargo, North Dakota; Fort Collins, Colorado; and Salinas, California. *Beta* germplasm research, much of which is for the development of disease resistant germplasm, is conducted at each location. A number of universities have active sugar beet research projects. However, none are involved in genetic resource activities, which are considered to be a national priority and, therefore, a USDA responsibility. In addition to the germplasm projects at the USDA stations, a number of sugar beet seed companies have breeding programmes in Minnesota, Colorado, Wyoming, Idaho, Oregon and California.

National Plant Germplasm System (NPGS):

This is a federal and state cooperative effort. Its activities are supported, at the federal level, by the Agricultural Research Service (ARS) and the Cooperative State Research Service (CSRS) of the USDA and, at the state level, by the state agricultural experiment stations. Since the early 1970s, the primary responsibility for management and support has rested with the ARS. A major development in the management of the system has been the establishment of Crop Advisory Committees (CACs). Since the early 1980s, 39 CACs have been organized to represent the major U.S. commodities.

Crop Advisory Committees:

The CACs represent the germplasm user community and provide guidance and coordination to the NPGS. They are composed of scientists drawn from the federal, state and private sectors. The curator of each crop serves as a member on his specific crop's committee. The functions of the CAC are to provide advice, guidelines, policies and work programmes for all activities in the germplasm management of a specific crop (NPGS, 1982).

Sugar Beet Crop Advisory Committee:

The sugar beet CAC was established in 1983 as a committee of the American Society of Sugar Beet Technologists (ASSBT). It consists of representatives from the federal, state and industry sectors with expertise in genetics, plant breeding, plant pathology, and cytogenetics. All geographical sugar beet growing regions of the U.S.A are represented on this committee. Since its organization, all *Beta* germplasm activities in the U.S.A have been coordinated and supervised through this committee.

This paper will present these activities under the headings of preservation, acquisition, multiplication, evaluation, and enhancement.

Preservation

The Plant Introduction Station (NC-7) at Ames, Iowa is the site of the U.S. *Beta* working collection, whereas the *Beta* base collection is stored at the National Seed Storage Laboratory (NSSL) at Fort Collins, Colorado. There are more than 1200 accessions in the NC-7 collection and more than 900 accessions in the Fort Collins repository. Efforts are underway to back-up all accessions in the NC-7 collection with a base sample at the NSSL. Some accessions have been deposited in the NSSL base collection without having had a sub-sample sent to the NC-7 working collection. These are being sub-sampled and included in the working collection. In the future, as accessions are added to the *Beta* collection, a portion of the seed sample will be deposited in the base collection at NSSL for long-term storage. The remaining seed will be included in the working collection for seed multiplication, evaluation and user availability.

Storage at both facilities is at a temperature of 5° C and a relative humidity of 40%. Small quantities of seed are being stored in liquid nitrogen (at -196° C) at the NSSL. This is an experimental project and not all accessions are stored in liquid nitrogen.

Acquisition

There were approximately 350 accessions stored in the NC-7 working collection in 1983. These were largely old landraces collected about 50 years ago from western and eastern Europe, USSR, and most of the Middle Eastern countries. At that time, the NSSL housed approximately 480 *Beta* accessions, the majority of which were sugar beet releases from U.S. breeders, both public and private. Most of the vegetable types (garden beet) are included in the NSSL collection. Seed exchanges, collection expeditions, breeders' collections, and breeder releases have increased the NC-7 *Beta* collection to more than 1200 accessions and that of the NSSL to more than 900. The policy to back-up all the NC-7 collection at NSSL and to include all desirable germplasm in the NC-7 working collection has resulted in some duplication. The total U.S. *Beta* collection numbers approximately 1800 accessions.

Several valuable breeder collections have been added to the U.S. *Beta* collection. These have included both wild species and cultivated sugar beet. One of the most valuable collections was that preserved and maintained by J. McFarlane. This collection consisted mostly of wild types collected by G. Coons, D. Stewart and J. McFarlane (McFarlane, 1982). Other collections have been donated by both public and private breeders.

A significant number of accessions have been received through seed exchanges. These have included both wild and sugar beet types. Seed exchanges from contacts with scientists from USSR, China, Greece, Hungary, Poland, Israel and the United Kingdom have added to the diversity of the collection.

The largest and most significant additions to the collection have resulted from four collection expeditions (Table 1). Other additions have resulted from breeder releases, mostly from the public sector.

Table 1: *Beta* **collection expeditions conducted by USDA teams in collaboration with teams from each respective country (Doney, 1985; Doney, 1987; Doney 1989; Seiler, 1991).**

Country	Year	Species
Southern Italy	1985	ssp. *B. maritima*
Sardinia	1985	
Corsica	1985	
England	1987	ssp. *B. maritima*
Wales	1987	
Ireland	1987	
France	1989	ssp. *B. maritima*
Belgium	1989	
Denmark	1989	
USSR	1990	*B. corolliflora*
USSR	1990	*B. macrorhiza*

Multiplication

Once accessions are placed in a genebank, they are of little use if there is insufficient seed for the user community. Original collections generally lack sufficient seed for distribution. This necessitates a seed increase before sufficient quantities are available for distribution. It is essential that the seed increase be from a sufficient number of individuals to maintain the genetic variability of the parent population. Since most of the *Beta* germplasm is wind pollinated, isolation and control of pollination is essential.

Under the direction and supervision of the sugar beet CAC, a *Beta* seed multiplication programme was established at Logan, Utah, in 1985. Seed of biennial accessions needing multiplication are planted at the Logan site in early August of each year. Annual accessions are planted in the spring. The Logan site has rather severe winters but usually has sufficient snow cover to protect the over-wintering beet seedlings. Each accession is planted in a two meter long plot of four rows and thinned to 8-10 cm in the row. This provides between 50 and 80 roots per accession. Over-wintering is sufficient to induce the biennials to bolt and flower the following year. In June of the following year, tents of tightly woven fabric are placed over each plot to prevent contamination between accessions (Fig.1).

This method eliminates the need to harvest, store, and/or transplant roots. Roots are stronger, healthier, and produce more high quality seed when they are not disrupted by transplanting. There is a wide variation in quantity of seed produced, due to the wide genetic variability of the germplasm being multiplied. However, the better accessions produce up to 500 g of polished new seed. Each year, seed is increased for about 100 accessions. The new seed can be used for evaluation and by the user community. It is essential that this be an ongoing programme. It not only provides seed for evaluation purposes but also replenishes seed as it is used.

Figure 1: Isolation tents for seed multiplication at Logan, Utah. Onas Mays, caretaker.

Evaluation

The use of this stored germplasm will depend, in large part, on the amount of information that is available. Breeders are reluctant to spend much time and money to evaluate a large collection of germplasm whenever they are looking for a specific character.

Descriptor list:

The sugar beet CAC addressed this problem and, in 1983, developed a descriptor list of the valuable traits of the beet. The list was patterned largely after the descriptor list developed by the IBPGR *ad hoc* working group, in 1980. By following the IBPGR outline, the list became more useful in the international arena. All descriptors are not equally useful. Therefore, a subset of priority descriptors that are useful to the breeder was identified (Table 2).

Evaluation:

A team of scientists with expertise in each of the priority descriptors was identified and approached for participation in this endeavour (Table 2). Funding was obtained through the USDA-ARS, supplemented by the donated participation of several beet seed companies and the Beet Sugar Development Foundation (BSDF). Each year, each scientist evaluates between 25 and 60 accessions for the specific priority descriptors of their expertise. Some evaluations (field tests) lend themselves

to the evaluation of additional descriptors (horticultural characteristics). This has resulted in a more detailed description of the germplasm.

Table 2: Evaluator, location and priority character for evaluation

Evaluator	Location	Descriptor/Character
Anderson	St. Thomas, ND	Root Maggot
BSDF (Brown)	Kimberly, ID	Curly Top
Doney & Kern	Fargo-Moorhead	Agronomic
Hecker	Fort Collins, CO	*Rhizoctonia*
Lewellen	Salinas, CA	Beet Yellows Virus,Rhizomania
Smith	Fort Collins, CO	*Cercospora* Leaf Spot
Whitney	Salinas, CA	*Erwinia*, Rhizomania, Mildew
Yu	Salinas, CA	Nematode

Database

Each scientist enters his evaluation data on a floppy disk containing information about the accessions sent him for evaluation. The floppy disks containing the evaluation data are returned to the *Beta* germplasm curator located at the NC-7 repository, who downloads the information into the Germplasm Resources Information Network (GRIN). GRIN, located in Beltsville, Maryland, is the national database for all plant germplasm in the USA Anyone in the U.S. may access this database via a personal computer and telephone line. Foreign countries are also offered this service but telephone costs may be excessive. Information in the database may also be obtained by writing directly to the database manager:

USDA-ARS-BA-PSI-GSL-DBMU
Database Manager
BARC-West, Bldg. 001, Rm. 130
Beltsville, MD 20705

After five years of evaluation, the programme is beginning to be of value. A search of the database for the numbers of accessions with moderate to good resistance for the important sugar beet diseases and pests is given, as an example, in Table 3.

The database is also capable of identifying each accession, conveying its complete evaluation and description, and accepting seed orders. As the evaluation data continues to accumulate, the collection becomes more and more valuable to the user community.

Table 3: Number of accessions in the *Beta* collection evaluated as having moderate to good resistance for major sugar beet pests. Resistance is based on a scale of 1 to 9 with 1=very resistant and 9=very susceptible.

Descriptor	Selection	Number
Aphanomyces	<5	0
Beet Cyst Nematode	<5	0
Beet Yellows Virus	<5	42
Cercospora Leaf Spot	<5	24
Erwinia Root Rot	<3	125
Erysiphe (powdery mildew)	<3	115
Rhizoctonia	<5	15
Rhizomania	<5	57
Sugar beet Root Maggot	<5	18

Enhancement

As already noted, much of the evaluation has been for pest and disease resistance. Efforts are currently underway to transfer resistances found in wild forms of *Beta* to cultivated sugar beet. Of particular importance is the work involving the transfer of genes for resistance to *Rhizoctonia*, Beet Yellows Virus, and Rhizomania. Accessions demonstrating good resistance to *Rhizoctonia* have been incorporated into the breeding pool for *Rhizoctonia* resistant beets (personal communication, R. Hecker).

Genes for resistance to Rhizomania have been transferred to sugar beet breeding pools. Some of these have been released to the industry (Lewellen and Skoyen, 1988; Lewellen, 1991).

A much broader programme of incorporating genes from wild sources into sugar beet germplasm was initiated by R. Lewellen, in 1984 (Lewellen and Skoyen, 1991). He crossed 48 easy-bolting and 21 non-bolting *B. maritima* lines to the same sugar beet parent (Y54). After an open pollinated polycross of the easy-bolting crosses, all crosses were subjected to selection for root shape and non-bolting tendency. These selections were increased in an open-pollinated polycross to produce population R22. Additional selection in population R22 was achieved for BYV and Rhizomania resistances as indicated in Table 4.

Table 4: Selection pressure in Beet Yellows Virus and Rhizomania nurseries for population R22 (Lewellen and Skoyen, 1991).

Disease Nursery	Selection Pressure	Cycles	Selection Code
Beet Yellows Virus	Sucrose yield	1	R22Y
Beet Yellows Virus	% sucrose	1	R22S
Rhizomania	Root size	1	R22RI
Rhizomania	Root size	2	R22R2

The resulting selected populations were tested under severe disease conditions in replicated field trials in 1990 (Figs. 2 and 3). The severe BYV conditions caused a significant reduction in sugar yield, root yield and percentage of sucrose for the susceptible check cultivar (Fig. 2). The R22 population had a significantly lower percentage of sucrose than the sugar beet parent. The other selections (R22Y and R22S) were not significantly different in percentage of sucrose from the parent. Both populations selected under BYV conditions exceeded the sugar beet parent in root yield, with the R22Y selection significantly exceeding the parent in both root yield and sugar yield (Fig. 2).

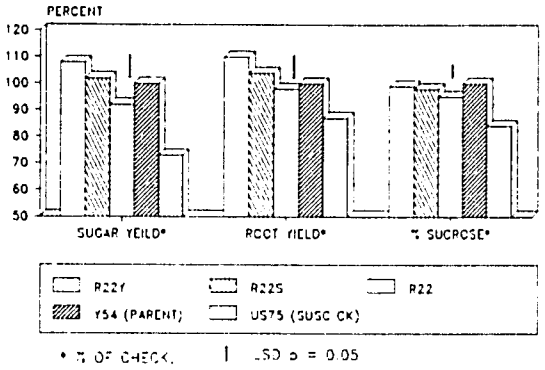

Figure 2: Sugar yield, root yield, and percentage of sucrose for a sugar beet check, sugar beet parent, and three selections from a sugar beet x *Beta maritima* cross. Data are from replicated field trials with severe beet virus yellows infection, in 1990.

The data in Figure 3 are the means of two field trials. A resistant and susceptible check were included in these tests. Highly significant differences were obtained between the resistant and susceptible checks under these severe Rhizomania conditions. The selected populations, although showing an improvement in percentage of sucrose in each succeeding cycle, were all significantly lower than the resistant check. Each selection cycle significantly increased root yield and sugar yield. The second cycle selection significantly exceeded the resistant check in root yield and sugar yield. These results demonstrate that genes for resistance and/or root yield can be transferred from wild types in a relatively short period.

Enhancement efforts at Fargo, North Dakota, by D. Doney have focused on the development of near-sugar beet type genepools from *B. maritima* sources. These can be used as new sources of genetic variability in sugar beet breeding. *Beta maritima* types representative of geographical regions are crossed to a sugar beet pollinator segregating for genetic male sterility. Genetic male sterility is used to ensure intercrossing and maximum recombination. Selection in the first cycles is for root shape and biennial types. Later cycles focus on sugar concentration. Several such dynamic populations have been developed and others are in the developmental stage.

Several single crosses were made between selected *B. maritima* lines and a sugar beet parent, in 1985. Three cycles of selection for root shape and non-bolting tendency have been achieved in these crosses. Initial crosses had very sprangled roots. After three cycles of selection, roots began to resemble sugar beet type roots.

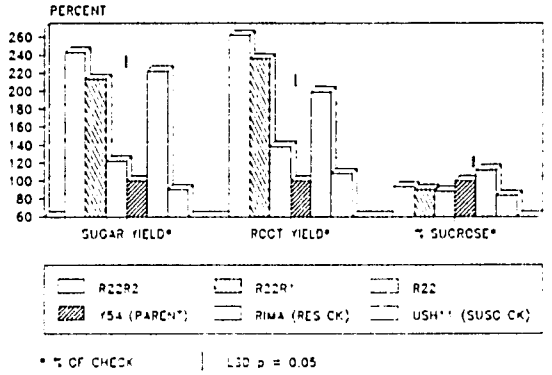

Figure 3: Sugar yield, root yield, and percentage of sucrose for a Rhizomania resistant and susceptible sugar beet check, sugar beet parent, and three selections from a sugar beet x *Beta maritima* cross. Data are the mean of two 1990 replicated field trials with severe Rhizomania infection.

Several of these populations were tested in a replicated field trial in 1989. All populations gave significantly lower sucrose concentrations than did the check hybrids. There was a significant difference between the cross populations in root yield and sugar yield. One cross significantly exceeded the check hybrids in root yield (Fig. 4).

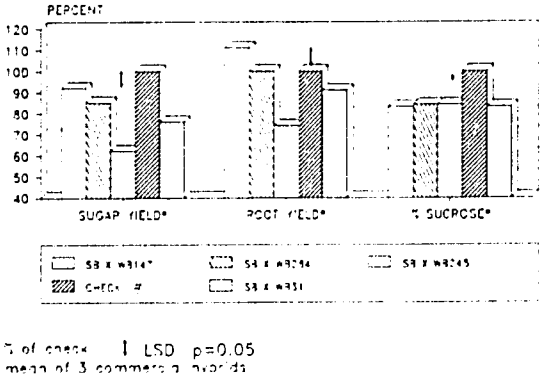

Figure 4: Sugar yield, root yield, and percent sucrose for sugar beet checks (mean of three sugar beet hybrids) and four sugar beet x *Beta maritima* crosses after three cycles of selection for root shape and biennial habit.

These data suggest that there are, in wild populations, genes for growth and/or root yield that may be beneficial in sugar beet breeding pools. Current sugar beet cultivars have been developed from a very narrow genetic background. This narrow base has been further reduced by the need for specific characters. There is, however, a wealth of genetic variability within the genus *Beta* that has not been utilized. The incorporation of additional genetic variability into sugar beet breeding populations is essential to the continued productivity of the sugar beet crop.

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Beta genetic resources activity in Asia

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Introduction

The availability of variable germplasm, its characterization and knowledge of different species and forms of any crop, is essential for a successful breeding programme. Sugar beet is a man-made crop that originated about 200 years ago from a limited range of beet types. Because of this, many scientists believe that the genetic variability in sugar beet is too limited for a successful and continued breeding programme (Bosemark, 1979). Wild species of our crop plants may not be of any direct use but because of their exposure, for centuries, to the vagaries and stresses of nature they have acquired many desirable characters through natural selection. Broadening of the genetic base through the collection of germplasm for different attributes is very important for our breeding programmes.

It is generally believed that different forms of beets, namely, leaf beets, fodder beets, garden beet and wild beets originated in NE Europe (De Bock, 1986). Wild forms of *Beta* show a wide distribution and are found in NW Europe along the Mediterranean coasts of Europe and Africa, and extend eastward to Iran, India, China, and other Asian countries.

Since wild relatives of crop plants are being destroyed in their natural habitat, due to continued industrialization and development, it is essential that such forms are conserved for future use. IBPGR, therefore, initiated the establishment of crop networks, in order to conserve valuable gene pools at the global level and also to study them systematically for future use. The World *Beta* Network was established during the first International Beta Genetic Resources Workshop, held at Wageningen in 1989. The main objectives of this international working group are, to rationalize genetic resources activities through task sharing, to enhance the access to collections for all potential users, to stimulate the use of germplasm in breeding and research programmes and to exchange information on the various aspects of the genus *Beta*. As a consequence of this task sharing responsibility the authors present this paper on the *Beta* genetic resources activities in the Asian region with the objectives of highlighting the current status and also drawing attention to problems, with a view to formulating future programmes for specific areas.

Organization

The organizational set-up of *Beta* genetic resources work in the four Asian countries, India, China, Japan and Iran, is generally under the control of either national research institutes for sugar crops or of a university in each country.

In India, there is an All India Coordinated Research Project (AICRP) on sugar beet whose headquarters are located at the Indian Institute of Sugarcane Research (IISR),

Lucknow (Fig.1). This Institute comes under the administrative control of the Indian Council of Agricultural Research (ICAR), part of the Government of India's Ministry of Agriculture. The *Beta* germplasm activities of collection, conservation, evaluation and documentation are undertaken by IISR, Lucknow. The AICRP has many other research centres located in different parts of the country. These conduct evaluation trials for root yield, quality characters and for biotic and abiotic stress conditions, keeping in view the specific requirements of the particular region. All the germplasm is preserved at Lucknow and is regenerated or multiplied at its sub-station located at Mukteswar, about 7000 feet above mean sea level in the Uttar Pradesh hill country.

Figure 1: The AICRP centres of research on sugar beet in India

In China, the Sugar Beet Research Institute of CAAS is responsible for sugar beet germplasm activities. The Sugar Beet Germplasm Resources Unit of CAAS looks after the work of seed collection, preservation and organization of sugar beet germplasm resources for the whole country. There are regional sugar beet breeding research stations and research institutes which belong to CAAS and the Department of Light Industry. These institutes are located in major sugar beet producing areas such as Heilongjiang, Jilin, Liaoning, Neimonggol, Shaxi, Ningxia, Gansu, Xinjiang, and Shanxi. They are responsible, mainly, for sugar beet breeding work in their regions.

Sugar beet germplasm activity in Japan is confined mostly to Hokkaido University and Hokkaido National Agricultural Experiment Station. The beet genetic resources work in Japan is done on a limited scale, mainly due to the small acreage of sugar beet (limited only to Hokkaido) and the absence of wild species of *Beta* in Japan. Germplasm of different crops are preserved by the Central Bank for Plant Genetic Resources, which is a part of the National Centre of Genetic Research of the Japanese Ministry of Agriculture, Forestry and Fisheries. There are several sub-banks which belong to this Central Bank. Of these, the Hokkaido National Agricultural Experiment Station is responsible for collecting, evaluating and reproducing the *Beta* germplasm. After the initial evaluation and characterization work is done at Hokkaido, the germplasm is transferred to the Central Gene Bank where it is registered and preserved.

In Iran the Sugar Beet Seed Institute (SBSI) located at Karadj is responsible for *Beta* genetic resources work. This institute was started in 1935 and is now considered as the base for Iranian beet breeding work.

Beta germplasm collections

The *Beta* germplasm collections in the four Asian countries, consist of the indigenous material available in each country, new breeding lines developed, and cultivated or wild *Beta* species received or exchanged from abroad.

Recently performed collection missions

Collection missions organized and performed by the different countries of the Asian region, and their future collecting programmes are summarized in Tables 1 and 2 respectively. For further details of leaf beet collections made in China, refer to the abstract of a poster by Sun Yi-Chu (pages 67 - 69).

During the last two years, *Beta vulgaris* ssp. *cicla* was collected in different parts of China by scientists of the Sugar Beet Research Institute of CAAS and the Biological Research Institute of Heilongjiang University. This subspecies is distributed in Central China and the Western Vast Region (Fig.2). Some plants have been found in villages with a history of sugar beet cultivation. Morphologically there was wide variation in the different samples collected. However, not much progress was made with this work due to limited manpower and funds. Collecting will be continued with the limited resources available.

Table 1: Collection missions recently performed by some Asian Countries for Beta genetic resources.

Sponsoring country	Collection area	Samples collected Type	Number
China	Central China	Leaf beet *B. vulgaris* ssp. *cicla*	8
Japan	Morocco, Spain and Gran Canaria (1990)	Wild beets *B. vulgaris, B. patellaris*	32
Iran	Khouzestan Ardabil area of Iran (1990)	*B. maritima, B. lomatogona B. trigyna*	Not available

Table 2: Future plans of some Asian countries for collecting *Beta* species

Country	Planned collection type	Planned collection area
India	*Beta* germplasm	N.W. and N.E. hills, along Ganges and Indus Rivers and in plains of Uttar Pradesh and West Bengal
China	Leaf and wild beets	China
Japan	-	Areas between Black and Caspian Seas in USSR
Iran	Wild *Beta* species	North, North-west, West, and South-west Iran

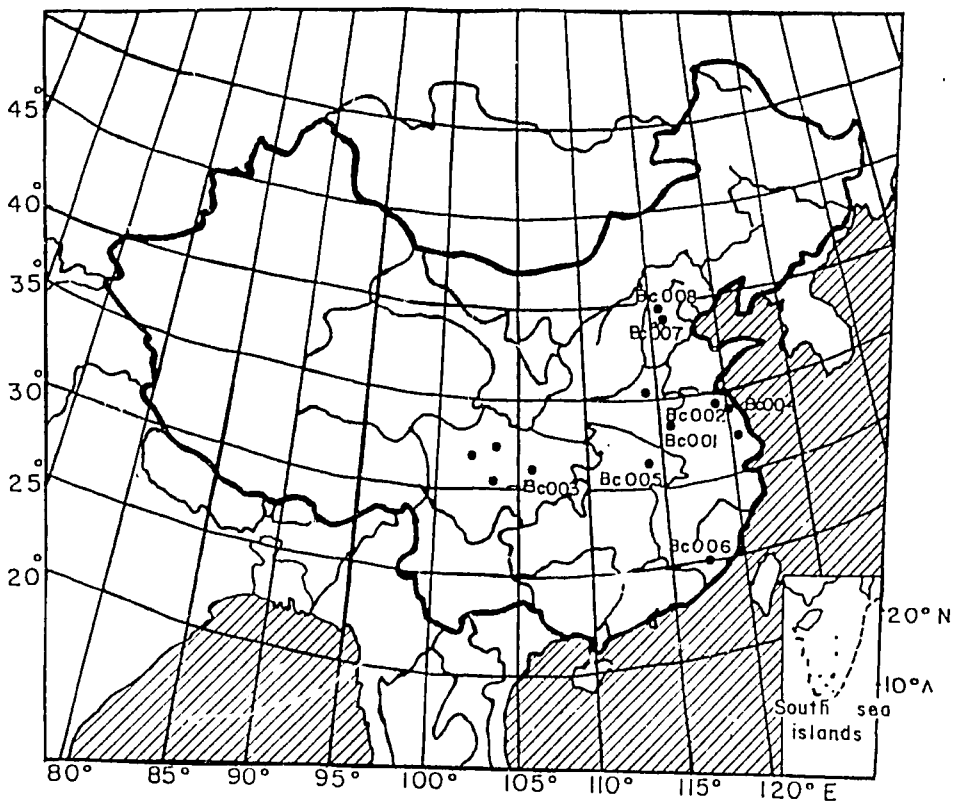


Figure 2: Distribution of leaf beets in China

An exploration for collecting wild species of *Beta* in the coastal areas of Morocco, Gran Canaria and the inland and coastal areas near Zaragoza (Spain) was undertaken by Japanese scientists from June 25 to July 15, 1990 (Fig. 3). During this trip, 32 accessions were collected, comprising *Beta* species from section *Vulgares* (now section *Beta*) and *Patellares* (now *Procumbentes*). They constituted both multigerm and monogerm types. At the time of exploration, with few exceptions almost all the *Beta* plants were dead or at the end of their growing stage. As such, it was difficult to clearly observe their morphological characteristics. It was observed that the population size of weedy,

wild *Beta* species was very small and only limited numbers of plants could be sampled, especially in Morocco. The Japanese scientists are planning, during 1991 and 1992, to study the morphological characteristics of the collected plants. The seed characteristics of these wild *Beta* species were studied and are given in Appendix 1. Details of this collection mission are given in Appendix 2. The Japanese scientists expect to visit, in the near future, the area between the Black and Caspian Seas in the USSR to collect different forms of *Beta* which may be available there.

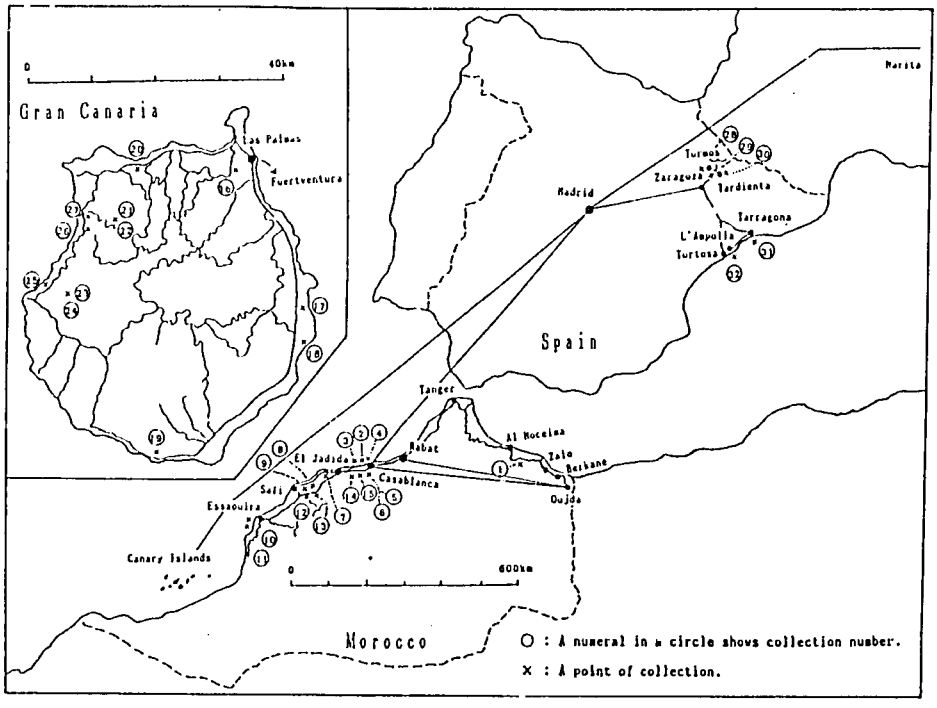


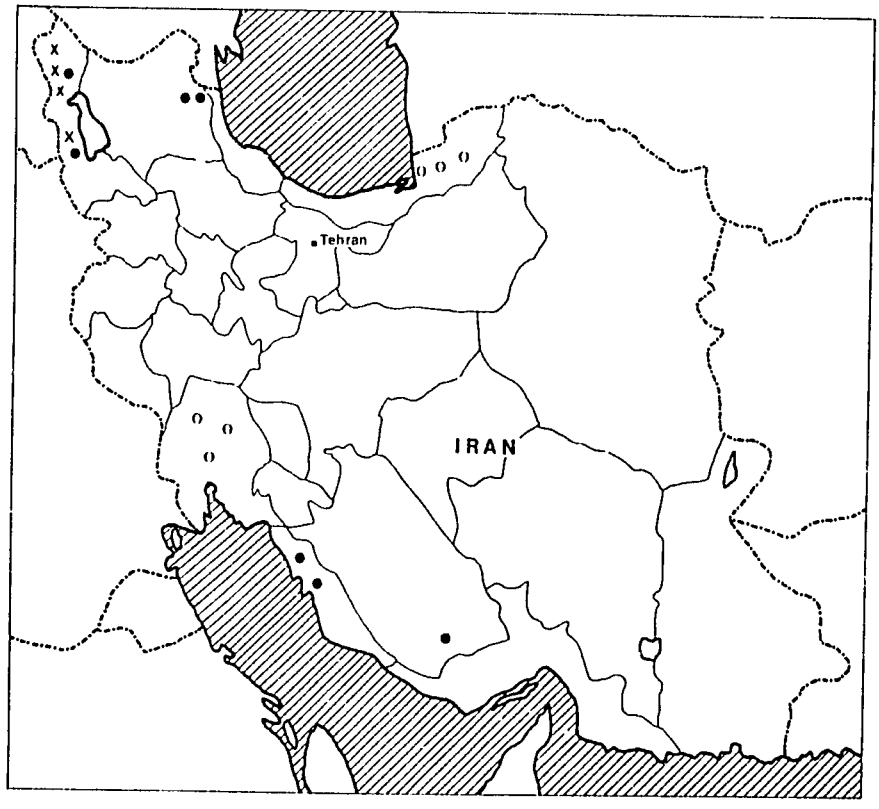
Figure 3: Exploration course and points of collection of *Beta* wild species in Morocco and Spain in 1990

Collection missions were organized in Iran during 1990 and 1991. Two missions went to each of the following areas: Khouzestan, Ardabil and Bousher. During these trips seed of *B. maritima* and *B. lomatogona* were collected (Fig. 4). Collection missions to the Bakhtaran and Minab areas of Iran were also undertaken. No collection mission for *Beta* has been systematically undertaken in India as yet. However, a joint Indo-US programme has been proposed to collect *Beta* germplasm in both the N. W. and N.E. hills, along the Indus river and also in the plains of Uttar Pradesh and West Bengal. This programme will be undertaken as soon as funds are made available.

Exchange of cultivated and wild Beta germplasm

Most of the *Beta* germplasm, both of cultivated and wild species, at present available in Indian collection, is exotic. It comprises diploid multigerm open pollinated varieties, monogerm inbred lines, cytoplasmic male sterile (cms) lines and their maintainers, multigerm pollinators and diploid and polyploid hybrids. Apart

from these, seeds of some wild species of *Beta* have also been received from abroad. This germplasm has been received from different countries of the world. Notable among them are Belgium, Denmark, Sweden, Germany, France, Poland, USA, UK and USSR. From this collection many multigerm inbred lines have been developed and new varieties have been produced, some of which have been recommended for commercial cultivation in India.



• B. lomatogona o B. maritima x B. trigyna

Figure 4: Distribution of wild beets in Iran

In Japan, between 1963 and 1987, 80 strains of wild *Beta* germplasm were introduced from the Netherlands, Germany, France and USA (Table 3). However, with a few exceptions of exchanges for breeding purposes, almost no exchange of cultivated germplasm was carried out between Japan and foreign countries.

Since 1950, many sugar beet varieties have been introduced into China from Poland, Germany, Japan, Sweden, USA and USSR. Until 1989-90, 98 accessions from China had been reported to the IDBB. These include local varieties and introductions from abroad which have acclimatized to the regional climatic conditions. In 1990, 41 sugar beet germplasm lines were introduced into China from CGN and the University of Birmingham (UK). Iran has also exchanged beet germplasm from different countries. The details are given in Table 4.

Table 3: Wild *Beta* species introduced from foreign countries into Japan between 1963-1987*

Species	Number of strains introduced from:					Total no. of strains
	Nether-lands	Germany	France	USA	Unknown	
B. adanensis	-	-	-	-	1	1
B. atriplicifolia	2	-		1	1	4
B. cicla	1	-		-	-	2
B. corolliflora	2	-		-	2	4
B. intermedia	1	-		-	-	1
B. lomatogona	-	-		1	-	1
B. macrocarpa	3	1		1	2	7
B. macrorhiza	-	-		-	2	2
B. maritima	5	7	1	4	6	23
B. patellaris	4	1	-	2	3	10
B. patula	1	1	-	1	-	3
B. perennis	1	-	-	-	-	1
B. procumbens	-	1	-	1	2	4
B. rapa	-	-	1	-	-	1
B. trigyna	1	1	1	1	1	5
B. vulgaris	-	5	-	-	2	7
B. webbiana	1	1	-	-	2	4
Total	22	18	4	12	24	80

* This information does not include collections, made in 1991 by Japanese scientists, in Morocco, Spain and Gran Canaria.

Table 4: Exchange of cultivated and wild *Beta* germplasm by Iran.

Name of Species	Number of Accessions	Donor	Character	Receptor
B. intermedia	3	CGN	---	S.B.S.I.
B. vulgaris	2	FAL	*Cercospora* resistance	S.B.S.I.
B. vulgaris	2	USA	Curly top resistance	S.B.S.I.
B. maritima	1	S.B.S.I.	---	CGN
B. lomatogona	1	S.B.S.I.	---	CGN
B. vulgaris	13	S.B.S.I.	---	CGN
B. maritima	1	S.B.S.I.	---	Sun Yi-chu
B. lomatogona	1	S.B.S.I.	---	i f
B. vulgaris	4	Sun Yi-Chu	---	S.B.S.I.

Beta germplasm conservation

The current status of germplasm conservation methods and seed increase programmes used in different Asian countries, is described below.

Conservation methods

Generally two important methods of germplasm conservation are currently being followed in Asian countries; they are seed and *in vitro* conservation. Very little or no work has been done on cryopreservation.

Seed conservation

The general practice of conserving sugar beet germplasm, in the four Asian countries under consideration, is in the form of dry seeds (Table 5).

In India, the active collections are preserved at the Indian Institute of Sugarcane Research, Lucknow, by storing the dry seeds at room temperature or under sealed polythene covers and refrigerated at 4° C. Further excellent facilities for medium and long term seed storage are available at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi. This is a national facility where germplasm of different crops is being stored. In China, the active collections are stored at the Sugar Beet Research Institute of CAAS, while germplasm for long term storage is kept in the State Gene Bank at Beijing where good seed storage facilities are available. Both short and long term preservation, in Japan, is undertaken by the Central Gene Bank of Japan. Storage conditions for short term preservation are a temperature of -1° C and relative humidity (RH) of 30% ; for long term preservation the seed is stored at -10° C and 30% RH. The active collection is stored at 4° C and 40% RH, at Hokkaido National Agricultural Experiment Station. In Iran the breeding material is stored in cold rooms.

Table 5: *Beta* germplasm conservation methods used in some the Asian countries

Country	Seed Storage				*In vitro* Methods
	Room Temp.	At 4°C	From: -1°C to -4°C	From:. -10°C to -18°C	
India	+	+	-	+	Proposed
Iran	+	+	-	-	Is being used
China	+	+	+	+	-do-
Japan	-	+	+	+	-do-

In vitro conservation

This form of conservation of sugar beet germplasm has been initiated in the four Asian countries under consideration.

In Iran, excised true seeds have been cultured on suitable nutrient medium under *in vitro* conditions. This method has helped to improve germination by up to 70% in *B. lomatogona* which generally has a problem of poor germination. Researchers have also succeeded in obtaining haploid plants through ovule culture. In India *in vitro* conservation of cms and tetraploid lines has been initiated. It is hoped that this will be helpful in the off-season multiplication of these two types of genetic material. Researchers at Hokkaido University have developed an *in vitro* multiplication method using leaf discs from young *Beta* seedlings. This technique has proved very useful for a wide range of sugar beet genotypes and also for *Beta maritima*. At Hokkaido National Agriculture Experiment Station work is in progress for the mass multiplication of sugar beet using a kind of tissue at the reproductive stage. The CAAS in China has been using anther and unpollinated ovule cultures for propagation. Also, haploid seedlings have been produced from tube cultures.

Seed increase programme

In India sugar beet seed multiplication is undertaken at Mukteswar in the Uttar Pradesh hill country. Every three years a set of genotypes is multiplied under cotton tents or cages 6' x 6' x 6' in size (approx. 8m^3) housing 50-60 plants, or of 6' x 14' x 6' (approx. 28m^3) housing 120-150 plants. In China, seed of a particular germplasm line is multiplied as soon as its germination level drops below 50%. About 120-160 plants per genotype are kept in the field and 3-5 kg of seed are harvested per sample (or genotype). In Iran, regeneration of sugar beet breeding lines is carried out every year as required. This is done by the use of isolation chambers and isolation cages. In the seed increase programme, generally, difficulty is encountered in the germination of some wild species. Japanese scientists have reported difficulties in the germination of the seed of section *Patellares*.

A publication by the World *Beta* Network group on specific methods and recommendations for the multiplication of *Beta* germplasm (especially the wild species) would be useful.

Beta germplasm documentation

All the four Asian countries, whose work is reported in these pages, have computer facilities for the documentation of their germplasm.

Hardware and software facilities

At IISR, Lucknow, there is a well equipped computer facility: three PC XTs with 640 KB of memory and MS-DOS version 4.1, one PC AT with 1024 KB, and MS-DOS version 4.01; printers and one xy plotter; and the software packages dBase III, Lotus 1-2-3, and WS-4. There are programs for the design of experiments, programs of important genetic models, Hikkiks, Unix systems and Fortran, Basic and Cobal compilers. In addition there is a well trained scientist (statistician) to help with documentation and data analysis work.

The Chinese database has a Chang Cheng (Great Wall) 0520 computer. Iran has a well equipped hard- and software facility in the form of a PC XT 640 KB RAM with 20 MB memory, one Epson printer and two monitors (Monochom and Ei color). Software

includes a database facility, a sugar beet seed information database and a graphic facility. There is no special genetic resources information system at the Hokkaido gene bank. The Central Gene Bank of Japan, however, is fully equipped with such facilities.

Status of documentation

Most of the documentation of *Beta* germplasm in the Asian countries is now computerised. All the *Beta* germplasm collections available (either indigenous or exotic) in the four Asian countries are now being documented by computers on passport and seed tables using the format of the International Database for *Beta* (Frese and van Hintum, 1989). In India six subspecies of *Beta* are reported to occur naturally (IBPGR, 1988). They are *B. vulgaris* spp. *vulgaris*, *B. vulgaris* ssp. *conditiva*, *B. vulgaris* ssp. *altissima*, *B. vulgaris* ssp. *maritima*, *B. vulgaris* ssp. *orientalis*, and *B.vulgaris* ssp. *palonga*.

B. vulgaris ssp. *palonga* is a new *Beta* subspecies reported from India. Three species of *Beta*, *B. maritima*, *B. lomatogona* and *B. trigyna*, have been found to occur naturally in Iran. *B. vulgaris* ssp. *cicla* is indigenous in China. In Japan, however, no wild species of *Beta* have so far been reported to occur naturally. The accessions available or documented in the IDBB, from Asian countries, have been listed in Table 6.

Table 6: Details of accessions by country

Botanic Name(s)	India	China	Iran	Burma	Iraq	Pakistan
Beta sp. unknown.	-	-	-	-	-	-
B. vulgaris	-	5	42	1	2	7
ssp. *maritima*	4	-	2	-	-	-
var. *maritima*	-	-		-	-	-
var. *atriplicifolia*	-	-	-	-	-	-
ssp. *orientalis*	1	-	-	-	-	-
ssp. *palonga*	1	-	-	-	-	-
ssp. *cicla*	1	-	-	-	6	-
var. *cicla*	3	3	-	-	1	-
var. *flavescens*	1	-	-	-	-	-
ssp. *vulgaris*	112	-	3	-	-	1
var. *conditiva*	-	2	-	-	1	1
var. *crassa*	-	1	-	-	-	-
var. *altissima*	2	62	-	-	-	2
B. macrocarpa	2	-	-	-	-	-
B. lomatogona	1	-	1	-	-	-
B. macrorhiza	1	-	-	-	-	-
B. trigyna	2	-	-	-	-	-
Total:	131	73	48	1	10	11

Evaluation of *Beta* germplasm

In India a set of 25-30 sugar beet germplasm lines are evaluated every year at Lucknow and at some other centres of the coordinated project. Important aspects evaluated are: morphological characters, economic attributes, response to biotic stress (diseases such as *Sclerotium* root rot and *Cercospora* leaf spot), and abiotic stresses (salinity or alkalinity and high temperature). Table 7 summarizes the results of such evaluations. Studies on the genetic diversity of sugar beets in relation to some biochemical parameters (such as invertases and DNA and RNA content) have been carried out. Also, characterization of diploid germplasm through isozyme analysis has been initiated recently.

In China, the Sugar Beet Research Institute of CAAS has completed a study of 310 sugar beet germplasm lines according to botany, phenology, agronomic characters, technical quality and resistance. In Japan, evaluation of about 20-30 sugar beet germplasm lines has been done every year for the last 5-6 years. This evaluation is done with respect to three important attributes: morphological characteristics, physiological or ecological characters (such as disease and insect resistance, bolting resistance) and productivity. Japanese scientists collected wild *Beta* species in Morocco and Spain during 1990. They intend to study the botanical characteristics and to evaluate this collection. In Iran, evaluation of yield and sugar attributes of experimental varieties is done every year on a large scale. Further evaluation of breeding material for resistance or tolerance to major diseases, namely, Curly Top, *Cercospora* leaf spot and powdery mildew has been done in the field and in greenhouses. Bolting and salinity tolerance tests have also been carried out and the summary of these results is presented in Table 8.

Table 7: Evaluation and identification of genotypes for different attributes under sub-tropical conditions, in India.

Objectives	Achievements	Genotypes identified
A. Evaluation for productivity	Identification of high yielding genotypes	M. Magnapoly, Kawe Gigapoly, Virtus, Solid Monogen, IISR Comp-1, LKC-2, LS-6, Cutora, Ramonskaya-06
	Identification of high sugar types	LS-6, LKC-4, Pant S-10, CLR pb-II/79, AJ poly-2. M. Marinapoly, Polyrave-E, KWS-E, Italmono
B. Evaluation for biotic stresses	*Sclerotium* tolerance	IISR Comp-1, LS-6, Ramonskaya-06
	Cercospora tolerance	LS-6, M.R. poly
C. Evaluation for abiotic stresses	Salinity tolerance	II-SR Comp-1, Pant Comp-3, Ramonskaya-06. CLR pb II/79 M.R. poly
	High temperature tolerance	IISR Comp-1, IISR-2, LS-6, AJ-3, OPH, Ramonskaya-06

Table 8: Evaluation of *Beta* germplasm in Iran for different attributes

Objectives	Work done	Achievements
Curly top resistance	Total 719 lines tested in Fasa and Darab	8 tolerant lines have been selected
Cercospora leaf spot resistance	317 lines tested in the field in Ghaemshar from 1987 to date	12 tolerant lines have been selected
Powdery mildew resistance	50 lines tested in the field in Badantaran from 1988 to date	12 tolerant lines have been selected
Salinity tolerance	200 lines tested in green house in different E.C.	93 tolerant lines have been selected
Bolting	Breeding materials are tested in Khouzestan every year	A commercial variety has been introduced
Economic attributes	More than 400 different breeding materials are tested in different locations for their yield and quality traits	Many commercial varieties have been released

Enhancement and utilization of *Beta* germplasm

Current status

In India, sugar beet breeding is in its infancy. The initial stage of our breeding programme was short of germplasm lines. However, with the available diploid open-pollinated varieties inbreeding programmes were started and 40 multigerm inbred lines, 15 of which are good general combiners, have been developed. Many composites have also been developed and have shown promise. IISR Comp-1 and LS-6 are two varieties which have been identified as highly promising and have been recommended for commercial cultivation. They have good root yield, moderate sugar yield, and tolerance to *Sclerotium* root rot. These varieties have also shown tolerance to high temperature conditions (40°C - 45°C). In the last 7-8 years some monogerm cms lines and their maintainers have been received from USDA (Salinas, Colorado and Fargo). They are being used in breeding programmes to produce hybrids. One experimental three-way cross hybrid (LK-HY-1) has shown promise. In addition to the sugar beet germplasm, large numbers of commercial varieties from Europe and America have been received. Some of these have performed very well in multilocation trials conducted

under the AICRP on sugar beet and have been recommended for commercial cultivation in India. They are M. Magnapoly, M. R. Poly, Marocpoly, Kawe Gigapoly, Virtus, Mezzanopoly and Tribel. Wild species of *Beta* have not as yet been used in India in breeding programmes. The acreage under beet is very low. There is one cane-cum-beet sugar factory in India. Another is being built in the State of Punjab.

In China, since 1950, many sugar beet varieties have been introduced from abroad and have formed the basis of their breeding programme. The first varieties bred from this material have been released and have made a notable contribution to sugar production. Some progress has also been made with the use of wild beets in breeding programmes. A variety named Jiangsu-686 has been developed by crossing sugar beet AJ-1 with leaf beet (*B. vulgaris* ssp.*cicla*) and it has become popular in the Jiangsu region. Recently, a new variety was developed by making a three-way cross of *B. patula*, a *Beta* ssp. *cicla* from Turkey and sugar beet. This variety is being tested regionally. *B. vulgaris* ssp. *maritima*, *B. corolliflora* and *B. procumbens* are also being used in Chinese breeding programmes.

Sugar beet germplasm has been used in Iran since 1935 for developing varieties suitable for their climatic conditions. The SBSI of Iran has developed many beet varieties for summer and winter cultivation. They have developed diploid and triploid hybrids, both multigerm and monogerm, using cms lines. The varieties thus developed are being cultivated in different areas of Iran and have made a useful contribution in terms of production and productivity.

Future germplasm needs of Asian countries

Through the efforts of many countries, IBPGR, FAO and lately the World *Beta* Network, considerable amounts of germplasm have been collected, and documented. Now there is an urgent need for exchange or sharing of this germplasm and its evaluation for different characteristics in different ecogeographic situations. Many sugar beet growing countries have a sub-tropical climate and, therefore, there is an urgent need for the systematic evaluation of the available germplasm under sub-tropical conditions. Of all the germplasm of different crops conserved by gene banks, hardly 2-3% has been used for the welfare of society. Therefore, it is felt that the World *Beta* Network and similar international organisations should now plan programmes such that more of this conserved material may be evaluated for different characters and put to effective use in breeding programmes.

Conclusions

Role of the World Beta Network

The formation of the World *Beta* Network through the efforts of IBPGR and CGN is a positive step in bringing together curators, researches and users of *Beta* germplasm and thus enabling them to work in close collaboration for their mutual benefit. Further, the creation of the International Database for *Beta*, has helped in the documentation of the *Beta* germplasm available in different parts of the world and thus enables the researchers to know what type of material is available in genebanks and where it is being conserved. This has opened a new area of international scientific collaboration in germplasm research.

Possible contributions of the national programmes to the Network

The national programmes have contributed passport data of all their available germplasm to the IDBB. Some countries have organized collection missions which have helped to contribute germplasm which may be of much use in future. By task sharing, the national networks can, with their own resources, evaluate germplasm for different attributes at different locations in different countries. In so doing a large quantity of information on the characterization of the available germplasm can be generated. It is suggested, therefore, that the World *Beta* Network should try to identify important characteristics for which the germplasm can be evaluated on a regional and a global basis. The national networks can take responsibility for evaluating the germplasm, and so reduce the time required for evaluation and also generate invaluable information. The national networks can also help in regeneration and multiplication of the germplasm for the benefit of all. Serious thought should be given to these two proposals.

Expected benefits of the Network to the Asian countries

The creation of the World *Beta* Network has helped the Asian countries in three important ways. Firstly, all the available germplasm has been systematically documented through one system. Secondly, it has enabled the curators and breeders to exchange germplasm and valuable information on beet breeding. Thirdly, it has opened a new avenue to Asian scientists for international collaboration on *Beta* germplasm and sugar beet breeding through personal meetings and contacts. This is a positive benefit of the Network. Sugar beet breeding and cultivation in Asia have a long way to go.

Summary

The broadening of the genetic base through collection of germplasm is very important. The wild species of *Beta* may not be of any direct use. However, because of their exposure, for centuries, to vagaries and stresses of nature they have acquired many desirable characteristics through natural selection. The Asian region is considered to be a secondary centre of origin of the genus *Beta*. Thus, it is important to know about the occurrence of different species of *Beta* in this region. Collections carried out in the Asian region show that six types of beet belonging to section *Beta* (*B. vulgaris* ssp. *vulgaris*, *B. vulgaris* ssp. *altissima*, *B. vulgaris* ssp. *maritima*, *B. vulgaris* ssp. *cicla* , *B. vulgaris* ssp. *orientalis*, *B. vulgaris* ssp. palonga) and *B. lomatogona* of section *Corollinae* occur naturally. Recently, leaf beets (*B. vulgaris* ssp. *cicla*) have been collected in China. In 1990, Japanese scientists collected 32 new accessions from sections *Beta* and *Procumbentes* during their collection tour of Morocco, Cran Canaria and Spain. Exchange of wild and cultivated germplasm between Asian countries and Europe and North America has enriched the gene pool with different attributes. All the four countries have good facilities for short and long term seed conservation. *In vitro* conservation has also been initiated at some places. Documentation of germplasm is computerized. Good hard- and software facilities are available. Germplasm has been evaluated, in a phased manner, for productivity, disease resistance (viz. *Cercospora*, Curly Top, *Sclerotium* and *Rhizoctonia* root rot) and abiotic stresses such as salinity, alkalinity and high temperature. All the four countries have used the available germplasm to develop new varieties useful in their regions. Details of collection, conservation, documentation, evaluation and utilization activities in the four Asian countries are described in this paper.

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Appendix 1

Amount and characteristics of the seeds collected in Morocco, Gran Canaria and Spain by Japanese scientists in 1990.

Coll. No.	Weight of collected seed (g)	100 kernel weight (g)	Seeds per fruit[1] Range	Seeds per fruit[1] Average	Seed ball type	Collecting area[2]
1	43.8	4.17	2-4	2.9	Multi.	Morocco, M.
2	08.6	2.55	2-4	3.2	Multi.	Morocco, A.
3	17.1	2.73	2-4	2.8	Multi.	Morocco, A.
4	02.4	2.20	2-4	2.8	Multi.	Morocco, A.
5	95.5	1.18	1-3	2.1	Multi.	Morocco, A.
6[3]	10.6	3.52	2-5	3.3	Multi.	Morocco, A.
7	03.5	1.57	2-4	2.5	Multi.	Morocco, A.
8	03.2	1.70	2-3	2.1	Multi.	Morocco, A.
9	03.4	2.18	1-4	2.5	Multi.	Morocco, A.
10	01.7	2.32	2-5	3.3	Multi.	Morocco, A.
11	00.4	1.06	1-3	1.7	Multi.	Morocco, A.
12	03.4	1.61	2-7	3.8	Multi.	Morocco, A.
13	01.2	0.92	1-3	2.0	Multi.	Morocco, A.
14	02.7	0.89	2-4	2.9	Multi.	Morocco, A.
15	02.3	1.59	2-4	3.1	Multi.	Morocco, A.
16	02.9	1.54	-	1.0	Mono.	Gran Canaria, N.
17	02.4	1.30	-	1.0	Mono.	Gran Canaria, S.
18	17.0	2.32	-	1.0	Mono.	Gran Canaria, S.
19	03.9	1.28	-	1.0	Mono.	Gran Canaria, S.
20	06.3	1.92	-	1.0	Mono.	Gran Canaria, N.
21	01.5	2.05	2-5	2.8	Multi.	Gran Canaria, N.
22	00.3	0.87	-	1.0	Mono.	Gran Canaria, N.
23	02.5	2.05	3-5	3.9	Multi.	Gran Canaria, N.
24	03.2	1.65	-	1.0	Mono.	Gran Canaria, N.
25	00.7	1.02	-	1.0	Mono.	Gran Canaria, N.
26	02.3	1.31	-	1.0	Mono.	Gran Canaria, N.
27	01.5	0.69	-	1.0	Mono.	Gran Canaria, N.
28	02.4	1.51	2-3	2.8	Multi.	Spain, I.
29	03.5	0.72	2-3	2.1	Multi.	Spain, I.
30	11.8	1.53	2-4	2.8	Multi.	Spain, I.
31	13.4	2.34	2-5	3.2	Multi.	Spain, M2.
32	00.7	2.23	2-5	3.3	Multi.	Spain, M2.

Note:

1 Results obtained from investigating 20 seed-balls.

2 M.: Mediterranean seacoast area
A.: Atlantic seacoast area,
N.: Northern part of the Island, a wet area,
S.: Southern part of the Island, dry area,
I.: Inland area near Zaragoza
M2.:Mediterranean seacoast area near Tarragona.

3 Collected from bolting fodderbeet plants for comparison with No.5 seed sample, collected at the same place.

Appendix 2: List of Beta wild species collected in Morocco and Spain, 1990

Coll. No.	Date Month	Genus & Species1)	Sample P/In2)	Status3)	Location4), altitude, shortest distance from sea coast	Stage5)	Texture	Topography6)	Site7)	Drainage8)	Notes
1	29 June	Sect. Vulgares	3 / 1-2	Weedy	9km W of DRIOLR-H along road 38, 60m, 20km.	7-9		Plain	Level	Good	Collected at sugar beet field. Med.seacoast in Morocco.
	* Multigerm, creeping, large seed ball, large and long lignified perianth of 3-5mm length, 6-7 stems of ca. 70cm/plant.										
2	1 July	Sect. Vulgares	30-50/ 3- 5	Weedy	11km SW of CASABLANCA along road 130, 3m, 0.2km.	9	Sandy	Undulating	Slope	Good	Under a low stone fence. Atl. seacoast area in Morocco.
	* Multigem, creeping.										
3	1 July	Sect. Vulgares	30-50/ 3- 5	Weedy	do. ca.800m southward from Coll.No.2 point, 5m, 1km.	9	Sandy, reddish	Undulating	Level	Good	do.
	* Multigerm, creeping.										
4	1 July	Sect. Vulgares	1/ 1	Wild	8km SW of CASABLA.NLA along road 130, ATN-DIAB, 1m, 20m.	5-6	Sand	Plain	Level	Moderate	At a bathing resort. Atl. seacoast area in Morocco.
	* Multigerm, creeping.										
5	2 July	Sect. Vulgares	20-30/ 2- 3	Wild or weedy	10km S of CASABLANCA along road 8, 60m, 10km.	8-9		Undulating	Level	Moderate	At dumping place, near Coll.6 Atl. seacoast area in Morocco.
	* Multigerm, erect, 4-5 red streakad stems/plant, with enlarged root like a sugar beet, with smaller seed than Coll.No.6.										
6	2 July	B. vulgaris. L	Mass/ 1- 2	Cultivated	do.	5-6		do.	do.	do.	At a fodderbeet field. Atl. seacoast area in Morocco.
	* Unusual bolting fodderbeet plants, multigerm, with yellowish root. Material for comparison with Coll.No.5.										
7	2 July	Sect. Vulgares	10/ 2- 3	Wild or weedy	132km SW of CASABLANCA along road 121, SIDI-MOUSSA,50m,1Km.	8-9		Undulating	Level	Moderate	Among border plants . Atl. seacoast area in Morocco.
	* Multigerm, creeping.										

Coll. No.	Date Month	Genus & Species1)	Sample P/In2)	Status3)	Location4), altitude, shortest distance from sea coast	Stage5)	Texture	Topography6)	Site7)	Drainage8)	Notes
8	2 July	Sect. Vulgares	2- 3/ 2- 3	Wild or weedy	58km N.E. of SAFI, along road 121, 90m, 0.5km.	5-6		Undulating	Level	Good	Under a low stone fence. Atl. seacoast area in Morocco.
	* Multigerm, creeping.										
9	2 July	Sect. Vulgares	2- 3/ 2- 3	Wild or weedy	54km N.E. of SAFI, along road 121, 130m, 0.5m.			Undulating	Level	Good	Atl. seacoast area in Morocco.
	* Multigerm, creeping.										
JW10	3 July	Sect. Vulgares	20-30/ 2- 3	Wild or weedy	25 km S.W. of ESSAOUIRA, along road 8 and a pass SIDI-KAUKI, 30m, 0.1km.	4-9		Undulating	Level	Moderate	At a private courtyard Atl. sea shore in Morocco.
	* Multigerm, creeping.										
JW11	3 July	Sect. Vulgares	Mass / 2- 3	Wild	27km S.W. of ESSAOUIRA along road 8 and a pass, 1m, 5m.	1-5 9	Reddish	Flood plain?	Level	Moderate	Beside an inlet. Atl. sea shore in Morocco.
	* Multigerm, creeping, very small in plant size, sometimes 6-10cm in flowering stem length and 3cm in leaf length.										
JW12	4 July	Sect. Vulgares	4 / 4	Weedy	196km N.E. of ESSAOUIRA along road 8, 118m, 30km.	7-9		Undulating	Level	Good	At a small open place in a corn field. Atl. seashore in Morocco.
	* Multigerm, creeping, 4-5 red streaked stems of 20-40cm/plant.										
JW13	4 July	Sect. Vulgares	2 / 2	Wild or weedy	163km N.E. of ESSAOUIRA along road 8, EL-AGAGCHA, 123m, 37km.	7-9		Undulating	Level	Good	At a roadside of Route 8. Atl. sea shore in Morocco.
	* Multigerm, creeping, 6-7 stems of ca 15-25cm/plant.										
JW14	July	5 Vulgares	Sect. 4	4 / weedy	Wild or 44km N.E. of EL-JADIDA along road 8, 10m, 10km.	9	Sandy	Undulating	Level	Good	At a roadside of Route 8. Atl. sea shore in Morocco.
	* Multigerm, creeping, 4-6 stems of ca 50-60cm/plant.										
JW15	5 July	Sect. Vulgares	? / 2	Wild or weedy	30km S.W. of CASABLANCA along road 8, 20m, 10km.	9		Undulating	Level	Good	At a roadside of Route 8. Atl. seashore in Morocco.
	* Multigerm, creeping, ca. 70cm in stem length.										

Coll. No.	Date Month	Genus & Species1)	Sample P/In2)	Status3)	Location4), altitude, shortest distance from sea coast	Stage5)	Texture	Topography6)	Site7)	Drainage8)	Notes
JW16	7 July	Sect. Patellares	20-30/ 5-6	Wild or weedy	7km S.W. of LAS PALMAS, 155m, 7km.	9		Undulating	Slope	Good	At a roadside. N. side of G. CANARIA.
	* Monogerm, creeping.										
JW17	7 July	Sect. Patellares	50-60/ 5-6	Wild or weedy	23km S.E .of LAS PALMAS along Highway and a pass, PLAYA DEL BURRERO, 3m, 0.5km.	9	Sand	Plain level	Level	Good	At a roadside. S. side of G. CANARIA.
	* Monogerm, creeping.										
JW18	7 July	Sect. Patellare	Mass 5-6	(Wild)	29km S.E .of LAS PAMLAS along Highway and a pass, ARINAGA, 5m, 0.5km.	9	Gravel	Undulating	Level	Good	At foot of small hill. S. side of G. CANARIA.
	* Monogerm, creeping.										
JIW19	7 July	Sect. Patellares	Mass/ ca.10	Wild or weedy	68km S.W. of LAS PALMAS along motorway, 10m, 0.5km.	7-9	Sandy	Undulating	Slope	Good	At side of motorway. S. side of G. CANARIA.
	* Monogerm, creeping, with B. procumbens like shaped leaves.										
JW20	8 July	Sect. Patellares	Mass ca.10	(Wild)	17km W of LAS PALMAS along motorway, SAN FELIPE, 3m, 0.3km.	9	Sandy, reddish	Undulating	Slope	Good or level	At side of motorway. side of G. CANARIA.
	* Monogerm, with stem length of ca. 1.5-2.0m.										
JW21	8 July	Sect. Vulgares	2-3 / 1	(Wild)	12km SbE of GARDAR along mainroad and a local road, CAIDEROS, 312m, 5.7km.	9	(Gravel)	Mountaneous	Slope	Good	At a roadside near a craggy cliff. N. side of G. CANARIA. Couldn't find any Beta at the cliff.
	* Multigerm, creeping, 6 stems of ca. 40cm/plant.										
JW22	8 July	Sect. Patellares	5-6 / 2-3	(Wild)		do.	do.	do.	do.	do.	At the same point as JW21.
	* Monogerm, creeping, 5-6 stems of ca. 30-40cm/plant.										

Coll. No.	Date Month	Genus & Species1)	Sample P/In2)	Status3)	Location4), altitude, shortest distance from sea coast	Stage5)	Texture	Topography6)	Site7)	Drainage8)	Notes
JW23	8 July	Sect. Vulgares	4 / 4	(Wild)	7km EbS of PLAYA DE LA ALDEA along mainroad and a local road, SAN CLEMENTE, 230m, 7km.	9		Mountaneous	Slope	Good	Under a white roadside wall. N. side of G. CANARIA.
	* Multigerm, creeping, growing mixed with JW24 population.										
JW24	8 July	Sect. Patellares	Mass / 5-6	(Wild)	do.	9		do.	do.	do.	At the same point as JW23.
	* Monogerm, creeping.										
JW25	8 July	Sect. Patellares	3 / 1	(Wild)	84km SW of LAS PALMAS along mainroad, LA FAJANA, 360m,5m.	6	Sand	Mountaneous	Slope	Good	Among rocks at a seashore. N. side of G. CANARIA.
	* Monogerm, creeping, small in size.										
JW26	8 July	Sect. Patellares	2-3/ 2-3	(Wild)	78km SW of LAS PALMAS along mainroad, 6km LAS PALMAS side from the JW25 site. 530m, 10m.	6	Sandy	Mountaneous	Slope	Good	At a pass side. N. side of G. CANARIA.
	* Monogerm, creeping.										
JW27	8 July	Sect. Patellares	Mass ca.10	(Wild)	34km WbS of LAS PALMAS along mainroad, AGAETE, 80m, (2km).	7	(Gravel)	Mountaneous	Slope	Good	At a road side. N. side of G. CANARIA.
	* Monogerm, creeping, with small seed and B. webbiana like shaped leaves.										
JW28	10 July	Sect. Vulgares	4 / 3	Wild or weedy	79km NE of ZARAGOZA along road N123 and a pass beside a canal, SOTONERA, 451m, 176km.	6		Plain	Level	Good	S. side of a dam. Inland SPAIN.
	* Multigerm, erect, 1-3 red streaked stems of ca.1m or more/plant.										
JW29	10 July	Sect. Vulgares	Mass / 4-5	Wild or weedy	72km NE of ZARAGOZA along road N123 and a pass beside a canal, ALMLIDEBAR, 430m, 170km.	6		Plain	Level	Good	At a field beside a canal. Inland SPAIN.
	* Multigerm, erect.										

Coll. No.	Date Month	Genus & Species1)	Sample P/In2)	Status3)	Location4), altitude, shortest distance from sea coast	Stage5)	Texture	Topography6)	Site7)	Drainage8)	Notes
JW30	10 July	Sect. Vulgares	ca.50/ 4-5	Wild or weedy	79km NE of ZARAGOZA along road N123 and a pass beside a canal, TARDIENTA, 430m, 163km.	6		Plain	Level	Good	At open place near apartment. nland SPAIN.
	* Multigerm, erect, a stem/plant and similar to bolting sugar beet plant but with thin red streak at the base of stems usually, also dark red streaked plants involved.										
JW31	11 July	Sect. Vulgares	Mass/ ca.10	Wild	TARRAGONA, 2m,5- 20m.	6		Plain	Level	Good	At a seashore in TARRAGONA. Med. seashore in SPAIN.
	* Multigerm, erect, 1-5 stems of ca. 1m or more/plant.										
JW32	July	11 Vulgares	Sect. 1	? / weedy	Wild or 64km SW of TARRAGONA along road E15, L'AMPOLLA, 50m, O.lkm.		6	Sand, gravel	Plain	Level	Good At side of a house. Med. seashore in SPAIN.
	* Multigerm, erect.										

Note Med. = Mediterranean Sea; Atl. = Atlantic Ocean

1)Estimated.
2)P: Population size, Mass: More than 100 plants, In: Number of plants seeds collected.
3)Classified as follows: wild, weedy, landrace, improved, breeder's line, others.
4)S: south, N: north, E: east, W: west, NbE: north by east.
5)1:Young seedling; 2: Vegetative growing stage; Reproductive growing stage: 3: before flowering, 4: at flowering stage, 5: seed setting stage, immatured, 6: fully matured; 7: End of growing, a part of leaves or tems still living; 8: fully dead; 9: dead and dried up.
6)Classifid as follows: swamp, flood plain, plain level, undulating, hilly, mountaineous, others.
7)Classified as follows: level, slope, summit, depression.
8)Classified as follows: poor, moderate, good, excessive.

Regional *Beta* genetic resources activities: East European region

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Introduction

The East European region includes the following countries: the Soviet Union, Czechoslovakia, Bulgaria, Hungary, Poland and Romania. About 2.8 million ha of beets (sugar beet, fodder beet and table beet) are cultivated in the region. The greatest area of cultivated beets is in the Soviet Union.

Monogerm hybrid varieties based on male sterile lines were bred and cultivated in this region later than in the USA and western Europe. Until recently, multigerm varieties and monogerm population varieties have been cultivated; the latter in the Soviet Union. As a consequence of this, countries of the East European region have a great potential of multigerm and monogerm populations bred and growing under very differentiated agro-ecological conditions. The value of these populations is high.

A breeding tradition of about 100 years exists in the Soviet Union, Czechoslovakia and especially in Poland. Polish varieties produced by private seed companies such as A. Janasz, K. Buszyczynski and Czechoslovak varieties Dobrovice A and C are of note. The AJ varieties were exported to many countries in Europe and even to China, Japan and the USA before the Second World War. Therefore, their genetic material can be found in many varieties and breeding material scattered all over the world. Similarly, fodder beet breeding developed very early in this region. Multigerm population varieties of different agronomic value have been grown. The table beet production area is also remarkable because of culinary traditions in countries of this region.

The East European region is also an area of origin and diversity of wild species of the section *Corollinae* (Bulgaria, Romania and the Caucasus region of the Soviet Union) and the species *B. maritima* (Bulgaria and Romania). At present, wild species can be found there in their natural habitat but some of them are endangered because of human activities. In all these countries, except for Romania, collections of the genus *Beta* exist. Figure 1 illustrates the composition of the total collection held by East European countries. The state of *Beta* collections is presented separately for each country in the following sections of this paper.

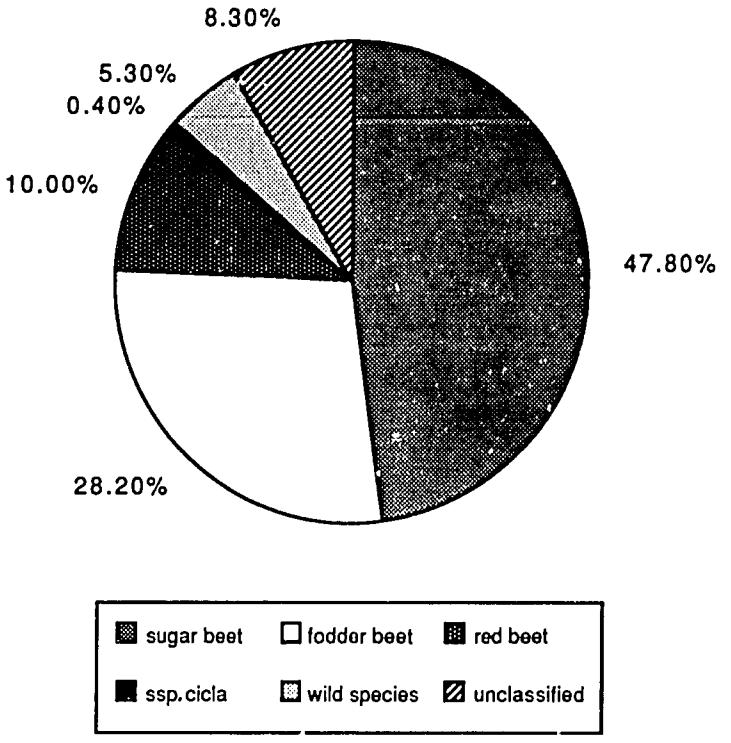

Figure 1: Percentages of different types of beet in the East European region

The Soviet Union

The N.I. Vavilov All-Union Scientific Research Institute of Plant Industry (VIR) possesses the largest collection of cultivated plants and their relatives in the world. It numbers more than 250 000 samples. An important circumstance that promoted the development of studies on plant genetic resources in the Soviet Union was that this institute was headed by N. I. Vavilov, who initiated the global effort to collect cultivated plant diversity. During recent years, scientists of VIR organized more than 200 expeditions in all zones of the USSR. As a result, plant breeders received many local varieties, populations and wild species from Siberia, the far eastern region, Kazachstan, the Caucasus and other areas.

The work on the *Beta* collection focuses on:

- collecting materials during expeditions in the country and abroad,
- quarantine evaluations of collected samples at special test locations,
- investigation of collected materials in the different regions of the country,
- maintenance and seed storage.

Between 1969 and 1990, four expeditions have been organized in the region of origin and diversity of the section *Corollinae* species (Armenia, Karabach, Daghestan

and Azerbaidjan). The most recent was an international expedition with team members from the USA, the Netherlands and Germany. Seed samples of *B. corolliflora*, *B. tomatogona*, and *B. macrorhiza* were collected.

Multiplication of accessions is done in two year cycles on isolation plots at the different experiment stations. Annual species are grown in the experiment garden.

Evaluation of accessions is conducted at experiment stations located in the different region of the Soviet Union. The subjects of evaluation are agronomic features such as bolting resistance, disease resistance and resistance to abiotic factors. Biochemical evaluation is carried out at the VIR and the regional experiment station laboratories.

The documentation of accessions is compiled in catalogues. To date, computers have not been available. At present, and in cooperation with the Centre for Genetic Resources at Wageningen, the data of the VIR *Beta* collection are being transferred onto a database management system. According to the information received from the International Database for *Beta* (IDBB), the *Beta* collection held at the VIR consists of 835 accessions, of which 95.8% belong to cultivated beets. The active collection is stored at the VIR. Seed for long term storage is kept at the National Storage of Plant Seeds at the Kuban Station.

Bulgaria

The K. Malkow Institute of Plant Production and Genetic Resources, at Sadovo, is responsible for the national *Beta* collection. The collection consists of 244 accessions of cultivated beets, two of *B. trigyna*, one of *B. macrorhiza*, one of *B. corolliflora* and one of *B. lomatogona*. Among the cultivated beets, one accession originates from Bulgaria. The curator of the beet collection is in contact with the Sugar Beet Institute, near Shumen, where breeding and research programmes on sugar beet are conducted. They have promised to give the data for some Bulgarian varieties and breeding lines to the IDBB.

Bulgaria is a region of diversity of wild species from the section *Corollinae* and species *B. maritima*. These wild species can still be found in nature. During the June 1988 plant exploration in Bulgaria, three samples of *B. trigyna* and two of *B. maritima* were collected.

Documentation of passport data of 244 accessions has been completed and sent to the IDBB, CGN, Wageningen. During the recent cooperative programme with the Sugar Beet Institute near Shumen, samples of wild beets from section *Corollinae*, originating from Matnica, Shumen, Pliska and Preclav, were received. These materials were grown and investigated at IHAR in Bydgoszcz (Poland). Morphological observations and cyto-embryological investigations indicated that the ecotypes from Bulgaria are mainly hybrids, presumably between *B. trigyna* and *B. corolliflora*.

Czechoslovakia

In Czechoslovakia, the national collection of the genus *Beta* is located at two institutes: the Research and Breeding Institute of Vegetable Crops (187 accessions of table beets), and the Research and Breeding Institute of Beets, at Semcice (317 accessions of sugar beet and 102 accessions of fodder beet). The collections are of mainly

Czechoslovak material. No collection missions abroad have been organized, for financial reasons, and because of the isolation of these institutes from international contacts.

Multiplication of collected samples is conducted in several breeding stations, in the institute at Bucany and in Praha-Ruzyne. The evaluation of the agronomic features is done on small plots at a few locations with foreign varieties used as standards. Resistance to diseases is tested on artificially infected plots. At Semcice, in recent years, very severe natural infection of yellowing virus has been observed.

For long-term storage (at -10° C to -20° C) seeds are sent to the genebank at the Research Institute of Plant Production in Praha-Ruzyne. The active collection is stored at the institute in Semcice at 0° C to -2° C. The accessions of the *Beta* collection are used in the breeding programme of the institute. On request, seed samples are sent abroad. Documentation of collection data is compiled by the national Genetic Resources Automatic Information System (EVIGEZ - EC1033SRBDSO051S). The passport data of the sugar and fodder beet accessions from this collection are not recorded in the IDBB.

Hungary

All activities (i.e. collecting, multiplication, characterization and preservation of *Beta* germplasm) are centred at the Research Centre for Agrobotany at Tapiozele. The collection of *Beta* germplasm is continuously being completed by inland multicrop collecting missions and seed exchanges. The collected samples are local types, often developed from old cultivars. Between 1987 and 1990, 22 samples have been collected (4 sugar beet, 7 fodder beet and 11 beet root). Multiplication of *Beta* samples is conducted in farmers' back gardens or, in the case of Hungarian local types, in fields near the place of origin. The general technique applied is the traditional two year cycle system. Some attempts have been made to introduce autumn planting and the leaving of roots in the field during winter but with little success. In many cases, local types are not sufficiently winter-hardy and a high proportion of plants die because of frost damage. During the last four years, 157 accessions were multiplied. Evaluation of collections, using the IBPGR descriptor list, has started recently.

Dried seed samples are stored for medium and long term storage in air tight glass jars at 0° C and -20° C as active and base collections, respectively.

Documentation of passport data of 197 accessions has been completed. Evaluation data have not yet been computerized. The software used was dBase III Plus. *Beta* germplasm seed samples are freely available and will be distributed on request.

Poland

The *Beta* collection was established, in 1981, at the IHAR at Bydgoszcz, as a unit of the National Department of Plant Genetic Resources at the Plant Breeding and Acclimatization Institute at Radzikow. This Department coordinates, finances and stores information in the central database and provides long term storage facilities for genetic resources of crop plants in Poland. The collection at Bydgoszcz includes wild species of the sections *Procumbentes, Nanae, Corollinae,* and *Beta*. Cyto-embryological and biochemical investigations have been conducted with these wild species and crossing with sugar beet and between wild species has been carried out, especially in the sections *Corollinae* and *Procumbentes*. The results yielded unique information about

the degree of the affinity between species, about apomixis in the section *Corollinae* and about the possibility of obtaining hybrids with sugar beet. Lines resistant to mosaic virus have been obtained from crosses between sugar beet and *B. corolliflora*. This research programme is partly financed by the USDA.

The main motivation for organizing *Beta* collections was the change in the breeding methods for sugar- and fodder beets. Breeding of monogerm hybrid varieties based on male sterile lines caused a rapid disappearance of multigerm population varieties from the market and the National Variety List. Therefore, it became essential for ongoing breeding and for the future to collect the population varieties with a broad range of variation. The activities focussed on are: collection, maintenance, evaluation of wild species and cultivated beets and the documentation of the resulting data. There are 29 accessions of wild species of sections *Procumbentes, Nanae, Corollinae* and *Beta*, 91 accessions of sugar beet and 78 accessions of fodder beet in the collection.

IBPGR-recommended *Beta* descriptors were chosen for the evaluation of collected materials. Evaluation and seed multiplication are done at Bydgoszcz (wild species) and at the Breeding Station at Konczwice. Cultivated beets are evaluated on 10 m^2 plots in two replications with standard varieties. For virus testing, the ELISA test is used. The most valuable genotypes are propagated by *in vitro* culture.

A three year evaluation cycle has been completed for 14 wild species and 70 cultivated sugar- and fodder beet accessions. Two years ago, a pre-breeding programme was started. It concerns:

- The possibility of using male sterility from *B. maritima* in sugar beet breeding.
- The crossing of annual, monogerm, wild *B. orientalis* with sugar beet, selection of biennial offspring of this cross and studying monogermity originating from this wild species.

New accessions obtained from foreign counterparts (in Belgium, Bulgaria, Greece, UK, the Netherlands, the USA and elsewhere) were added to the collection. No collection mission was organized abroad. In Poland wild *Beta* species do not occur naturally and landraces can not be found.

For long term storage (-20° C) 142 accessions were sent to the National Department of Plant Genetic Resources at Radzikow. The active collection is stored at Bydgoszcz at temperatures between 0° C and 4° C. Passport and seed stock data of 95 accessions were sent to the IDBB. Data are being completed successively and will continue to be sent to the IDBB. For data documentation, an IBM PC/AT computer and dBase III Plus software are used. The collected material is used in sugar- and fodder beet breeding at IHAR.

Romania

A national *Beta* genetic resources collection does not exist in Romania. The Department of Sugar Beet of the Institute at Fundulea (founded in 1981) is engaged in breeding new varieties for Romanian conditions. Besides this work, in recent years some attention has been given to wild species of the genus *Beta*. Seed samples received from several botanical gardens were multiplied on a small scale.

It has been decided to collect wild species existing in Romania. There is evidence that some wild species can be found in the delta of the Danube and several uncultivated places. This material will be used for crossing with cultivated beets in the resistance breeding programme in order to obtain initial materials for resistance to *Cercospora* and Rhizomania. It will be great improvement if, starting from this material and from population varieties originating from Romania, a national collection could be founded. Dr. Z. Stanescu is interested in wild species of the genus *Beta* and will take the necessary initiative.

International cooperation between the countries

From the 20th to the 25th of September, 1980, a meeting of curators of *Beta* and *Brassica* collections was held at Radzikow (Poland), as a part of scientific cooperation between COMECON countries. During this meeting the descriptors for *Beta, Brassica rapa* and *Brassica napus* were established and subsequently published at the VIR, Leningrad. Also, the exchange of seed samples between collections was conducted. As was described above, the work on *Beta* collections has advanced to different degrees in the different countries. In all collections, improvements, changes and some assistance from IBPGR and IDBB are needed. It should be stressed that the countries of this region are, at present, in the state of deep economical crisis and organizational change of their national economies. Some limitations in funding of scientific research can be expected but, currently, it is difficult to predict to what extent this may occur. In spite of the current situation, it is hoped that all curators will try to preserve the germplasm in each country. The establishment of the International Database for *Beta* and of the World *Beta* Network was of great advantage to the *Beta* collections and to breeders. The meetings of the Network are of great importance for the rationalization of the international and national germplasm activities and give the opportunity for personal contacts and discussions between *Beta* curators from different parts of the world.

Beet genetic resources activities of some Mediterranean countries (Spain, Greece and Turkey)

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Introduction

This paper summarizes the *Beta* genetic resources activities of Turkey, Greece and Spain which are the major distribution areas of section *Beta* and the sections *Corollinae, Nanae,* and *Procumbentes.*

The information received from G. Perez, of the National Institute for Agricultural Research (INIA) in Spain, indicates that there is no specific programme or national *Beta* genetic resources project in that country. In Greece, the activities are organized by H. Cortessi of the Hellenic Sugar Industry (HSI) working in close cooperation with the Greek Gene Bank (GGB). In Turkey, *Beta* genetic resources activities are coordinated by A. Tan of the Plant Genetic Resources Research Institute (PGRRI), within the framework of the National Plant Genetic Resources Research Project.

Activities

Collection

No specific beet collecting missions were organized in Spain or in Greece. However, three *Beta* accessions were collected by GGB during a polycrop collecting mission to Crete in 1990. As reported by the International Database for Beta (IDBB), ten accessions of *Beta* have been included in the GGB holding since 1989. It is reported from Spain that some *Beta* species were collected during the horticultural crop expedition programme. Furthermore, some accessions of *Beta* were collected from Spain by the Centre for Genetic Resources of the Netherlands (CGN) in 1989 (CGN Annual Report, 1989).

In 1989, a special collection and survey programme for wild *Beta* species was carried out in the transitional region between central Anatolia and the western Black Sea region. This was done in cooperation with a sugar company. During this expedition six accessions of section *Corollinae* species, and 13 of section *Beta* species were collected. Eight accessions of section *Beta* from the eastern Black Sea region were also collected. In 1990, an expedition was organized jointly with the CGN to cover the 18 provinces in southern and western Anatolia and the western Anatolian transitional region where species of sections *Beta* and *Corollinae* (excluding *B. macrorhiza)* occur. There were 151 accession collected during this expedition. As much as possible of the available genetic diversity was sampled for each population and more data related to plant habitat was collected in order to enable studies on relations between plant and ecogeographical characters.

It is planned to collect landraces growing at high altitudes in Greece because such landraces may have a great potential as sources of bolting resistance. Additionally, collecting on Ipiros and Thrace for section *Beta* species, and some sites for collecting

section *Nanae* species should be considered. The reported reasons for further collection of B. *nanae* are:

- very few areas have been explored for collection;
- the species faces a severe threat of extinction; and
- an insufficient amount of seed per sample was collected previously.

From the report of the IDBB, north-western, north-eastern and inland Spain seem not to have been explored. For the further systematic collection programme, the valuable inland types of wild beet and landraces and the wild beets along the coast should be found in order to contribute to the Spanish *Beta* collection.

In Turkey, a further *Beta* collection programme is planned to fill the gaps in areas where *Beta* species, both wild and landraces, possibly occur and which are not yet represented in the PGRRI *Beta* collection. Special attention will be given to *B. macrorhiza* because insufficient collections were made previously.

Preservation

Genebank facilities are used to preserve the base and active collections of *Beta* accessions in Spain, Greece and Turkey. Base collections are preserved in long-term storage at -18°C in Spain, at between -18°C and 20°C in Turkey, and at -22°C in Greece. Whereas, for medium-term storage, the cold room facilities are working at 0°C in Turkey and Greece, and at -2°C in Spain.

Beta accessions held by the genebanks total 115 in Spain, 741 in Greece and 328 in Turkey (IDBB Report, 1991).

Multiplication and regeneration

Wild species of *Beta* generate more problems than do cultivated beets and require special attention during seed preparation and seed increase. All species of section *Beta* and some species of section *Corollinae* (with the exception of apomictic species such as *B. trigyna*, and *B. intermedia*) need isolation. *Beta nana*, *B. macrorhiza* and the species of section *Procumbentes* have to be increased under controlled conditions. These materials need more attention because of their hard-walled fruits and lesser adaptation to cultivation.

In Greece, isolation cages were used to avoid contamination with respect to genetic purity of increased seed of the cross pollinated species. Spatial isolation was mainly used in Turkey.

Seeds of *Corollinae* species usually fail to germinate. The hard cover of the seeds has to be removed manually or by acid scarification, to prompt germination. Therefore, seed preparation of those species is very laborious.

In Spain, approximately 15 % of the collection was multiplied and in Greece about half of the accessions have been regenerated. The PGRRI in Turkey is in the process of multiplying/regenerating that part of its *Beta* collection that has not already been done. Some *Corollinae* material of the Dutch/German *Beta* collection and some *Beta* section *Beta* material of the Birmingham collection have also been multiplied by the PGRRI.

Documentation

Beta accessions are documented using database system facilities at the genebanks in Spain, Greece and Turkey. The facilities are CYBER 180/810 in Spain, APPLE II Europlus in Greece and PC/AT in Turkey. For documentation of plant genetic resources material information, the GGB use dBase II and PGRRI uses dBase III. By using their systems and facilities, 756 and 328 *Beta* accessions have been registered and documented at GGB and PGRRI, respectively.

The survey information was also kept by PGRRI for future expedition programmes. Passport information of all collections from Turkey was requested by PGRRI, from the IDBB, for the determination of previous collection sites, and the status of these collections.

Evaluation

The information received from Spain and Greece indicates that there is no specific evaluation programme in these countries. However, some characteristics were noted during the HSI regeneration programme.

In Turkey, wild *Beta* accessions are continuously assessed for characteristics. At PGRRI, almost 150 accessions have been processed and classification and taxonomic identification of some wild accessions has been completed since 1989. Furthermore, biosystematic studies are also conducted.

Conclusion

The *Beta* genetic resources programmes of Spain, Greece, and Turkey should be implemented more actively since those countries have valuable germplasm sources for *Beta* research and breeding projects. Particular emphasis should be given to the determination of future activities and necessary steps for the enhancement of *Beta* programmes.

Regional *Beta* genetic resources activities: Northwest European region

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Most (if not all) indigenous *Beta* germplasm in Northwest Europe is represented by *Beta maritima*. Most collections of *Beta maritima* have been made recently by Devon Doney for the USDA in collaboration with national counterparts. These collections will be described separately. A summary of other *Beta* germplasm activities follows.

Belgium

Organisation:	Ministry of Agriculture (RvP) for fodder beet germplasm ICI/SES for sugar beet germplasm Storage at +3 °C or -20 °C 200 accessions
Seed increase:	Some multiplication, but difficulty with isolation
Documentation:	IBM, Lotus 1,2,3 available but not currently used
Evaluation:	Some evaluation for root shape, bolting, tare, fanginess, disease resistance
Breeding:	Some use of germplasm

France

Organisation:	INRA-Dijon 2700 accessions of 180 ecotypes including 145 of *B. vulgaris* ssp. *maritima*
Collection:	In France in 1989 by Laby and Doney
Seed increase:	Some multiplication
Documentation:	dBase III, with minimal passport data
Research:	Cytoplasmic and nuclear molecular genetics

Switzerland

Organisation:	Federal Agricultural Research Station, Nyon 70 accessions of *B. vulgaris* ssp. *cicla* var. *flavescens* from the Rhone Valley, Lyon/Geneva. Collection is due to be duplicated at Braunschweig.
Collection:	Swiss chard landraces collected since 1980, and continuing
Seed increase:	Regeneration when required after germination tests. Isolation a problem.
Documentation:	Passport data only are computerized.
Breeding;	A new variety of Swiss chard developed ("Berac"). Now developing a monogerm hybrid variety.

Germany

Organisation:	Klein Wanzleben (former GDR) with assistance from Gatersleben. Most work with wild beet is concentrated at Klein Wanzleben. Special collection of cytoplasm types.
Collection:	Participation in possible collecting in southern USSR with the Vavilov Institute, CGN and FAL, Braunschweig in 1991/2.
Seed increase:	Wild perennial species maintained. Some *in vitro* regeneration.
Documentation:	In common with IDBB.
Evaluation:	Screening (RFLP) for cms, screening for virus resistance (BMYV, BYV and BMV).
Research:	Fundamental aspects of nematode resistance in wild species (with University of Kiel), RFLPs and wild species (with University of Halle/S).

Netherlands/Germany

Organisation:	National *Beta* genetic resources programme (Netherlands and Germany). Germplasm held at Braunschweig and Wageningen. 1804 accessions.
Collecting:	1990 - Armenia/Daghestan (8 accessions), W/SW Turkey (146 accessions) Joint with Vavilov Institute, CGN, FAL. 1992 - second expedition to Daghestan /Azerbaidjan, and Turkey. 1993-5 - proposed to Spain, Italy and Balkan countries.
Seed increase:	828 accessions available for exchange (over 50g). Seed increase taking place for 800 accessions (44% hard-seeded species). Major problems with *B. nana* and *B. macrorhiza*.
Documentation:	Passport data duplicated at CGN and FAL, DBMS based on ORACLE.
Research:	Conducted by CPO on rhizomania and *Polymyxa* and nematode resistance. Also new sources of cms. Biosystematic and taxonomic research on section Beta is carried out at the Agricultural University of Wageningen in association with CGN.

United Kingdom

Organisation:	HRI, Wellesbourne holds a small number of accessions of garden beets. Kew genebank holds UK *B. maritima* duplicated by USDA. University of Birmingham holds a working collection of wild and cultivated beets, most accessions of which are duplicated at GGB, Plant Genetic resources Research Institute, Izmir or CGN/FAL.
Collection:	UK *B. maritima* has recently been collected by Devon Doney (USDA) in collaboration with Kew genebank.
Seed increase:	None at Kew. Some University of Birmingham accessions have been multiplied in collaboration with breeding companies, CGN and PGRRI, Izmir. More still require regeneration.

Documentation:	Passport data held in dBase IV, with data duplicated at CGN.
Evaluation:	Evaluation for disease resistance (*Polymyxa*, BMYV) is carried out in collaboration with Broom's Barn Experiment Station.
Research:	Research at University of Birmingham includes some tissue culture and genetic manipulation (with British Sugar), use of isozmes, RFLPs and RAPD for studying genetic variation, and the use of transposon tagging for gene isolation and cloning (with Broom's Barn). RFLPs as molecular markers have been developed with Lion Seeds (formerly Nickerson's).

Agenda

Tuesday, 25-06-91

9.00 - 9.20	Opening and welcoming addresses by *Prof. M. Dambroth* (FAL) by *P. Perret* (IBPGR)
9.20 - 9.30	Election of the chairmen and secretary
9.30 - 10.30	Business report of the *Beta* Coordinating Committee (BCC) and progress report of the IDBB by *L. Frese*
10.30 - 11.00	Coffee break

Regional Beta genetic resources activities

11.00 - 11.30	Beet genetic resources: North American activities by *D. Doney*
11.30 - 12.00	*Beta* genetic resources activities in Asia by *H.M. Srivastava*
12.00 - 12.30	Regional *Beta* genetic resources activities: East European region by *L. Dalke*
12.30 - 13.45	Lunch
13.45 - 14.15	Beet genetic resources activities of some Mediterranean countries (Spain, Greece and Turkey) by *A. Tan*
14.15 - 14.45	Regional *Beta* genetic resources activities: Northwest European region by *B.V. Ford-Lloyd*

Working session on collaborative activities in 1992 and 1993

14.45 - 17.30	Data exchange, collecting missions
16.00 - 16.15	Coffee break
16.15 - 17.30	Joint seed increase programme, germplasm evaluation
17.30 - 18.00	Poster installation
18.00	Barbecue at the Forum of the FAL

Wednesday, 26-06-91

Working session on collaborative activities in 1992 and 1993

9.00 - 10.30	Utilization and research

10.30 - 11.00	Coffee break
11.00 - 12.00	Programme and funding of the third Network meeting. Election of the BCC and end of the working session.
12.00 - 13.15	Lunch

Lectures on biosystematics and taxonomy

13.15 - 14.00	Ecogeographical differentiation and ecotype formation by *J. Smartt*
14.00 - 14.30	Genetic variability for life-histories of *Beta maritima* by *H. van Dijk*
14.30 - 15.00	Morphology of North Atlantic wild *Beta* by *D. Doney*
15.00 - 15.30	Patterns of morphological and isozyme differentiation in wild taxa of *Beta* section *Beta* by *J. Letschert*
15.30 - 16.00	Coffee break
16.00 - 16.30	Isozyme and RFLP research on wild and cultivated forms of section *Beta* by *B. V. Ford-Lloyd*
16.30 - 17.00	Molecular analysis of the *Beta* genome by *C. Jung*
17.00 - 17.30	Male sterility and DNA polymorphism in *Beta maritima* *by J. Cuguen*

Thursday, 27-06-91

Lectures on biosystematics and taxonomy

9.00 - 09.30	Characterization of *Beta* species by DNA- and isozym analysis by *H. Budhan* (not presented)
9.30 - 10.00	Species relationship in the genus *Beta* as revealed by crossing experiments by *B. Jassem*
10.00 - 10.15	Coffee break

Poster session and report writing

10.15 - 11.15 Presentation of evaluation and research projects.

- Results and conception of collaborative activities of evaluation of *Beta* gene resources of Klein Wanzleben 1983 - 1991
  by *R. Melzer*
- Application of *in vitro* methods for conservation, multiplication and production of *Beta*- DH-lines
  by *K. Lohmann et al.*
- Genetic stability in tissue cultures of beet assessed by isozyme and RFLPs

by *B. V. Ford-Lloyd*

- Germplasm collection of leaf beet (*B.vulgaris* ssp. *cicla*) in China
  by *Sun Yi-Chu*
- Application of mapping systems for analysis of the geographical origin of collected material
  by *T. Hazekamp* and *L. Frese*
- Breeding and improvement of sugar beet for subtropical climates
  by *H.M. Srivastava*

11.15 - 12.15	Meeting of the EC-working group (research project proposal DGXII)
12.15 - 13.30	Lunch
13.30 - 16.00	Visit to the Institute
16.00 - 17.00	Consideration of the report and its approval by the participants. Other matters.

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Presented Papers

Ecogeographical differentiation and ecotype formation

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Introduction

When a species such as *Beta vulgaris* L. (*s.l.*), which has a wide geographic distribution, is examined morphologically, differences may or may not be apparent between forms of different geographical provenance. It is necessary for those who are interested in such variation to have an adequate understanding of the biosystematics of their target taxon and appreciate the real nature and extent not only of phenotypic variation but also of the underlying genetic variation which produces it. For this reason, a good taxonomic treatment is essential for those interested in the genetic resources field. It greatly facilitates the setting up, organization and management of gene banks to have such a system available.

Table 1: Taxonomy of the genus *Beta*

Section	Species	Subspecies
Beta	*B. vulgaris*	*maritima*
		macrocarpa
		atriplicifolia *
		trojana *
		adanensis
		patula *
		orientalis *
		cicla (leaf beets)
		vulgaris (root beets)
Corollinae	*B. corolliflora*	
	B. macrorhiza	
	B. lomatogona	
	B. trigyna	
	B. intermedia	
Nanae	*B. nana*	
Procumbentes	*B. procumbens*	
	B. patellaris	
	B. webbiana	

* These former specific epithets may have potential value in future in denoting geographical populations.

Table 2: Genetic resources of cultivated beets (*Beta vulgaris* L.) in terms of Harlan and de Wet gene pools

Gene Pool		Taxa	
Primary (GP1)	division A domesticated	Sect. *Beta*	*B. vulgaris* ssp. *vulgaris* *B. vulgaris* ssp. *cicla*
	division B		*B. vulgaris* ssp. *maritima* *B. vulgaris* ssp. *macrocarpa* *B. vulgaris* ssp. *adanensis*
Secondary (GP2)		Sect. *Corollinae*	*B. corolliflora* *B. macrorhiza* *B. lomatogona* *B. trigyna* *B. intermedia*
		Sect. *Procumbentes*	*B. procumbens* *B. patellaris* *B. webbiana*
Tertiary (GP3)		Sect. *Nanae* Genus *Spinacia*	*B. nana*

As is well known, there are a number of different approaches to the development of taxonomic systems. However, the classical typological approach and that using the biological species concept are those which can best be used in the present context. The biological species approach has considerable appeal to those involved in collection, evaluation and utilization of plant genetic resources since it indicates the relative accessibility of the genetic resources residing in a particular species and congeneric forms of potential value (Table 1). This concept has been developed very usefully by Harlan and de Wet (1971) in the system which categorizes biosystematic relationships in terms of relative accessibility to the target crops and the biological species of which it is a part (Table 2).

Historically, the approach to classification of *Beta* has been typological. This has resulted in the establishment of several sections within the genus, with one or more species within each section. Study of crossing relationships between and within sections indicates that, in section *Beta*, crossing barriers are not very strongly developed. Those which do occur appear, when studied, to have a relatively simple cause such as simple reciprocal translocation, which can arise quite commonly within accepted "good" species. The result of these studies has been the reduction in rank of former species to subspecific or even lower ranking in the hierarchy (Table 1). The view of Ford-Lloyd (1986) that within section *Beta* of the genus there is effectively a single (biological) species, *Beta vulgaris*, with three wild subspecies (*maritima*, *macrocarpa*, and *adanensis*) and two groups of domesticated subspecies (*vulgaris* and *cicla*) is a welcome simplification. He also recognizes the possibility of further simplification, in the reduction to two wild and a single domesticated subspecies: ssp. *maritima* and ssp. *macrocarpa* (wild) and ssp. *vulgaris* (domesticated).

The relationship between a formal taxonomic treatment (Table 1) and that organized according to the gene pool system of Harlan and de Wet (1971) (Table 2) is one which should be worth further exploration and development. There are two components to the primary gene pool (GP1). The first consists of the domesticated forms *B. vulgaris* ssp. *cicla* and *B. vulgaris* ssp. *vulgaris* (the A segment) and the second (the B segment) comprises subspecies *maritima, macrocarpa,* and *adanensis*. Species of the secondary gene pool (GP2) comprise some, if not all, members of the sections *Corollinae* and *Procumbentes*. More exploration of these relationships by crossing and isozyme studies could be of obvious value. Apomixis in the *Corollinae* is a considerable complication but electrophoretic studies in addition to crossing studies, such as those of Jassen (1980) would be of particular value in the clarification of the situation. The GP3 appears to comprise section *Nanae* and the genus *Spinacia*.

Ecogeographical differentiation and ecotype formation

In taxonomic terms, ecogeographic variation in wild populations of *Beta vulgaris* is recognized at the subspecies level in ssp. *maritima* (L.) Thell., *macrocarpa* Thell. and *adanensis* Ford-Lloyd and Williams. These have characteristic ranges which overlap somewhat. The *maritima* forms are to be found largely along the Atlantic coasts of Europe and north Africa and in the Mediterranean, while ssp. *macrocarpa* predominates in the western Mediterranean basin and ssp. *adanensis* is centred in the eastern Mediterranean area. It is useful to examine the relative time scale on which the present distribution of these different forms was achieved. That of the Mediterranean forms is of very long standing in contrast to that of ssp. *maritima* which has achieved much of its present distribution only in the present interglacial period. This means that, in northern and western Europe, most of the present populations could have been in place for ten thousand years or less. With the recession of the ice sheets, populations close to their edges would be drawn into the ecological vacuum and continually drawn northwards until the position of the ice sheets stabilized. In the areas originally occupied i.e. in the south, selection pressures favouring adaptation to the new climatic circumstances would then produce waves of migration northward following the retreating ice sheet.

The question is posed as to how this migration was achieved. The answer is straightforward. The seed-balls of *Beta* are resistant to salt water and could be carried on ocean currents such as the Gulf Stream (Dale and Ford-Lloyd, 1985) in a generally northerly direction. This, coupled with prevailing south-westerly wind reaching gale force at times (especially at the autumnal equinox), could have provided (and may still provide) a very effective means of dispersal. The ocean current thus moves propagules over relatively long distances and strong winds distribute them on the shore, above the high water line and even inland. Although sea beet is self-incompatible, germination of the multigerm seed-balls should frequently produce sexually compatible combinations (Dale and Ford-Lloyd, 1983). This circumstance should reduce the frequency of abortive colonization in favourable ecological conditions. These conditions could extend well beyond the shoreline to inland habitats at low and moderate elevations. Strong winds could provide effective dispersal from a shore line source to such habitats.

Assuming that relative climatic stability had been achieved and that suitable habitats had been reached and colonized, selection pressure, lead by increasing severity of competition, would tend to increase closeness of adaptation to the immediate environmental conditions. There are two, or possibly three, ways in which this might have come about: by the generation of adaptive clines or the evolution of ecotypes or, perhaps, even a combination of both. The seashore environment tends to be

one with, often, very marked ecological gradients. Optimal levels of adaptation to such gradients are achievable with a xenogamous or random mating system, as demonstrated in another strand plant, *Plantago maritima*, by Gregor (1938).

It is, by and large, the seashore populations of *B. vulgaris* which have been most extensively collected and studied. Variation in these populations has been observed in vegetative and reproductive morphology but it is the morphological features of the reproductive system which are of the greatest value in distinguishing populations, largely on a geographical basis (e.g. *B. vulgaris* ssp. *adanensis* and *B. vulgaris* ssp. *macrocarpa* in the Mediterranean, Buttler, 1977). There is little apparent patterning of vegetative morphological variation either in a geographical or broad ecological sense. To put it another way, the adaptation of the species to the seashore environment has not entailed partitioning of the habitat and the production of clear-cut and recognizable ecotypes but rather the development of numerous different genotypes capable of coping with the range of environmental conditions likely to be encountered in a complex habitat such as the seashore.

It might be expected, however, [illegible] tion pressures operating on inland populations could produce forms diverg[illegible] bly from those of the seashore, thus producing distinct ecotypes. Quite probably that such inland populations provided the base populations for domestication. It is commonly noted that inland beets are ruderal and characteristic of disturbed habitats. The disturbed nature of the seashore habitat has evoked an adaptive response in coastal beets and, in a rather curious way, this response is also adaptive in agricultural habitats. Inland populations are much more widely dispersed and are smaller than those of the coast. This makes them more difficult to investigate, but may well prove to be a productive avenue to explore. It is worth noting that one of the consequences of domestication was the establishment of an agricultural ecotype which diverged initially from its wild prototype and ultimately generated the impressive range of morphotypes we see in the leaf beets, chards, fodder and sugar beets of the present time. The maintenance of these different morphotypes is only effected by strict control of breeding. The selected populations are allowed to inter-pollinate, while the products of out-pollination are rigorously eliminated from the population (as far as possible). Without this domestication, populations would neither have produced the present range of morphotypes nor would these have been maintained over the centuries. This situation provides a clue to the apparent lack of differentiation of the seashore population into specialized ecotypic variants adapted to partitioned component subhabitats of the admittedly complex seashore habitat. Xenogamy, as expressed in *B. vulgaris* ssp. *maritima*, would clearly militate against development of ecotypes on the seashore but could permit it in small, isolated inland communities where gene flow through pollen from external sources would be minimized.

Adaptive response to the complex ecological demands of the habitat on maritime beet is probably achieved by a different mechanism. Buttler (1977) described morphological variants in respect of growth form within *B. vulgaris* ssp. *macrocarpa* and noted that production of these variant forms had no clear-cut genetic base. Presumably, the different growth forms were responses evoked from the genotype by different environmental conditions, i.e. through phenotypic plasticity. While appropriate growth forms might be evoked by a range of different environmental pressures, different genotypes could well respond in a similar fashion to a similar ecological pressure, producing convergence in phenotype. In other words, there could be a homoeostatic response. Phenotypic plasticity is the morphological expression of a well-buffered genotype. In this context, ecophysiological adaptability is another form of expression of this kind of genotype.

In the absence of compelling evidence for the evolution of distinct and distinctive ecotypes within the seashore communities of the *vulgaris* ssp. *maritima*, the pattern of ecogeographic variation can best be understood as a system of clines. The major northward migration could have established a primary geographical cline, while movement from the high-water mark inland would tend to generate a secondary topographical cline, up the beach and perhaps some way inland. The existence of ecological and climatic gradients, coupled with phenotypic plasticity or ecophysiological adaptability, is compatible with the observed patterning of variation which is apparent in the Atlantic, and especially the North Atlantic, populations of maritime beet. The almost random nature of the distribution of morphological variation in these populations contrasts strongly with that in the Mediterranean populations, where there is some apparent patterning of variation. The evolution of this pattern of ecogeographical variation has been concisely summarized by Frese (1991).

The ecotype concept and *Beta vulgaris*

A good definition of an ecotype is that it is a sub-division of an ecospecies whose members are generally fertile in crosses, both within the ecotype and between ecotypes, but which retain their individuality because of particularly suitable environmental adaptation.

In point of fact, there are other features of ecotypes in the original Turessonian sense of the word (Turesson, 1925). Ecotypes are fixed genetically and they may occur sympatrically in different habitats within the same geographical area. There are conditions which can be considered as conducive to ecotype formation. These include:

- Sharply discontinuous habitats
- Relative uniformity within habitats
- Predominantly autogamous breeding system
- Low levels of recruitment to population by seed
- Low levels of gene flow to population through pollen
- Perennial life form

Consideration of these factors in relation to the situation in *Beta vulgaris* shows that rather few of these criteria are met. In the case of *B. vulgaris* ssp. *maritima*, the breeding system is xenogamous while life form is perennial or biennial. In the Mediterranean populations, autogamy is possible but the life form is annual. Although the Mediterranean populations have been *in situ* for longer than the North Atlantic populations no more evidence has come to light of ecotype formation in this region. In xenogamous populations of the Atlantic coasts, under the most favourable conditions for pollination, winds in the morning will tend to move pollen from the lower to the upper shore and inland. At midday the breeze will drop and in the afternoon and evening breezes will tend to move pollen from the upper part (and perhaps further inland) to the lower shore, tending to promote panmixis. In such circumstances, not only will ecotype formation be inhibited but the existence of clines will only be maintained by strong selection against conferring any adaptive disadvantage in specific microhabitats.

It is reasonable to conclude, therefore, that in the seashore habitat, conditions are not favourable for ecotype formation in *Beta*. In circumstances where gene flow into the population is drastically reduced, by isolation, for instance, ecotypic development becomes a possibility. Under domestication the products of extra population matings are strongly selected against, and it is not difficult to visualize natural situations

where natural selection might operate strongly against the products of matings from outside the population. Adaptation to extremely saline conditions, very high altitude or other extreme environmental conditions may, possibly, only be maintained by minimizing effective gene flow. Ecotype formation can arguably be considered to have taken place under domestication and to have met the terms of the definitions satisfactorily. Under natural conditions ecotype formation, where it does occur, may be somewhat cryptic in nature and be manifested in genetically controlled, ecophysiological rather than morphological differences. Chemotaxonomic studies such as isozyme polymorphisms may give useful indications as to whether any such differentiation has, in fact, taken place.

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Genetic variability for life-histories in *Beta maritima*

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Introduction

People cultivating and breeding beets usually consider the species *Beta vulgaris* to be biennial; a perfectly biennial species, if there were not, every now and then, troublesome annual individuals. Floras record that *Beta maritima*, the wild ancestor of the cultivated beets and, therefore, the same species, is a perennial. These floras must be correct, because one can sometimes find very old individuals with stems like tree roots. To decide how to characterize this species or its genotypes, if there is genetic variation at this level, the words annual, biennial and perennial have to be defined. Table 1 shows the authors' definitions:

Table 1. A survey of the main life cycles

	Flowering in the first year	No flowering in the first year
No survival after first flowering	Annual	Biennial
Survival after first flowering	Perennial (early flowering)	Perennial (late flowering)

Both "flowering in the first year" and "survival after flowering" are meant in the sense of a physiological potential at sufficient day-length but without any vernalization. With both potentials present the individual will be an early flowering perennial even if the environment is not sufficiently favourable to realize one or both of them. A biennial or a late flowering perennial cannot flower in the first year because the necessary hormones are lacking, and, in the same way, annuals and biennials cannot survive after flowering because there is no investment in reserves for a new growing season. In addition to the four possibilities of Table 1, other developmental parameters may be of interest. If a plant flowers, does this flowering start early or late after germination or early or late in the second season? If a plant keeps its vegetative status, does it form a single rosette? Does it bolt without flowering? Will it form side rosettes or perhaps lateral stems? In this paper the authors restrict the discussion mainly to the time of flowering; firstly, the year in which first flowering happens and secondly, its earliness.

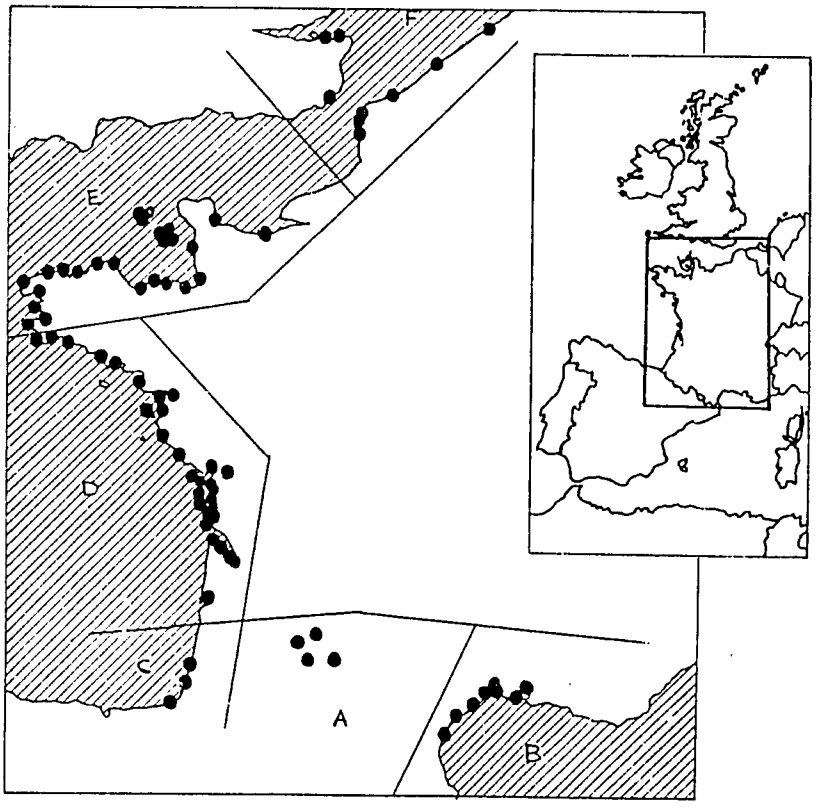

Figure 1: Geographic origin of populations sampled in 1989

Results

Bolting and flowering in the first year in natural populations

Almost 100 wild beet populations were sampled in 1989. Seeds were collected separately for each female plant. Because beets are self-incompatible, the seed collection consists of a number of half-sib families from each population. The number per population ranges from 1 to 30. Figure 1 shows these populations. They are mostly situated in France, but there are also some British, Belgian and Dutch populations. Beets almost always grow near the coast, or near salt or brackish water. An exception is the region in the south-west of France where wild beets are present far from the sea (but not far from the seed production fields).

When seeds of the available populations are sown in a greenhouse at 20° C and 16 hours day-length in sufficiently large pots, a certain percentage of the plants bolt and flower. This percentage ranges from 0%, for the northern populations, to almost 100% for the inland and some Mediterranean populations. The map has been divided into six regions (Fig.1):

A:	inland populations
B:	Mediterranean coastal populations
C,D,E F:	Atlantic coastal populations,

The percentage of flowering in the first year in each region is given in Table 2:

Table 2. Percentages of first-year flowering and the average time between germination and flowering within the regions of Fig. 1

Region	Total sample size	% first-year flowering	Days to flowering
A	40	98	50
B	60	75	65
C	20	0	--
D	570	6	63
E	195	0	--
F	88	0	--

Plants from inland populations flower, on the average, two weeks earlier than those from coastal populations. There is no significant difference between Mediterranean and Atlantic coastal populations.

Genetics and physiology of bolting and flowering in the first year

The first question that should be asked after obtaining the results mentioned above, is whether or not the well known "bolting gene", B, is responsible for first-year bolting and flowering in these natural populations. Furthermore, the genetic basis, if any, of the variation in earliness of flowering is of interest. The B gene was discovered in the 1920s and has been shown to be located on the same chromosome as the root color genes, yellow and red. Together they form the YRB linkage group. B is said to be partially dominant over b. This is something that needs to be investigated more precisely because the authors have found several very early bolting heterozygotes. The product of the B gene is still unknown after all these years. The gene is not directly involved in the synthesis of the hormone gibberellin, although bolting is accompanied by high levels of this hormone. Application of gibberellin (GA_3) to a non-bolting plant forces bolting, but without flowering. After the treatment is stopped, new rosettes form on the stem (perched rosettes). In fact, the B gene cancels the need for vernalization. Vernalization happens over a rather long period (at least several weeks) of low temperatures, preferably between 0° C and 10° C. The genotypes BB and Bb are able to bolt and flower after a period of constantly high temperatures, but the genotype bb needs vernalization in order to flower. A second external factor which is necessary for bolting, but which cannot be replaced by the B gene, is a long day (at least 14 hours for a non-vernalized plant). The interaction between all factors is as follows:

Vernalization or genotype BB/Bb	Long day	Application of gibberellin	
+	+	-	==> bolting and flowering
+	-	-	==> no bolting, no flowering
-	+	-	==> no bolting, no flowering
-	-	-	==> no bolting, no flowering
-	+	+	==> bolting without flowering
+	-	+	==> bolting and flowering*
-	-	+	==> bolting without flowering

* depends on vernalization intensity

In the absence of the B gene, both vernalization intensity and day-length are quantitative variables; they can, to a certain extent, replace each other's effects. Their common mechanism must be the formation of gibberellin, but during vernalization some other process of unknown character also occurs. The B gene is a major gene for the quantitative character, time of flowering. Theoretically it is possible that a series of minor genes can replace or mask the B gene. To be able to be sure about the role of the B gene in natural populations, genetic markers are necessary. Three allozyme loci, ICD (isocitrate dehydrogenase), GOT-1 and GOT-2 (aminotransferases), are good candidates for marker genes. They are situated in the YRB-linkage group at about the right distance from R, but at which side (the same side as B or the opposite one) is not yet known and has to be determined. So far, crossing results are not in contradiction with a B/b polymorphism in coastal populations; crosses between first-year-flowering plants giving either 100% or about 75% immediate flowering.

Earliness of flowering in the first year

Among plants that flower in their first year, there is much variation in the time between germination and flowering. The earliest plants started flowering 33 days after germination, but others needed more than 100 days. Mean temperature during the experiments, which could not always be controlled very strictly, considerably influenced the actual duration, but with the use of common families in different experiments, the heritability of earliness could be estimated. Two generations of artificial selection for earliness in plants from the inland populations, which were already rather early, gave heritability estimations of > 0.8 and 0.72 respectively. Using plants from coastal populations the heritability after one generation of artificial selection could be estimated at 0.82. Although these results are not very precise because of the scaling differences between experiments, it can be concluded that variation in earliness under controlled conditions is largely due to genetic differences.

Need for vernalization

Plants that do not flower in their first year need a certain vernalization intensity to initiate flowering. In one of the authors' experiments, plants from 28 populations all over the range of the populations sampled were moderately vernalized by placing three-month-old greenhouse plants outside in early April, a rather cold month in Lille in 1990. Apparently this intensity was sufficient for most, but not all, of the plants. Table 3 indicates how many plants started flowering in each month after this cold period. Populations are combined according to the subdivisions used in Fig. 1.

A north-south cline seems to exist for the need for vernalization. The two plants from the Mediterranean coast which had not already flowered in the greenhouse, flowered immediately after the cold period outside. Contrarily, plants from the north flowered either late or not at all. All plants from region A, in this experiment, had already flowered in the greenhouse.

Flowering in the second year

A long term study on flowering periods and survival has been carried out with plants from all available populations. In June 1990, seeds of all populations were sown in the greenhouse. According to Table 2, some of these plants flowered immediately (92 plants, 630 plants remaining in the vegetative state). In the greenhouse, day-length was not controlled, and temperature during winter was kept between 2° C and 12° C.

Almost all plants survived the winter. Two plants that had flowered, and two plants that had not yet flowered, died before spring. Bolting and flowering has been recorded for the plants of both categories. Plants that did not flower in their first year are discussed first. The earliest plants of this group started bolting at the end of February. Apparently the intense vernalization compensated for the still rather short days at that time of the year (less than 12 hours). First flowering among these plants was recorded in the first 10 days of April; the last plants started in the last 10 days of June. All plants finally flowered. Figure 2 shows the distribution of flowering over 10 day periods.

Table 3. Start of flowering after a moderate vernalization intensity.

Region	Sample size	Flowering starts in					No flowering
		May	June	July 1-15	July 16-31	August	
B	2	1	1	-	-	-	-
C	9	-	7	1	-	-	1
D	67	3	25	26	11	-	2
E	78	-	26	37	12	1	2
F	65	-	-	6	24	11	24

Of the 90 plants that had already flowered in 1990, 89 plants flowered again in 1991, clearly showing the potential of this species to flower several times in an individual's life-span. These plants flowered, on average, about 10 days later than the plants of the first category (Fig. 2). Two questions are as yet unanswered concerning these results: are the differences in earliness in the second year based on genetic differences and, if so, are the same genes that influence earliness in the first year, also responsible for earliness in the second year? In order to answer the first question, crosses are being made between the 20 earliest plants and between the 20 latest plants. The earliest flowering among the plants that had not yet flowered in the first year, was found in plants from origin D (Fig. 1). A direct relationship with the gradient found for the need for vernalization (Table 3) was lacking. Even some Mediterranean plants were among the latest ones.

Sensitivity to day-length

Biennials and late flowering perennials have two major mechanisms for regulation and synchronization of their flowering period: need for vernalization and sensitivity to day-length. Which one of these mechanisms exists depend on the species. Beets, like some other species have both mechanisms but the need for vernalization depends on the genotype. A major gene for insensitivity to day-length, like the B gene for the absence of the need for vernalization, seems not to exist in these species. Perhaps such a gene could be found in related species growing at lower latitudes (*Beta patellaris* seems to be indifferent to day-length). Nevertheless, the possibility of the presence of minor genes influencing day-length sensitivity is still open. In order to investigate the existence of such genes of small effect, an experiment was carried out in which the photoperiod was manipulated, simulating a day-length increasing linearly by three minutes per day, from 11.5 to 16 hours. Families selected for very early flowering at a photoperiod of 16 hours, and a cross between two plants

that were relatively early, at a photoperiod of 12 hours, were used. Significant differences in the point of change of slope could not be established. The family selected for earliness at a photoperiod of 12 hours appeared to be relatively early at shorter photoperiods and relatively late at longer photoperiods, pointing to the possibility of a genetic component. This observation has not, however, a very strong statistical basis, so that a next generation, enabling a heritability estimation, is required to give more insight into this phenomenon. Figure 3 gives a summary of the results in the form of regression lines of flowering time on photoperiod. Increasing the photoperiod shortened flowering time considerably in the range of 11.5 to 14 hours. At longer photoperiods factors other than the photoperiod appeared to be limiting.

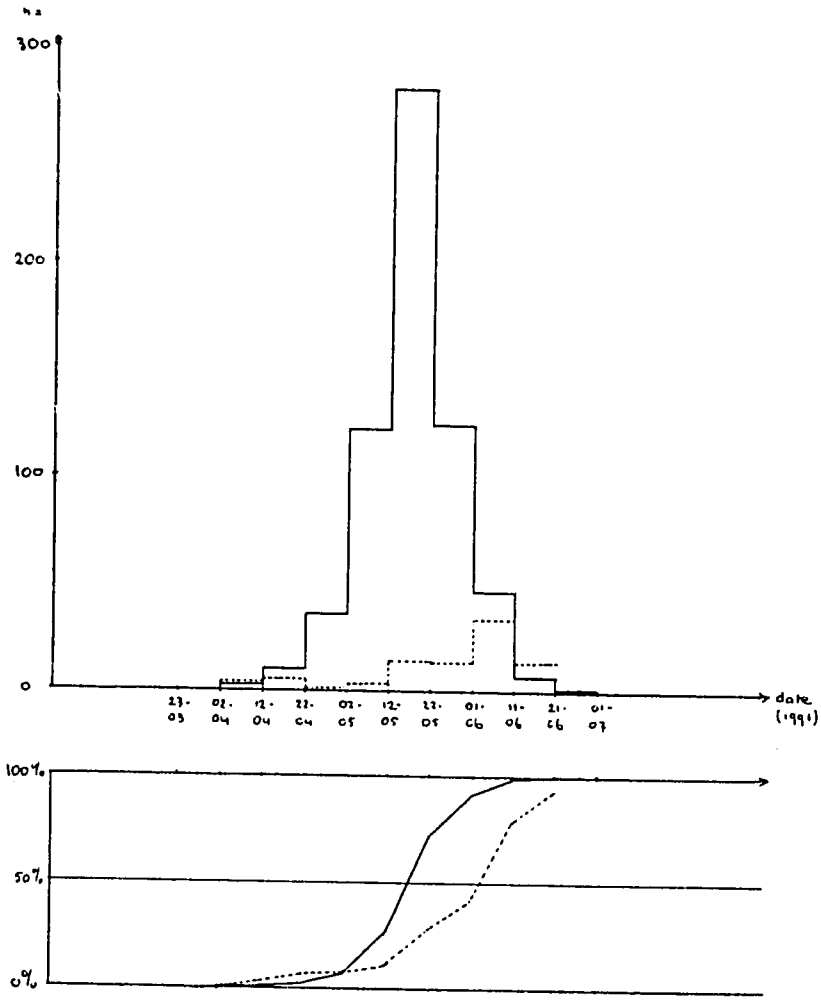

Figure 2.: Flowering in the second year.

Solid line represents plants that did not flower in their first year; dashed line represents plants that flowered in the first year.
a: histogram in real numbers, b: cumulative percentage

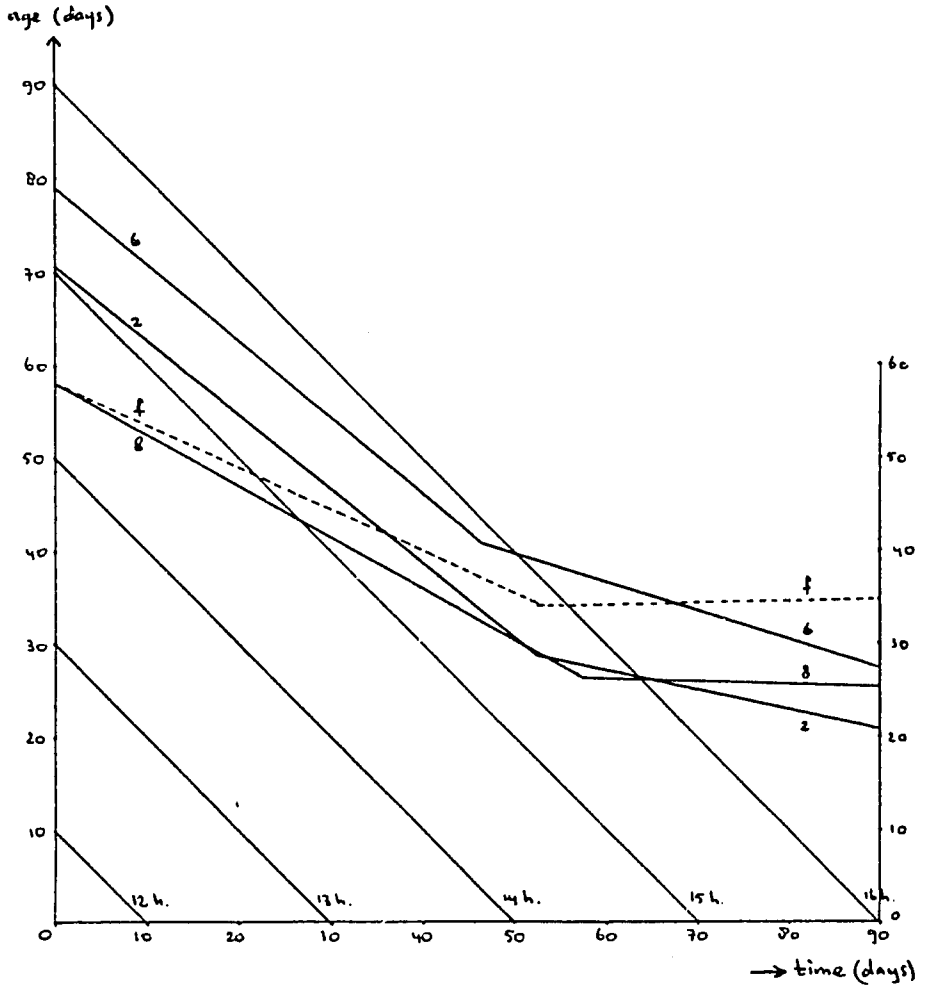

Figure 3: First signs of bolting as a function of age and photoperiod.

Families 2, 6 and 8 have been previously selected for earliness at a 16 hours photoperiod; family f is the descendant of a cross between two plants that bolted relatively early at a photoperiod of 12 hours.

Discussion

Supposing that germination of wild beets happens in early spring, what are the advantages and/or disadvantages of flowering in the same year? An advantage could be the certainty of seed production if winter survival is rather low. A second advantage is that of expanding populations due to the favourability of early reproduction. A possible disadvantage of first-year flowering and seed production is that it can have negative consequences for winter survival and seed production in later years. This disadvantage will be even more manifest if climatic conditions do not favour seed ripening in the first year, for instance in northern regions. In that case, first-year flowering will be strongly selected against.

The apparent absence of the B gene in the north (if it is this gene that causes the differences observed) could be explained by the above mentioned disadvantages. The fact that inland populations are almost fixed for the B gene is due to a low winter survival. In that region, the species can be considered as a weed with a high probability of being removed by ploughing and cleaning up of the fields and gardens in which it is growing. This can also explain selection for early flowering, even when this diminishes seed production because of a smaller plant size. The presence of rather high frequencies of the B gene in Mediterranean populations is less easy to explain. The first demographic observations lead to the conclusion that germination in these populations takes place, almost exclusively, in autumn. If young plants can be vernalized (a factor which has to be checked) then the B gene is neutral or nearly neutral in these populations. There are no indications of more disturbance than in northern populations. Differences in vernalization requirement could easily be interpreted as being an adjustment to the local climate, ensuring that southern plants can bolt in their relatively moderate climate, and that northern plants do not bolt too easily in their colder environment.

The significance of variation in flowering time during the second year, is not clear. There is certainly an optimum, set by the need for a sufficient time of development before flowering, and a time afterwards, long enough to assure seed ripening. Furthermore, a certain synchronization is necessary within populations since beets are outbreeding, so that plants that are extreme with respect to flowering period will be less well pollinated. Altogether there must be a strong stabilizing selection for flowering time.

Taking into account all kinds of variation observed (a part of which certainly has a genetic component while in other cases this has yet to be demonstrated) the species seems to have a rather high potential for rapid evolutionary change in life-history characters. Some other species have a similar variability for the characters described, e.g. *Daucus carota, Verbascum chapsus, Holcus lanatus* and *Melilotus alba*. A question that remains, when referring to Table 1, is whether the definitions given for annuals and biennials apply to plants growing under natural conditions. In the greenhouse, survival after flowering is high, but plants selected for earliness in the first year are difficult to keep alive. This could mean that, indirectly, natural selection for strict annuals could succeed. An alternative possibility, however, is that survival after flowering, by forming reserves for a following year, is a phenotypically plastic character. This would mean that under favourable conditions resources are used for reserves, whereas in unfavourable conditions all resources are used for seed production as in strict annuals. A more detailed study of biomass allocation during flowering and seed ripening will be necessary to be able to distinguish between these two possibilities.

Morphology of North Atlantic *Beta*

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Introduction

In recent years, the preservation and utilization of wild relatives of our major crop plants has been of great importance. Fear of wide-spread epidemics, due to the uniformity and narrow genetic background of our present crop cultivars, and of the apparent erosion and loss of native habitats of wild types, has had a significant impact on the need to collect and preserve wild type germplasm. Of the *Beta* species, *Beta vulgaris* ssp. *maritima* has received the most interest. This sub-species offers the greatest potential for sugar beet improvement. It crosses readily with sugar beet and produces fertile offspring in the F_1. While most of the Mediterranean *B. vulgaris* ssp.*maritima* plants are annual in growth habit, North Atlantic maritime beets are mostly biennial. The biennial habit is more suitable for evaluation, as well as for crossing and transferring of genes to sugar beet breeding pools.

North Atlantic maritime beet occurs all along the northern Atlantic eastern seaboard. It occupies a very narrow niche between high tide and 10 to 20 meters inland. Its adaptation to this harsh environment has, through natural selection, accumulated many strong growth genes as well as genes for various stress resistances. The accumulation of these genes over the centuries has developed a gene pool different from that of cultivated sugar beet. Characters very valuable to sugar beet production have been found within this gene pool, e.g., male sterility, Rhizomania resistance, *Cercospora* resistance, mildew resistance. (Whitney, 1986; Lewellen, *et al.*, 1987; Doney and Whitney, 1990). Destruction of native habitats and the resultant threat of extinction of wild populations prompted a systematic collection effort of North Atlantic *B. vulgaris* ssp. *maritima*.

Collections

Three collection expeditions (Southern Italy, Sardinia and Corsica in 1985; England, Wales and Ireland in 1987; and France, Belgium and Denmark in 1989) for maritime beet germplasm have been conducted over the past six years. The major purpose was to collect, sample and preserve the genetic variation existing in the native North Atlantic populations. Therefore, the entire coastlines of the above countries were explored. Since it was impossible to collect everything, a stratagem was developed to collect every 20 to 40 km or wherever a geographic barrier caused isolation. Collections were bulk (core) samples from as many representative plants as possible from each population as well as individual plant samples. These systematic collections, along with collections from earlier explorers, should represent the genetic variation that is present in the native populations of *B. vulgaris* ssp. *maritima* (Doney, 1985; Doney, 1987; Doney, 1989).

Large populations appear to be in no danger of extinction. However, elimination of small populations is possible and, in some cases, has already occurred. The most damaging activity to the survival of maritime beet is the grazing of livestock, particularly sheep. Other major factors eliminating wild *Beta* are intensive farming practices, concrete sea walls, ports, recreational parks, and other human activities

(Doney *et. al.*, 1990). These collection efforts were, therefore, very timely for the preservation of this germplasm.

Evaluation

Much of this germplasm has been increased under controlled isolation conditions and grown in evaluation field trials at Fargo, North Dakota. This paper will present a summary of the morphological evaluations carried out.

Materials and methods

Bulk populations from the England, Wales and Ireland collection expedition were grown in a field trial at Fargo, North Dakota during the summer of 1988. Each population was planted in a single 7-meter row. A commercial hybrid check (Hilleshög 5135) was replicated every 20 rows as a reference. Canopy measurements were taken in mid-August after maximum leaf expansion and prior to stalk initiation. The most mature non-senescent leaves (two per plant) were measured for leaf thickness, length and width; petiole length and width; leaf dry weight, leaf percent dry weight; and leaf osmotic pressure. Ten plants from each population were also grown in the greenhouse for root osmotic pressure and root soluble solids measurements. Analysis was conducted on frozen root tissue of two-month-old plants.

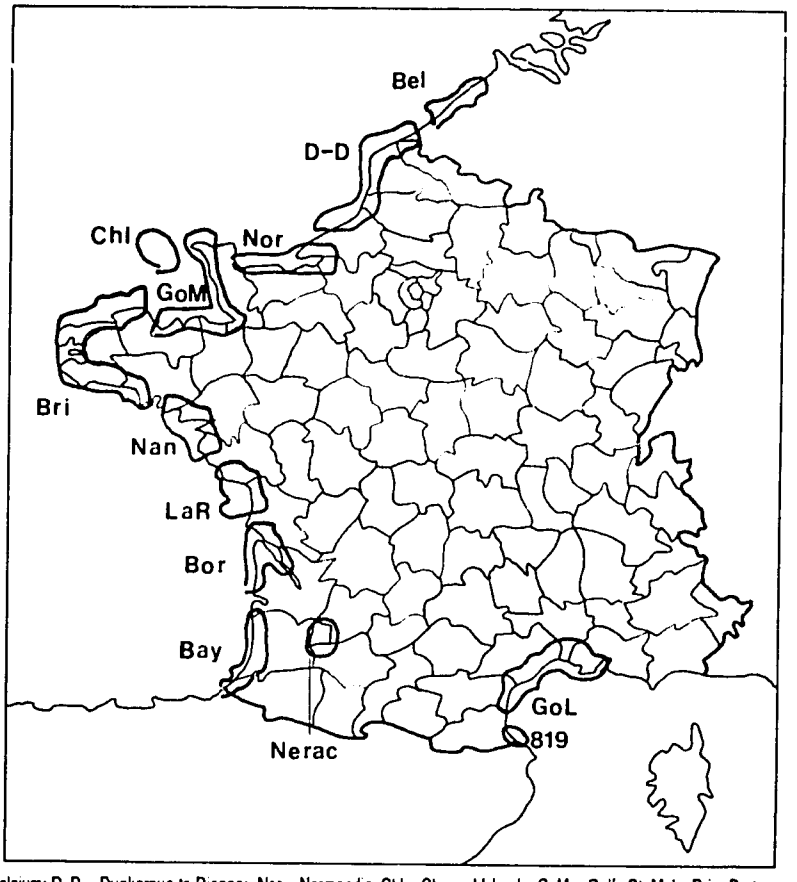
The entire French, Belgian and Danish collections, consisting of 3200 individual plants, representing 146 populations, were grown at Fargo, North Dakota, in 1990. Ten seeds of each individual plant were planted per 4-meter row. Rows were 1.1 meters apart. This resulted in between 5 and 10 plants per row. The entire collection was evaluated for growth, canopy and flower characteristics. In order to give a simpler presentation of this large amount of data, the data were summarized by geographical region (Fig. 1).

Results

Bolting

Fargo, North Dakota lies at a latitude of 46° 5' North. The day length should be similar to that of Nantes, France. Almost 100 percent of the plants collected in the Gulf of Lion (Mediterranean Sea coast) and those collected in and around Nerac bolted and produced seed at Fargo. Nerac is the area of sugar beet seed production in France. It is also the only location in France where wild beets were found inland. From collections obtained on the southern French Atlantic sea coast, the proportion of plants bolting was around 50 percent. This decreased from 50 percent in the south to less than 5 percent in the north coastal region of France (Fig. 2). In Fargo, no seed or flower buds were produced on the first year's growth of accessions from England, Wales, Ireland and Denmark, even though nearly all produced prostrate flower stalks. The Denmark collection had the least tendency to bolt.

As the wild beet spread north along the Atlantic sea coast, natural selection appears to have favoured the biennial habit. In their native habitat, many plants appeared to behave as perennials, i.e. produce seed stalks for several years. Many plants were observed with seed stalks that were obviously from several successive years. Seed was sometimes found on the first year's growth, but it was generally late and immature.

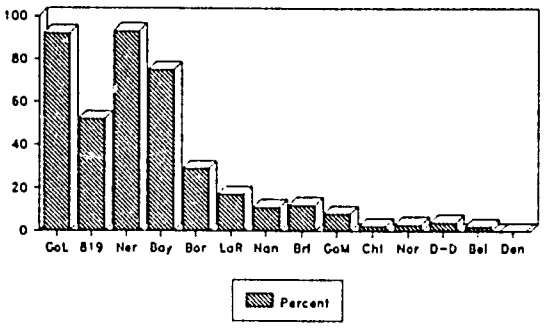


Bel = Belgium; D-D = Dunkerque to Dieppe; Nor = Normandie; Chl = Channel Islands; GoM = Golfe St. Malo; Bri = Bretagne; Nan = Nantes; LaR = La Rochelle; Bor = Bordeaux; Bay = Bay of Biscay; Nerac = Nerac; GoL = Golfe du Lion

Figure 1: Geographic regions in which morphological data are combined and presented in graphic form. Data were obtained in 1990 in field trials at Fargo, North Dakota.

Segregation

There was a wide range of variability within populations. Some were very uniform, suggesting a common ancestry. These populations were generally small and appeared to be of recent establishment or origin. A great deal of variability was present in populations that appeared to be old and well established. These populations were most prevalent on shingle, relatively undisturbed beaches and harbours. The Danish populations seemed to have the least genetic variability. Population 819 in the Gulf of Lion was maintained as a separate entity because of the large variety of plant types in this population. Visual observations of segregation of progeny from individual plants were made in the 1990 field trial. These data are summarized in Fig. 3. The least segregation was found in the populations in the Bayonne region. These populations were small and looked rather recent. Population 819 and populations in the Nerac region exhibited the most segregation for canopy characteristics (92 and 89 percent, respectively). In most of the populations, at least 60 percent of the plants were segregating. Most of the populations were therefore dynamic (i.e., experiencing continual crossing, recombination and segregation).



LAT = 46.5N

Figure 2: Percent of plants bolting within each region. Plants grown in 1990 at Fargo, North Dakota (latitude 46.5° N).

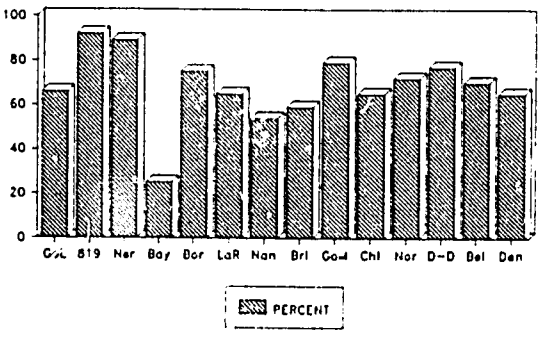


Figure 3: Percentage for each region of the individual plant progenies showing segregation.

Germs per seed-ball

Most plants had 3 to 6 germs per seed-ball. Some had as many as 8 germs per seed-ball, while several had only one (i.e., were monogerm). The average number was between three and four. Populations with the largest number were in the Bordeaux and La Rochelle regions (Fig. 4). The monogerm plants were found in the Normandy and Dieppe to Dunkerque regions of France and on the southern coast of England. The fact that monogerm plants were found on both sides of the English Channel, and nowhere else, suggests the possibility of a common ancestry.

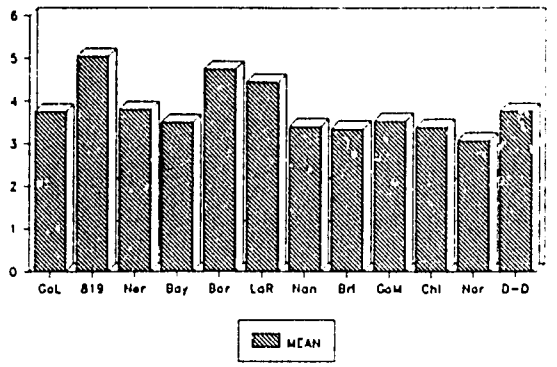


Figure 4: Mean germs per seed-ball for each region.

Growth habit

Growth habit was measured in the leaf rosette (Fig. 5) and flower stalk (Fig. 6). Since few of the plants bolted in the Denmark accessions, the data in Fig. 6 represent only a small portion of the total plants from Denmark. Few plants exhibited an erect growth habit in the leaf rosette. The Nerac and 819 populations were the only ones that exhibited significant erect leaf growth habit. There was a trend from procumbent to prostrate growth habit from south to north. The Danish accessions were almost completely prostrate in leaf growth habit (Fig. 5).

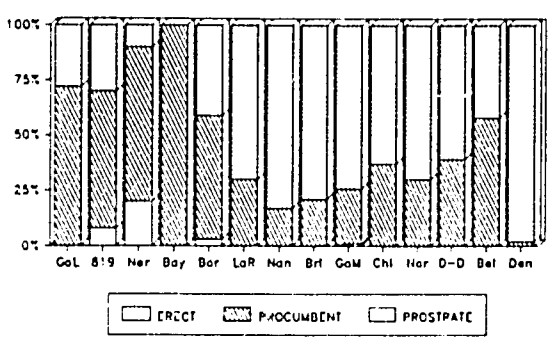


Figure 5: Percentages for each region of the individual plant progenies having erect, procumbent, or prostrate leaf rosette growth habit.

Flower stalk growth habit was similar to but more erect than leaf growth habit (Fig. 6). Eighty-two percent of the Nerac population was classified as erect. All other regions except the Gulf of Lion (60 percent) and Bayonne (50 percent) regions displayed very little erect growth habit. The procumbent growth habit for flower stalk was predominant throughout. Population 819 was segregating for all three types of flower stalk growth habit, with the highest portion of the plants displaying prostrate growth habit.

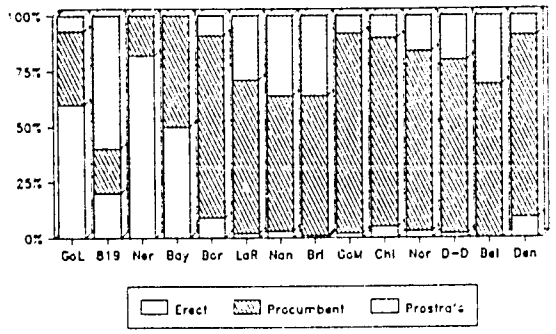


Figure 6: Percentages for each region of the individual plant progenies having erect, procumbent, or prostrate flower stalk growth habit.

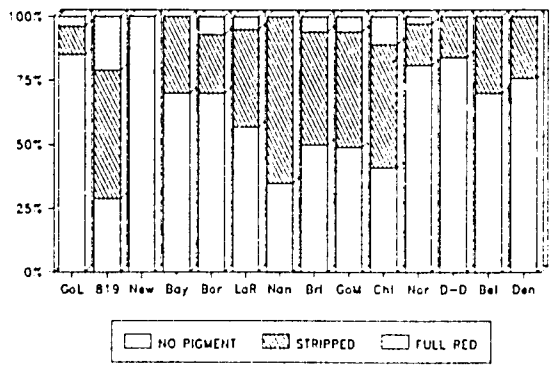


Figure 7: Percentages for each region of the individual plant progenies having none, striped, or red flower stem pigmentation.

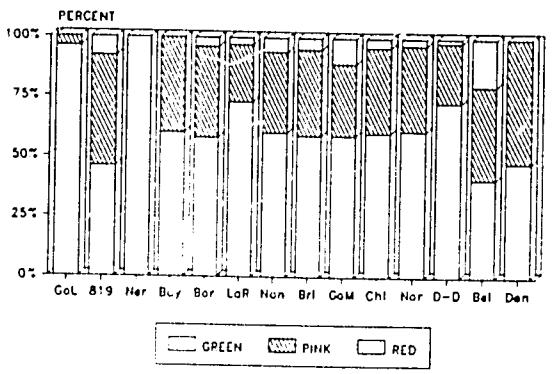


Figure 8: Percentages for each region of the individual plant progenies having green, pink, or red petiole colour.

Canopy pigmentation

Data were taken for flower stem (Fig. 7), petiole (Fig. 8), and leaf pigmentation in the French, Belgian and Danish collections. There was no segregation for canopy pigmentation in the Nerac population. All plants in this region were green and lacked any red colour in the canopy. The canopy pigmentation of most of the plants from the Gulf of Lion region also was green (Fig. 7 and 8). There were a few plants in the Gulf of Lion region with red flower stems and red veins in the leaves, but none had red petioles. A few plants with full red stems were found in most populations except in the northern regions of France, Belgium and Denmark. Populations with the highest percentage of striped stems were found in the central regions of France (Fig. 7).

A small percentage of plants with red veins was observed in most populations. The highest proportion of plants with red veins was in Denmark (25 percent). Most populations were segregating for green, pink and red petiole colour in a ratio of about 60, 35, and 5 percent, respectively (Fig. 8). However, there were no red petioles found in the Denmark, Bayonne and Nerac populations.

Leaf size

Leaf measurements were made in the England, Wales and Ireland populations. There were significant differences in leaf length, width and size and in petiole length and width, both within and among populations. However, in all cases the leaves were smaller in length, width and size than the sugar beet check. Petiole length and width of the wild populations were also smaller than sugar beet.

North Atlantic *B. vulgaris* ssp *maritima* leaves are much thicker than sugar beet leaves (Fig. 9 and 10). All populations had significantly thicker leaves than the sugar beet check, with some being more than twice as thick. There were significant differences for leaf thickness within and among populations. Populations with the thickest leaves came from south-western England and western Ireland (Figs. 9 and 10).

Osmotic pressure

There were significant differences between leaf osmotic pressures within and among populations. Most were equal to or higher than the sugar beet check (Fig. 11). Many populations were significantly higher. However, root osmotic pressure in two-month-old plants was not different from the sugar beet check (Figs. 12 and 13). Deviations from the sugar beet check (higher and lower) were about the same as would be expected based on a 5% probability level. These data suggest that the North Atlantic wild beets have root osmotic pressures similar to that of sugar beet but that their leaf osmotic pressures are higher. Presumably, these higher osmotic pressures in the leaves are the result of natural selection for survival in highly saline environments. Many populations surviving at or near high tide, where a constant spray of salt water covered the foliage, were observed.

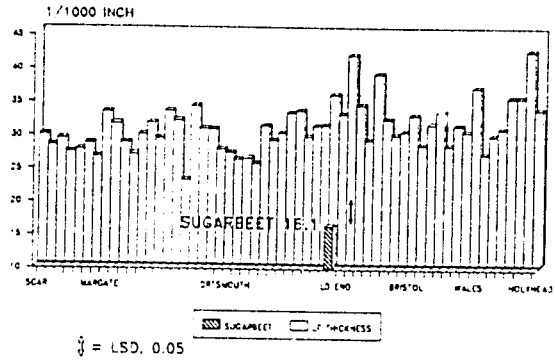


Figure 9: Mean leaf thickness (1/1000 inch) of sugar beet and the *B. vulgaris* ssp. *maritima* populations from England and Wales.

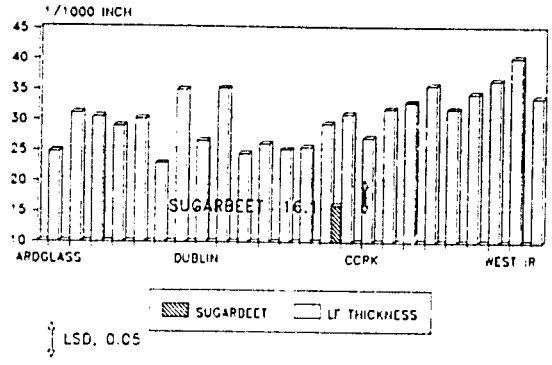


Figure 10: Mean leaf thickness (1/1000 inch) of sugar beet and the *B. vulgaris* ssp. *maritima* populations from Ireland.

Leaf thickness increased the weight of the wild beet leaves and a number of the populations had fresh leaf weights equal to or greater than the sugar beet leaf weight. However, leaf percent dry matter was significantly higher in sugar beet (14.7 percent) than in wild beet (9.3 to 13.6 percent). This resulted in a lower total dry matter per leaf in the wild beet leaves. *B. vulgaris* ssp. *maritima* generally had more leaves, which compensated for the small size of individual leaves. There was a significant negative relationship between leaf thickness and percent dry matter (r = -0.52) in the wild beets. This suggests that the thicker leaves of wild beet were due to larger and not more parenchyma cells (Doney and Theurer, 1983; Doney and Theurer, 1985).

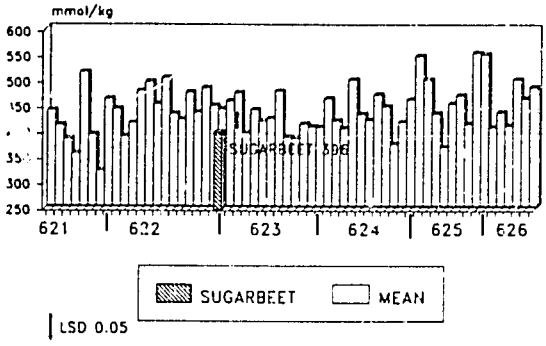


Figure 11: Mean leaf osmotic pressure for sugar beet and *B. vulgaris* ssp. *maritima* populations 621 through 626 from southern England.

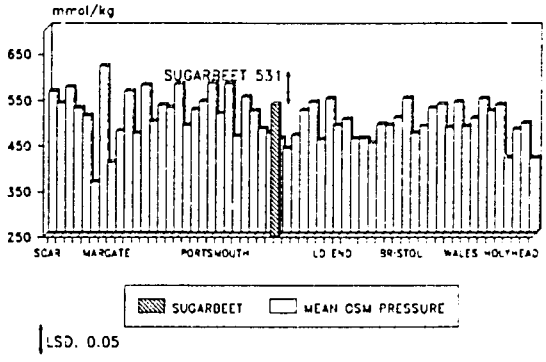


Figure 12.: Mean root osmotic pressure for sugar beet and the *B. vulgaris* ssp. *maritima* populations from England and Wales.

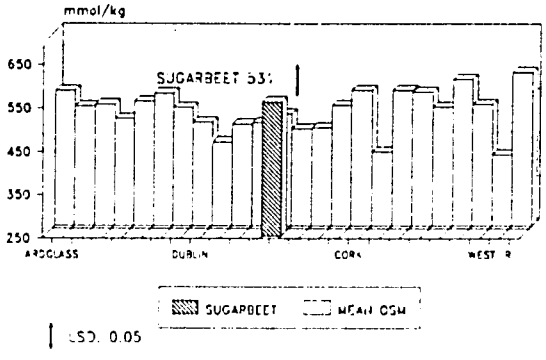


Figure 13: Mean root osmotic pressure for sugar beet and the *B. vulgaris* ssp. *maritima* populations from Ireland

Conclusions

North Atlantic *Beta* differs from Mediterranean *Beta* in a number of characteristics. The former is largely biennial compared with the largely annual nature of the Mediterranean *Beta*. North Atlantic beet has fewer leaf hairs, thicker leaves, less seed shattering and is more prostrate than Mediterranean beet. Weed beets found in the commercial fields of northern Europe do not appear to be outcrosses with North Atlantic *Beta*. This assumption is based on the following observations:

- North Atlantic wild beets are mostly biennial, whereas the weed beets are annual;
- North Atlantic wild beets are procumbent to prostrate in growth habit compared with the erect habit of weed beets;
- the wild beets found in the Nerac region more closely resemble the weed beets than any found on the Atlantic coast; and
- wild beets found in the Mediterranean regions are more characteristic of the weed beets and the wild beets found in the Nerac region.

The author concludes that the weed beets are from outcrosses with the wild types found in the Nerac region, which were probably introduced many years ago from outcrosses of sugar beet to Mediterranean wild beets.

An extraordinary amount of genetic variation can be found in North Atlantic *Beta* (Figs. 14, 15 and 16). The greatest genetic variation was found in large, old, undisturbed populations. Even these populations appeared to be dynamic (i.e., changing in genetic makeup), due to the crossing, segregation and recombination taking place. Natural selection from south to north appears to favour annual to biennial, erect to prostrate, increased leaf thickness, and increased cold hardiness. These evaluations were largely morphological and were not necessarily designed to identify desirable sugar beet characteristics. Desirable monogerm and male sterility traits were found, however. Independent evaluation of some of this germplasm has identified resistance to many of the most serious pests of beet. North Atlantic *Beta* represent a vast storehouse of germplasm that can be easily incorporated into cultivated beet. This storehouse has accumulated, due to natural selection in harsh environments, many stress resistant and growth genes that may be useful and necessary for the future of the beet industry or for other purposes as yet unknown.

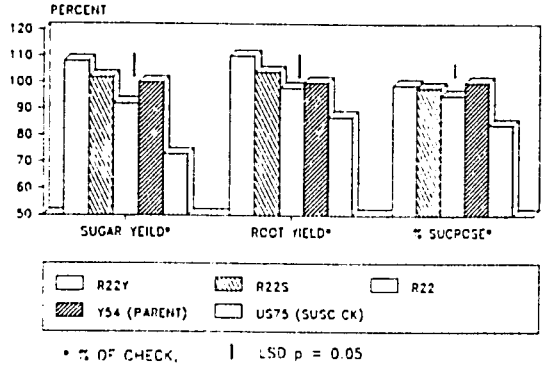


Figure 14: Relative performance of sugar beet x *B. maritima* crosses under severe BYV conditions

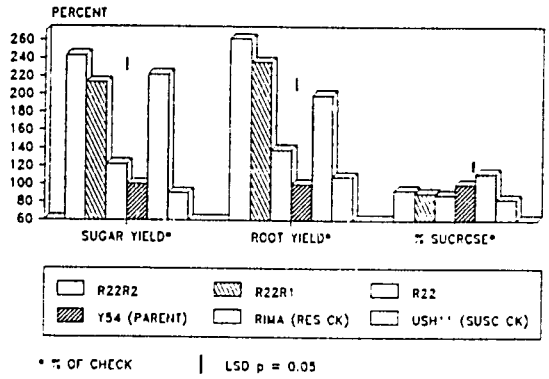


Figure 15: Relative performance of sugar beet x *B. maritima* crosses under severe rhizomania conditions

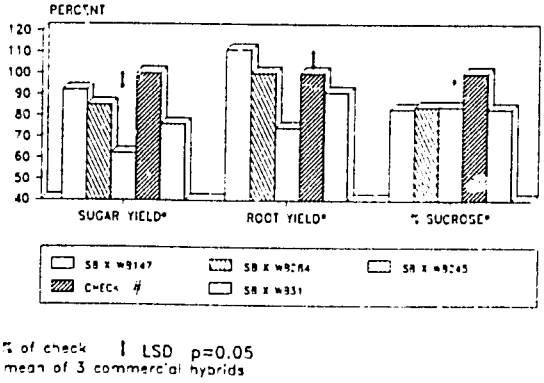


Figure 16: Relative performance of third cycle sugar beet x *B. maritima* crosses

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Patterns of morphological and isozyme differentiation in wild taxa of section *Beta*

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Introduction

This paper presents the distribution of allozyme variation in a number of gene bank accessions. The objective was, firstly, to know more about the distribution of allozymes over taxonomic groups and, secondly, to look for macro-geographic patterns in the distribution of certain allozymes. In the second part of this paper, the morphological divergence in a group of accessions from the East Mediterranean region and inland accessions originating from Iran, Iraq, India and Pakistan, is discussed. Due to morphological intergradation the taxonomy of this group has been in dispute. The morphological divergence is approached from a quantitative point of view. In addition, a comparison is made among the group of eastern Mediterranean taxa regarding the trait of early flowering.

Distribution of allozyme variation

In total, 72 accessions from the *Beta* gene bank collection have been analyzed. In most instances, plants used for electrophoresis were grown as population samples from the progeny of accessions collected during expeditions. From each of the accessions some 20 to 40 plants were screened. These plants were also evaluated in a field trial. The author looked, specifically, at known polymorphic allozymes of sugar beet (*Beta vulgaris* L.) and wild relatives of sugar beet (van Geyt and Smed, 1984; Abe and Tsuda, 1987; and Nagamine *et al.*, 1989). A genetic interpretation was performed to estimate allelic frequencies in the accessions. Since the accessions have been collected by many different people in different years and usually it is not known how many plants were sampled from the populations, an estimation of allelic frequencies is biased. It is, however, possible to acquire a general impression of the macro geographic distribution of variable allozymes.

Map 1 (see Appendix 1) depicts the origin of the accessions. *Beta vulgaris* ssp. *maritima* (L.) Arcang. was represented by accessions from the east coast of Ireland, a few accessions from the Netherlands, populations from Brittany and Charente Maritime in France, the coasts of Portugal and from SE Spain. A number of coastal and inland accessions from Sicily and scattered populations from the eastern Mediterranean have also been analyzed.

As is indicated on the map, the taxa *Beta macrocarpa* Guss., *Beta adanensis* Pamuk., and *Beta patula* Ait. were represented by a number of populations. *Beta macrocarpa* is distributed along the southern Mediterranean coast and is often found sympatric with *B. vulgaris* ssp. *maritima*. *B. adanensis* is a species from Greece and Turkey. B. patula is confined to Ilheu dos Desembarcadores, a small desert island near Madeira.

Before discussing the macro-geographic distribution patterns of a number of allozymes, it is necessary to remark on the degree of genetic variability that was encountered and which proved to depend largely on differences in the breeding system of the species involved. *B. macrocarpa*, *B. adanensis* and *B. patula* are said to be self-fertilizing (Dale and Ford-Lloyd, 1983). The allozyme genotypes of plant populations belonging to these species are usually monomorphic and very few heterozygotic genotypes can be found (Table 1). However, the populations of these species are not necessarily uniform. For instance, adjacent populations of *B. macrocarpa* in southern Spain are fixed for two different allozymes of acid phosphatase (locus Acp 1). It is clear that the outbreeding populations belonging to *B. vulgaris* ssp. *maritima* exhibit higher levels for heterozygosity, especially in the populations from Spain, Italy and Greece. The figures in this table are based on five polymorphic gene loci, namely Lap 2, Acp 1, Pgm 2, Icd and Mdh 2.

Table 1: Heterozygosity in populations of section *Beta*

Taxon/ploidy level	Country of origin	Observed heterozygosity	Standard deviation	Population observed
maritima	Ireland	0.11	0.06	6
	The Netherlands	0.09	0.12	4
	France	0.17	0.05	9
	Portugal	0.13	0.05	6
	Spain	0.30	0.30	2
	Italy	0.25	0.13	13
	Greece	0.36	0.14	5
adanensis	Greece	0.06	0.04	5
	Cyprus	0.02	0.03	2
	Turkey	0.02	0.00	1
macrocarpa 2n	Spain	0.00	0.00	4
	Portugal	0.00	0.00	2
	Tunisia	0.01	0.00	1
	USA	0.00	0.00	1
	Cyprus	0.00	0.00	1
macrocarpa 4n	Spain	0.54	0.07	2
patula	Portugal	0.01	0.00	1

Comparisons of the species from the point of view of isozyme divergence, showed that *B. macrocarpa* has unique allozymes at Acp, Lap and Pgm, and also characteristic allozymes at a number of other enzyme loci. *B. patula* and *B. adanensis*, on the other hand, share all of the common allozymes found in *B. vulgaris* ssp. *maritima*, but there are a few exceptions (see Table 2).

In Appendices 2, 3 and 4, the distribution of a number of allozymes is shown to illustrate the macro-geographic variation pattern. These allozymes can be considered as "rare", "localized" or "low frequency" allozymes as opposed to the 'normal' allozymes that are found widespread and usually with higher frequencies. The first category discussed in this paper contains those allozymes that are of restricted or localized occurrence.

Table 2: Allozymes observed in wild taxa of section *Beta*

Locus	*maritima*	*adanensis*	*patula*	*macrocarpa*
Acp_1	4,5	4	2	3
	(2,3 6,7)	(5)		(1)
Lap	3	3	3	1
	(2,4)	(2,4)	(2)	
Mdh_1	3	3	3	1
	(1,4,5,7)	(2)		(3)
Pgm_1	2	2	2	4
	(1,3)	(3)		
Pgm_2	2	2	2	2
	(1,3)			
Icd	1,2	1	1	1
Pgi	1,2	1	1	1
		(3)		
Skdh	2,3	2	2	2
	(1)			
Got_3	2,3	1	2	1
No. acc.	57	7	1	11

Note:
Common allozymes are on line 1; rare or low frequency allozymes are in parentheses on line 2. The number of accessions evaluated for each taxon is shown on the bottom line.

The category of localized allozymes is primarily centered in the Mediterranean populations. Most of this variation comes from the inbreeding taxa, e.g. Acp 1, in two *B. macrocarpa* populations from Spain; Lap 2' in a *B. adanensis* population from Rhodos; Mdh 2 and a variation for Pgi in a *B. adanensis* accession from Crete. Local allozymes are also found in Mediterranean *B. vulgaris* ssp. *maritima* populations, e.g. Acp 7, in Sicilian and Greek accessions and a slow electromorph, Mdh 7, in a Turkish and a Greek accession. The frequency of these allozymes within the population can be high (e.g. Acp 1 fixed in two *B. macrocarpa* populations in Spain), or less than 20 percent (e.g. Mdh 7 in the eastern Mediterranean accessions).

The second category of enzymes covers those that are of widespread occurrence but with low or moderate frequencies in the populations. A good example of this is the Mdh 5 electromorph for malic enzyme (Appendix 2). In a number of Atlantic and Mediterranean *B. vulgaris* ssp. *maritima* accessions as well as in *B. adanensis*, Mdh 5 is present. Though it is less frequent in the Atlantic *B. vulgaris* ssp. *maritima*, for some variable populations it occurs at medium-high frequency.

Another, faster migrating electromorph of Malic enzyme, Mdh 1, is more typically present in southern populations, and only very local and with very low frequency in two Atlantic populations (Appendix 3). This allozyme is fixed in *B. macrocarpa*. Almost all Sicilian *B. vulgaris* ssp. *maritima*, populations examined carried Mdh 1 in low to medium high frequencies. Tetraploid *B. macrocarpa* plants from the Canary Islands were all heterozygous for Mdh 1 and Mdh 3, the latter being the common *B. vulgaris* ssp. *maritima* allozyme. All of the tetraploid plants carried an additional third heterodimeric band for this enzyme. A similar distribution pattern as for Mdh 1 was observed for Lap 4 (distribution not shown). Lap 4 is an electromorph that migrates more slowly than does the normal Lap 3 allozyme. It has a very low frequency in two French populations, and it could not be observed in any other Atlantic populations. It

was found locally frequent in Sicilian, Greek and Turkish *B. vulgaris* ssp. *maritima* accessions.

So far, most of the allozyme variation has been shown to be concentrated in the Mediterranean populations. The North Atlantic *B. vulgaris* ssp. *maritima* populations generally show less variation but there are some that have a comparable degree of variability. A characteristic of Atlantic *B. vulgaris* ssp. *maritima* populations is the presence of a fast migrating acid phosphatase allozyme named Acp 2. Acp 2 is geographically restricted to the Atlantic *B. vulgaris* ssp. *maritima* populations (Appendix 4). None of the other Mediterranean beet populations that we examined displayed this enzyme band. It is clear from Appendix 4 that the frequency of Acp 2 is highest in the Irish populations, but there are also populations lacking this allozyme. It may reach fairly high frequencies in some French and Portuguese accessions. Acp 2 is not found in *B. vulgaris* ssp. *maritima* populations south of Lisbon. Unexpectedly, it was found to be fixed in all individuals of *B. patula* that were examined. The lanceolate leaves and the multigerm fruit balls make this species look quite different from the southern *B. vulgaris* ssp *maritima* populations, but the fixation of Acp 2 and other characteristic *B. vulgaris* ssp. *maritima* allozymes in this species provides substantial information about its phylogenetic history; it seems to have a close genetic relationship to Atlantic *B. vulgaris* ssp. *maritima*.

Morphological divergence in the eastern Mediterranean gene pool

Quite a number of (sub)specific taxa of section *Beta* originating from the SE Mediterranean coast and inland sites of Turkey, Syria, Iraq, Iran and as far east as India, have been described in the literature. Taxonomic discrimination in this group was described by Aellen (1938, 1967) and Buttler (1977). In order to gain a better understanding of the phenetic relations in this group, a number of accessions from this region were analyzed in a field trial. The variation pattern of the glomerule and the flower has been quantified as shown in Figure 1. In this figure the data of 54 accessions are plotted. From each accession 24 plants were studied. The ratio of flower tepal length and flower tepal width was plotted as the coordinate. The total number of flowers per cluster was plotted as the ordinate. As this character sometimes varies even within a single plant, 20 clusters per plant were counted.

Three entities seem to be present in this plot. The code seven accessions represent accessions from S.E. Turkey, the islands of Rhodos, Kos, Leros and Samos. They exhibit a low number of flowers per glomerule and relatively broad and short tepals. The glomerules are ebracteate or they have fleshy, succulent, obovate bracts attached to them. The growth habit is erect or prostrate. This group should be referred to as *B. adanensis*. The accessions numbered four are from northern Turkey, the mainland of Greece and some Greek islands. The mean number of flowers per cluster is variable but is generally higher. In all morphological characters this group resembles the wild *B. vulgaris* ssp *maritima* populations from other Mediterranean countries such as Italy, Yugoslavia and France. Accessions numbered three represent inland accessions from Iraq, Iran, India and Pakistan. These weedy accessions have tiny tepals and a low number of flowers per glomerule. They differ in some characters such as growth habit (erect) bolting time (early) and colour (very little pigmentation).

Accessions that were entered into the IDBB as *Beta orientalis* have been given a number eight in the scatter plot. *Beta orientalis* Roth is described as a species from India. Descriptions of this taxon in the literature suggest a close relationship with *B. vulgaris* ssp. *maritima* (Roth, 1821 and Aellen, 1938). Flower characters were not different from those found in *B. vulgaris* ssp. *maritima*. Any other distinctive

characters were lacking. In the opinion of the author, only *B. adanensis* is distinct enough to receive taxonomic recognition. The morphological divergence of all other accessions in the plot can be looked upon as continuous geographic variability of *B. vulgaris* ssp *maritima*. It must be noted that intergradations between and within the groups are common and it can be seen in the plot that accession means of different groups sometimes overlap.

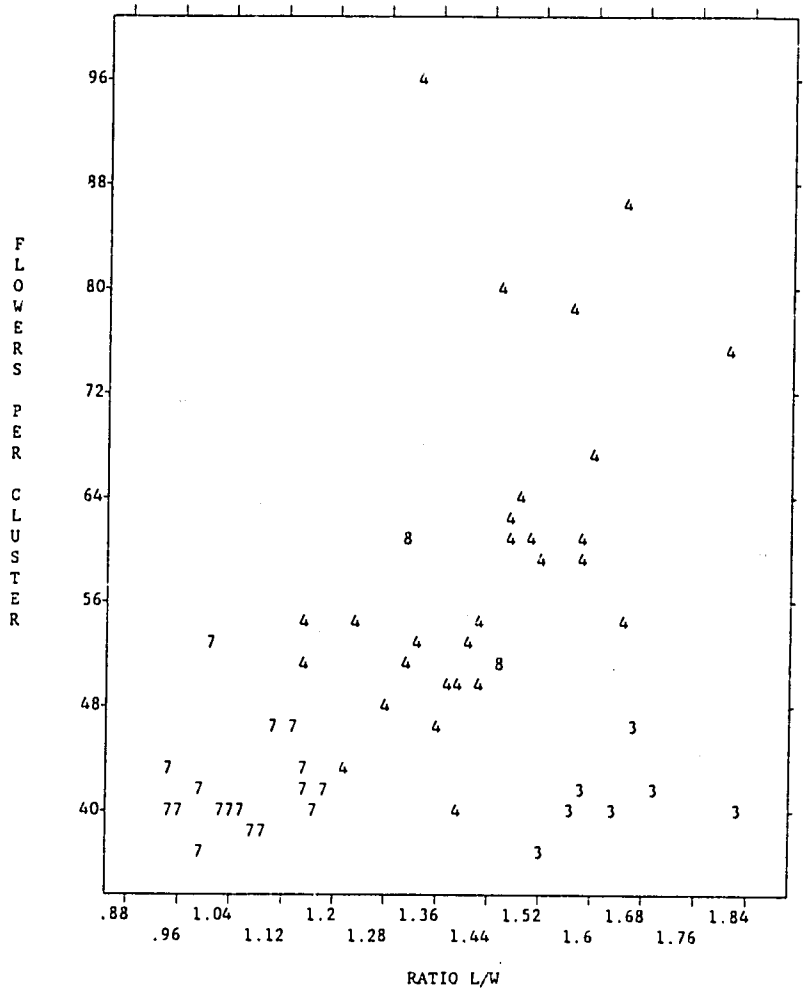

Figure 1: Plot of number of flowers per 20 glomerules (ordinate) with ratio of tepal length: tepal width (coordinate)

Numbers represent accession means. Accessions are coded as follows:
4: *B.vulgaris* ssp. *maritima* from Greece and Turkey;
3: *B. vulgaris* ssp. *maritima* from Iran, Iraq and India;
8: accessions received as *B. orientalis*;
7: accessions representing *B. adanensis*

Variation in early flowering in the eastern Mediterranean region

It is interesting to compare the variation in early flowering in the eastern Mediterranean *B. vulgaris* ssp. *maritima* and related annual species from this area. Large differences exist between populations of the former and the annual species *B. macrocarpa* and *B. adanensis*, in time of flowering (Appendices 5a and 5b). Whereas *B. vulgaris* ssp. *maritima* has a short period of vegetative growth in spring, both other species bolt almost immediately and flower very soon thereafter. The inland *B. vulgaris* ssp *maritima* from Iran, Iraq and India bolts earlier than does the Greek *B. vulgaris* ssp. *maritima*, but the start of flowering is almost the same. It is not easy to determine the response of plants to factors in their indigenous environment from field trials, but one might speculate about the kinds of adaptation to annual cycles of stress and about the differences between ecotypes and species in their inherited growth rhythms.

Species in the eastern Mediterranean environment face a long period of summer drought and high temperatures. Immediate flowering and seed set, as can be observed in *B. macrocarpa* and *B. adanensis*, ensures an escape from this unfavourable time. As a means of surviving the summer drought stress, perennial *B. vulgaris* ssp. *maritima* may rely on moisture extracted by the deeply extending roots. Some of these plants may survive only if there is a sufficiently large volume of soil from which to obtain moisture. Also, plants may be induced into a period of summer dormancy during which growth is slowed down. The perennials may show a quick reaction to sparse rainfall in autumn and growth can be more abundant at this time of the year.

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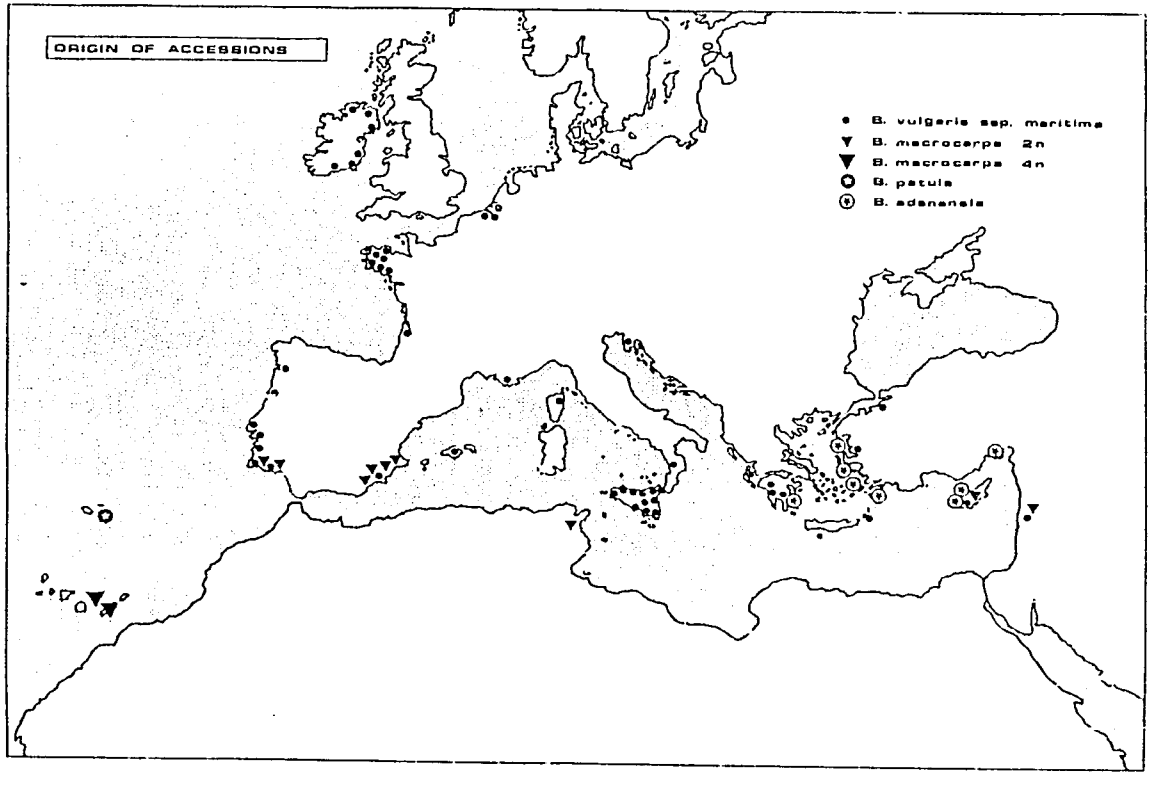
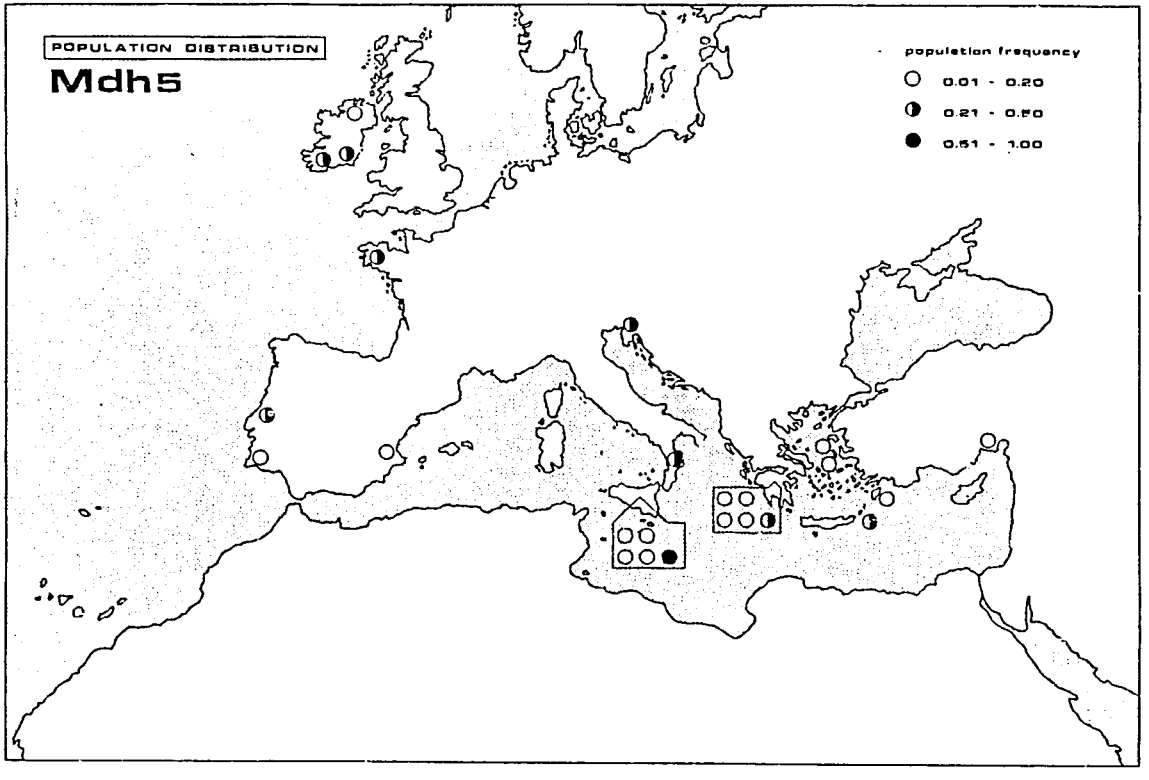
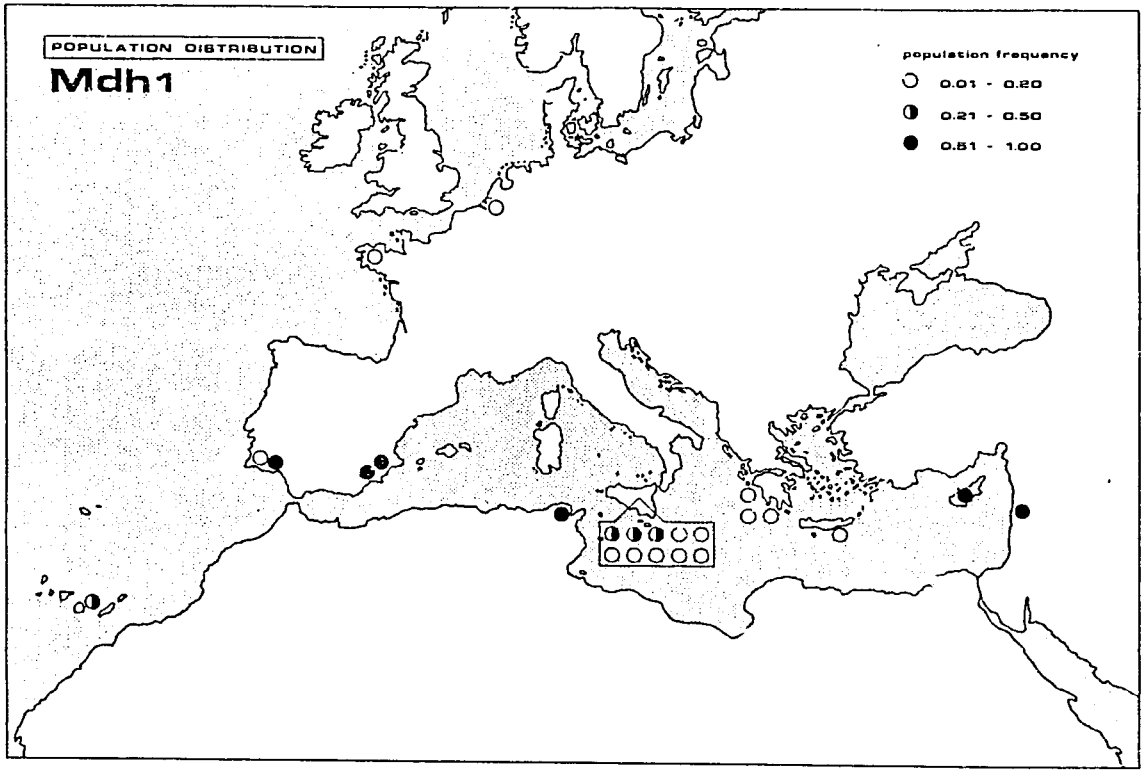
Appendix 1:


ORIGIN OF ACCESSIONS
B. vulgaris ssp. maritima
B. macrocarpa 2n
B. macrocarpa 4n
B. patula
B. adanensis

Appendix 2:


POPULATION DISTRIBUTION
Mdh5
population frequency
0.01 - 0.20
0.21 - 0.50
0.51 - 1.00

Appendix 3:


POPULATION DISTRIBUTION
Mdh1
population frequency
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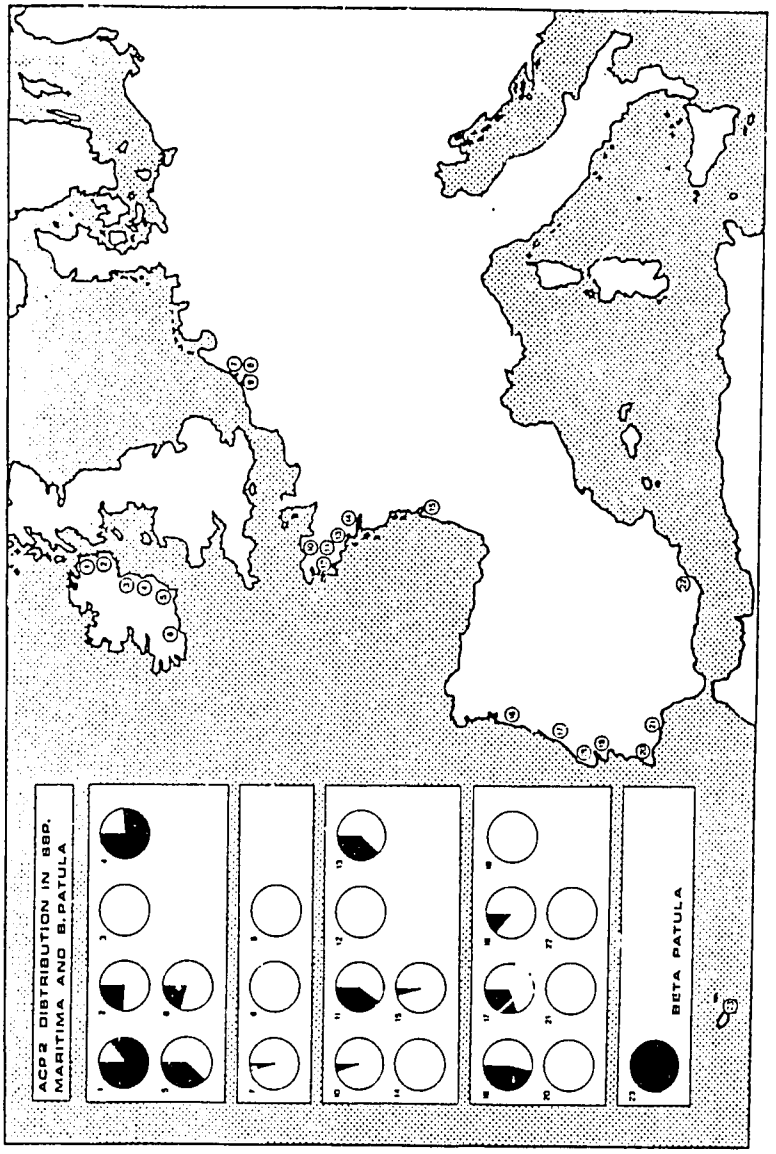
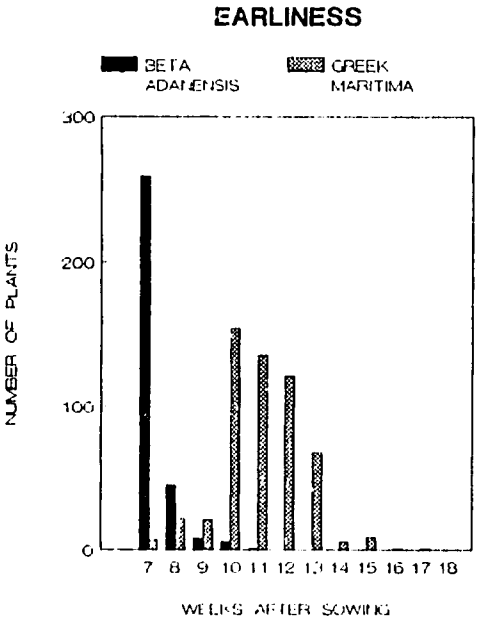
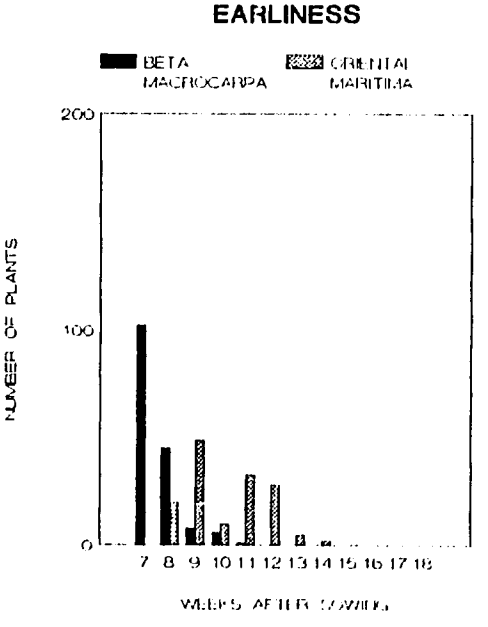
0.21 - 0.50
0.51 - 1.00



Appendix 4:

Appendix 5: Early flowering in 1. Greek *Beta maritima* and related *B. adanensis* and 2. Oriental *B. maritima* and related *B. macrocarpa*





Isozyme and RFLP research on wild and cultivated forms of section *Beta*

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Evaluation of genetic diversity can only be carried out effectively and accurately using isozymes or by analysing polymorphism in DNA. Isozyme analysis is relatively inexpensive and gives easily interpreted results but, even if large numbers of enzyme systems are studied, only a small portion of the genome is assessed. Analysis of DNA polymorphism by way of RFLPs can allow for the assessment of a greater proportion of the total genome and can provide easily interpretable results, depending upon the type of probe used (e.g. multi-locus or single locus). However, the procedure is longer, more technically demanding and more expensive than that for studying isozymes.

A new alternative for studying DNA polymorphism, by way of random amplified polymorphic DNA (RAPD), is now available because of the existence of the polymerase chain reaction (PRC). The technique depends upon the amplification of random DNA segments using oligonucleotide primers of arbitrary nucleotide sequence. When genetic polymorphism exists within a group of organisms, it is simply detected as DNA segments which are amplified, using the PRC, in some individuals but not in others and these segments are inherited in a Mendelian fashion. The advantages of RAPD over the other two techniques lie in the increased proportion of the genome which could be examined for variation and the relative ease with which variation can be detected:

- a single set of universal primers (constituting the probes) could be used for genomic analysis in a wide range of species;
- no preliminary work, such as isolation of cloned DNA probes, preparation of filters for hybridisations, or nucleotide sequencing, is required;
- no radioactive labelling is needed; and
- the length of time required is vastly reduced in comparison with RFLP work, v) it is much more feasible to analyse large numbers of samples in comparison with RFLPs.

Having established that genetic diversity and variation can be effectively determined by several techniques, for what purpose should they be used? From the point of view of breeding sugar beet, isozymes have already been used, for instance, to trace genes confering resistance to beet cyst nematode through a breeding programme. Several breeding companies have already developed nearly complete RFLP linkage maps for sugar beet, which they will be using as markers for agronomic characters in crossing programmes. We have already identified Mendelian segregation for RAPD markers in breeding lines of sugar beet, and some are linked to RFLP markers.

With regard to screening of beet genetic resources collections, isozymes have been used to make estimates of genetic diversity within various sugar-, fodder- and wild beet genepools, and also to identify levels of allelic variation within populations of wild beet, and even within cultivars of sugar beet. On a very limited scale (using a small number if probes) this author has used RFLPs to demonstrate different levels of variation within species and within cultivars. Perhaps the greatest progress has been made with RAPD analysis, where in a very short time it has been possible to detect

genotypic variation in beet material, which can characterize parental and F2 genotypes of sugar beet breeding lines.

Such techniques may hold out various possibilities for future beet research which may include the full analysis of diversity within cultivars, breeding lines, populations etc., and may even allow for the fingerprinting of varieties of beet. Can information from studies of isozymes and DNA polymorphism allow an assessment to be made of existing germplasm collections to determine whether further collections need to be made of particular types of beet in particular ecogeographical areas? Do we, for instance, have sufficient collections of annual beets from the Mediterranean, of section *Patellares* or *Corollinae* species, or of northern European *B. maritima*? Do multigerm fodder beets or garden beets hold more unique genetic variation than do sugar beets? Will it be possible to identify small numbers of accessions containing much variation, to be maintained as core collections? These are questions which it should be possible to answer using the techniques described.

Molecular analysis of the *Beta* genome

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Introduction

During the past four years, representative genetic maps have been developed for a variety of plant species. These maps are based on DNA markers revealing Restriction Fragment Length Polymorphism (RFLPs) after hybridization with genomic DNA which has been digested with discrete restriction enzymes. It is to be expected that the unique features of the marker system, independence of allelic and non-allelic interactions, early detectability and high resolution due to an almost unlimited number of RFLP markers (Tanksley *et al.*, 1989), will soon complement or replace customary phenotypic or isozyme marker systems and be used to locate and isolate economically important genes such as disease and stress resistance or quality traits. Also, quantitatively inherited characters (QTL) may be mapped with RFLP markers. Due to their simple banding patterns which can easily be evaluated, markers derived from unique sequences (single-copy markers) are preferred for mapping. However, 50-90% of the DNA of higher plants represents repeated sequences which, in most cases, probably possess no coding function. Probes of this DNA fraction generally give complex banding patterns due to multiple hybridization of DNA fragments. If the patterns are polymorphic between genotypes they can readily be used to characterize and, thus, identify individuals. This technique, designated "DNA-fingerprinting", has been applied in human research (Westneat *et al.*, 1988) but also in plants (Gebhardt *et al.*, 1989).

For the past three years the authors have been working with plasmid libraries from *B. vulgaris* and *B. procumbens*, mainly for the following projects:

- Establishing a linkage map of the *Beta* genome based on RFLPs and morphological and isozyme markers (Pillen *et al.*, in preparation).
- Selecting genome-specific DNA probes from *B. procumbens*, which can identify wild beet DNA introduced to sugar beet (Jung *et al.*, 1990).
- Isolating probes from repetitive DNA fractions which can be used for fingerprinting *Beta* genotypes. Because of the high degree of polymorphism between *Beta* species, the fingerprint data have also been used for taxonomic studies.

Genome-specific DNA probes

In the course of a project to map and clone a gene for nematode resistance from the section *Procumbentes* species we selected plasmid probes which are closely linked to that gene. These probes were selected by cross-hybridization experiments; 108 single-copy and repetitive probes which had been cloned from a sugar beet line carrying a fragmented *B. procumbens* chromosome, were hybridized to filter-bound DNA from *B. vulgaris* and *B. procumbens*. The principal results can be summarized as follows:

- There is an extremely low degree of homology between sugar beet and wild beet DNA. Approximately two thirds of the probes cloned from sugar beet did not hybridize at all with wild beet DNA and only 14 % gave identical bands with both species. Remarkably, corresponding results were obtained with single-copy and repetitive probes. Usually a high degree of conservation is expected for single-copy probes since they are derived from coding regions of the genome.

- There was a low degree of polymorphism between the three wild species. Single-copy probes as well as repetitive probes cross-react and give the same bands with DNA from the three *Procumbentes* species.

- Several probes have been selected that hybridize specifically with wild beet DNA but do not give any signal with sugar beet DNA. For example, the probe pCJ1012 hybridizes exclusively with repetitive DNA from the *Procumbentes* species. After hybridization with DNA from sugar beet/wild beet addition lines carrying monosomic chromosomes from the wild beets, each line is characterized by a discrete banding pattern. Obviously, this sequence is dispersed among all wild beet chromosomes. This implies that the monosomic addition lines may be identified after hybridization with that probe.

Fingerprint analysis

Repetitive sequences in the *B. vulgaris* genome amount to about 64 % of the total DNA as revealed by analyzing shot-gun cloned plasmid libraries. The arrangement of several major repetitive DNA elements (satellite DNA) has been studied in some detail (Schmidt *et al.*, 1991). These probes display typical ladder patterns which are characteristic for tandemly repeated elements. They are, however, not suitable for fingerprinting because the banding pattern is conserved between species. Therefore, probes from dispersed repetitive elements were isolated which give a polymorphic banding pattern after hybridization with restricted nuclear DNA. Two of these probes were hybridized with DNA from different *Beta* species together with a third probe containing sequences of a tandemly arranged element:

- pCJ57 hybridizes with a low-copy DNA element resulting in a polymorphic banding pattern. Generally 10 bands can clearly be resolved with Hind III-digested DNA.

- pCJ47 also hybridizes with a low-copy element. However, the banding pattern is more complex than that of pCJ57. Approximately 19 bands were scored for each individual, but the DNA of some genotypes gave a much higher number of bands. These additional bands were not included in the cluster analysis, in order to avoid misclassification.

- pCJ714 hybridizes with tandemly arranged, repetitive DNA resulting in a single, strong band. *Beta* accessions representing all sections and spinach that served as a control were included in the test. DNA was extracted from four individuals of each genotype, restricted with Hind III and separated in 0.75% agarose gels. Probes were labelled with a-32P-dCTP and hybridized with filter-bound DNA. Signals were generally detectable after three days of exposure.

A high degree of divergence was found between species of the different sections. The probe pCJ714 displayed a single strong band of the same size, shared by all species. Three ecotypes, two of *B. maritima* and one of *B. atriplicifolia*, gave a lower molecular weight fragment which was found in all individuals tested (Fig. 1). Since the higher

molecular weight fragment is conserved in all species tested, including spinach, this probe may be used to identify these three ecotypes.

The probe pCJ57 generated conserved bands in the low molecular weight range and a number of larger polymorphic bands (Fig. 2). Among the conserved fragments only two ecotypes displayed polymorphism; one *B. maritima* ecotype from Portugal (RNR 870906) and one of *B. orientalis* of unknown origin (RNR 892254). Interestingly, the *B. maritima* from Portugal also gave the differing pCJ714 band. Highly complex banding patterns are visible after hybridization with pCJ47, including again, conserved bands in the low molecular weight range. This probe also revealed inter- and intraspecific polymorphism between closely related species and may, therefore, be used for fingerprint analysis of sugar beet varieties, breeding lines and material derived from tissue culture. Polymorphisms between different individuals of the same sugar beet cultivar could also be detected with this probe.

Generally, the species of the sections III and IV displayed banding patterns different from that of the cultured species and the wild species of the sections I and II. In *B. nana* most of the conserved bands are missing and new bands appear which are highly characteristic for this species. This is consistent with results obtained with cDNA probes (Nagamine *et al.*, 1989). The *Procumbentes* species showed the lowest degree of homology to both probes, pCJ47 and pCJ57. Their reaction was comparable with that of the control species (spinach). These results are in accordance with those obtained by differential Southern hybridization with plasmid probes as mentioned above.

Cluster Analysis

A Dice similarity analysis was performed by Dr. A. Melchinger (Stuttgart-Hohenheim, Germany). This analysis was based on 32 polymorphic pCJ57 fragments and 35 polymorphic pCJ47 fragments (Fig. 3). It yielded three points of interest:

- The cultured beets included in the test displayed remarkably high similarity indices. Swiss chard, however, showed a low similarity to the other cultured beets.
- The wild species of the sections II, III and IV possessed a low similarity to the species of section I.
- No clustering took place among the wild species of section I. Specifically, the *B. maritima* ecotypes displayed high variability.

Summary and Conclusions

Several genome-specific DNA probes from *Beta* species were isolated. One probe is highly specific for wild species of section IV and may be used for identifying wild beet chromatin within cultured species, as in the case of alien addition and translocation lines. Such material has, for example, been selected in order to transfer genes for nematode resistance from wild species to sugar beet.

Two fingerprint probes showed high polymorphism between species of the genus *Beta*, but a low variability was found in sugar beets. Genomic probes may, therefore, be more suitable for taxonomical studies than plastid DNA for which the degree of variability between species was reported to be relatively low (Fritzsche *et al.*, 1987).

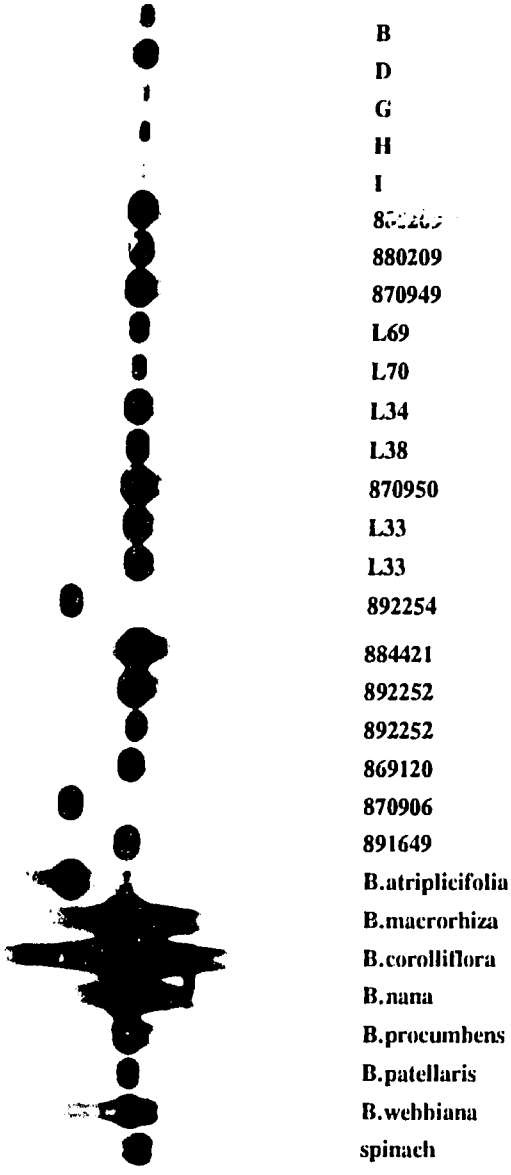

Figure 1: Hybridization pattern of the probe pCJ714.

DNA from different *Beta* species was restricted with Hind III and transferred to nylon membranes. The membrane was hybridized at 60° C with the P32 labelled probe in the presence of 5 x Denhardt's solution, 5 x SSPE, 0.2 % SDS with herring sperm DNA (200 µg/ml). The filter was then washed with 0.5 x SSC, 0.2 % SDS at 65° C and exposed for 2 days.

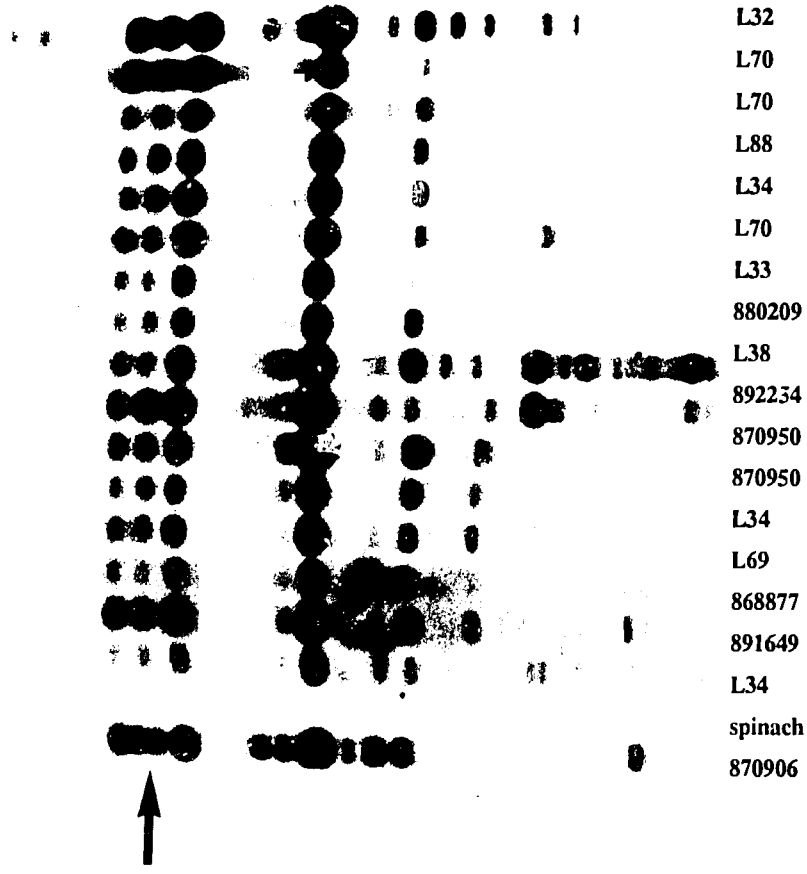

Figure 2: Hybridization pattern of the probe pCJ57.

Hybridization and post hybridization conditions were the same as described in Fig. 1

There is a strikingly low degree of DNA homology between section *Beta* and *Procumbentes* species. This phenomenon is not restricted to highly variable repetitive DNA but is also found among single-copy sequences. In contrast, little variability was found among the wild species of section IV. These findings demonstrate the separate position of the *Procumbentes* species within the genus *Beta*. From a molecular point of view it remains questionable whether or not these species belong to the genus *Beta*. Comparable studies made with tomato (*Lycopersicum esculentum*) and potato (*Solanum tuberosum*) resulted in a high degree of DNA homology. These species differ only by five translocations. With very few exceptions, the tomato probes hybridized perfectly with potato DNA (Bornierbale *et al.*, 1988). Work is in progress to confirm the results from the cluster analysis with additional previously selected fingerprint probes.

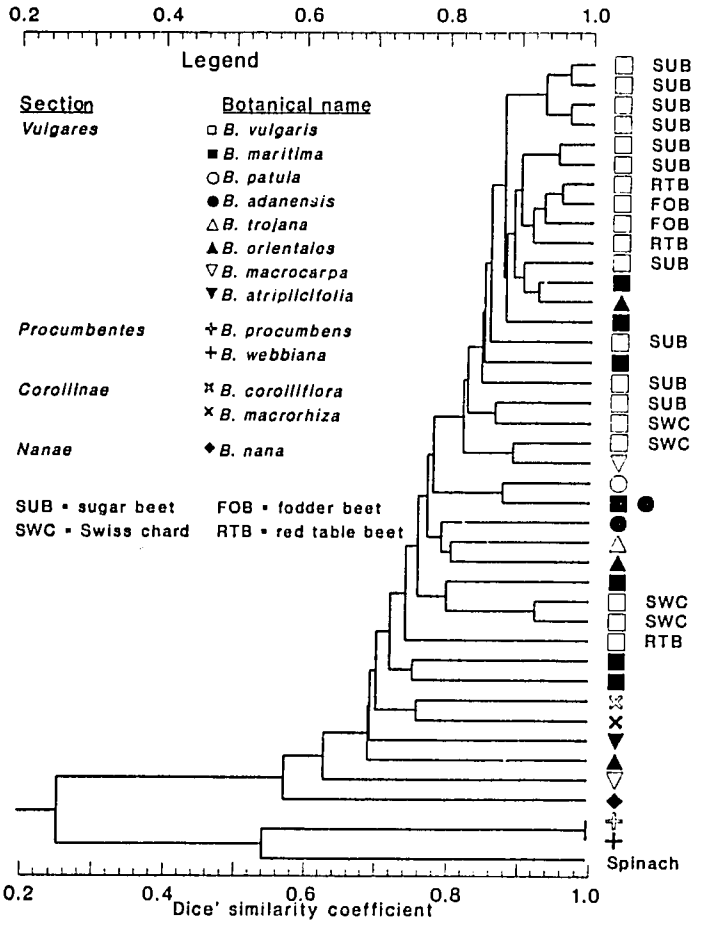

Figure 3: Preliminary cluster analysis with 39 *Beta* species and cultured beets based on 67 polymorphic restriction fragments which were scored after hybridization with the fingerprint probes pCJ47 and pCJ57. Two ecotypes (RNR 884421, 891649) displayed intraspecific variability and were classified in two new sublines.

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Male sterility and DNA polymorphism in *Beta maritima*

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Introduction

Knowledge of the distribution patterns of genetic diversity, together with the analysis of the evolutionary mechanisms underlying this distribution, are crucial for the development of an efficient genetic resources management strategy. Among the different genetic resources of interest for use in breeding programmes, cytoplasmic male sterility (cms) has proved its usefulness in seed production of hybrid varieties. So far, the only male sterile sugar beet cytoplasm commercially available is the one introduced by Owen (1945). Consequently, all hybrid sugar beet cultivars are based on this cytoplasm, generally referred to as the "Owen cms". This cytoplasmic uniformity is a potential danger because it may render sugar beet cultivars vulnerable to diseases, as has been the case for T-type cms in maize. Therefore, it is important to obtain new male sterility sources which could be used as an alternative to the Owen cms. The investigation of new accessions can be performed in the *Beta vulgaris* collections (Komarnitsky *et al.*, 1990), or in natural populations of wild beets (Mikami et al., 1985; Boutin et al., 1987; Halldén et al., 1988; 1990; Mann *et al.*, 1989).

Fundamentally, male sterility in the wild (i.e. the existence of high frequencies of male sterile individuals together with hermaphrodites in natural populations) is also of great interest in evolutionary biology. The existence of such gynodioecious populations has long been reported in many plant species, including taxa from the genus *Beta*. However, the occurrence of high frequencies of male steriles in natural populations is basically an unexpected situation because it is difficult to understand how natural selection could maintain a deleterious mechanism which stops the transmission of genetic information. Indeed, male sterile individuals only transmit their genes through ovules, whereas hermaphrodites transmit them through both ovules and pollen. Thus, the existence of male steriles in the wild is unexpected. How can the fact that gynodioecy is rather frequent in many plant species be explained? This paradoxical situation can be more easily understood when the genetic inheritance of the trait, together with the mode of transmission of the components involved, is considered. It is now clear that in most gynodioecious species, the expression of male sterility is governed by the interaction between two genetic components of the cell: on the one hand a sterilizing mitochondrial component, and on the other hand some nuclear restorer genes. Each component has its own specific mode of inheritance; biparental inheritance for nuclear genes and uniparental, maternal inheritance for mitochondria.

The differential modes of transmission of these sets of genetic information create the potential for a conflict of interest in the determination of the reproductive strategy of the individual. The selective interest of each set is to maximize its own propagation, i.e. to favour its specific mode of transmission. For cytoplasmic genes, pollen production is useless. A mitochondrial mutation producing male sterility which allows the reallocation of these "wasted" resources towards an increased production of ovules (which transmit the cytoplasm) will be selected for. On the other hand, natural selection acts on the nuclear genome (biparentally inherited) and favours genes that

restore male fertility. This conflicting relationship has been termed the "nucleo-cytoplasmic conflict" by Cosmides and Tooby (1981). The approach gives a very interesting basis for the understanding of the co-evolution between the different sets of genetic information of the cell. On this basis, many theoretical models to explain the existence of females at high frequencies within populations have been made. According to such models, no stable equilibrium can be reached within populations but an alternation of different phases is expected:

- A cytoplasmic phase, after the introduction of a male sterile cytoplasm without its specific restorer genes. This cytoplasm produces only females, whose frequency increases according to the female advantage, i.e. females producing no pollen can use the "saved" resources to increase their seed set.
- A nuclear phase, after the introduction of restorer genes of male fertility, with a consequent decrease in female frequency.
- An intermediate phase, with no females; restorer genes are fixed in the population. This latter phase lasts until the introduction of a new male sterile cytoplasm.

From these models it can be predicted that the distribution of genetic variability is not stable in space and time but must be viewed as a dynamic process. The dynamics will not be the same for nuclear and cytoplasmic genes.

The recognition, in natural populations, of the different episodes of this conflicting relationship between nucleus and cytoplasm requires the knowledge of the spatial distribution of both kinds of genetic information, nuclear and mitochondrial, within and among populations. Consequently, genetic markers of the subsets involved in the trait are needed. Techniques of molecular biology supply the tools for investigation of genetic polymorphism of the different genetic subsets. While restorer genes are not yet identified, population studies of mitochondrial variation are possible using RFLP analysis. The rest of this paper addresses the following questions:

- How does female frequency vary among populations?
- What is the polymorphism for the mitochondrial genetic information and how is it distributed in populations?
- What is the link between this polymorphism and the presence of male sterile plants?

Materials and methods

The populations studied are located along the French coast of the Channel and the French and Belgian coasts of the North Sea. In this region, *Beta maritima* is mainly located around the estuaries of coastal rivers.

In order to analyze the mitochondrial variability, restriction profiles of purified mitochondrial DNA were used, initially. This method efficiently reveals differences between mitotypes but is too expensive and too time consuming to be easily used on many individuals for population studies. For that reason, molecular probes on Southern tranfers are now being used to reveal mitochondrial variability in populations (Saumitou-Laprade *et al.*, in prep). Among the different probes that have been tested, the two most informative were kept for subsequent analysis: pbv4, a homologous probe obtained from cms Owen mitochondrial DNA (CPRO, Wageningen), and atp6, from maize.

Results and discussion

Inheritance of male sterility

Previous studies have shown that the inheritance of male sterility in *Beta maritima* is nucleo-cytoplasmic (Boutin *et al.*, 1987), with, among the plants analyzed, two different cytoplasms, that differ from those of the sugar beet:

- A cytoplasm producing a high frequency of female plants.
- A cytoplasm with restorer genes almost completely fixed, producing mainly hermaphrodite plants.

Variation in female frequency between estuaries

Female frequency varies widely between the estuaries (Table 1). At some estuaries, populations are highly gynodioecious (Authie, Canche, Wimereux and Slack River estuaries), while females are absent from the others.

Table 1: Distribution of mean female frequency among populations of different estuaries of the French coast of the Channel.

Estuary	Male steriles	Hermaphrodites
Somme	0.00	1.00
Authie	0.03	0.97
Canche	0.46	0.54
Wimereux	0.06	0.94
Slack	0.09	0.91
Aa	0.00	1.00
Yser	0.00	1.00
Zwin	0.00	1.00

Molecular mitochondrial variability in relation to male sterility

The two probes were used to analyze the mitochondrial variability of the gynodioecious populations at the estuaries of the Channel. A random sample of one hundred and nine individuals was made. Sexual phenotype and mitotype were identified for each individual. Two variants for the atp6 probe were found in the populations and were termed A and B respectively. Three variants noted as I, II and II were identified for the pbv4 probe. The frequencies of the variants of both probes are shown in Table 2. They differ widely between estuaries, revealing a low gene flow through seeds between the populations.

Table 2: Distribution of frequency of atp6 and pbv4 variants among gynodioecious populations of estuaries of the French coast of the Channel.

		Probe				
		atp6		pbv4		
Estuary	No. of plants	A	B	I	II	III
Authie	11	0.00	1.00	1.00	0.00	0.00
Canche	52	0.73	0.27	0.94	0.04	0.02
Wimereux	23	0.30	0.70	0.61	0.34	0.05
Slack	23	0.09	0.91	0.48	0.04	0.48

The combination of the variants for the two probes allows the definition of mitotypes. Only four of the six possible combinations were found. The mitotypes can be related to male sterility (Table 3). Male sterility is strongly associated with the atp6 A variant in which 67 % of the individuals are male sterile, instead of less than 5 %, as is the case for the other types.

Table 3: Distribution of sexual phenotype frequency among the different mitotypes.

	Sexual phenotype		
Mitotype	Male-sterile	Hermaphrodite	Total
AI	0.67	0.33	45
BI	0.05	0.95	42
BII	0.00	1.00	10
BIII	0.00	1.00	12

Distribution of phenotypic and mitochondrial information within gynodioecious populations of two estuaries.

The distribution of phenotypic and mitochondrial information within the populations allows the identification of the three different phases of the dynamics of male sterile frequency predicted by the theoretical models: cytoplasmic, nuclear and intermediate phases.

Within the gynodioecious populations of estuarine areas, sexual phenotypes are not randomly distributed but there is a high differentiation among populations. This differentiation is mainly due to the non random distribution of the mitotypes. This

reveals that gene flow through seeds is limited among populations of an estuary. Different situations have been encountered:

At the Slack River estuary, male steriles and hermaphrodites have different mitotypes. Females are found with the AI type whereas hermaphrodites are mainly found with BIII and BI types. The absence of hermaphrodites having the AI type suggests that restorer genes of this type are absent from this area. Hence, it may be assumed that this estuary is in the cytoplasmic phase of the dynamics of gynodioecy. The future of the populations will be of particular interest for testing predictions of theoretical models because, according to such models, female frequency may increase during this phase.

At the estuary of the Wimereux River, one of the two existing populations contains no females. The AI mitotype correlated with male sterility is absent. This population can be considered to be in the intermediate phase of the dynamics of gynodioecy. The other population is highly gynodioecious. Male sterile frequency locally reaches 78 %. Females are only found with the AI mitotype, but there are some hermaphrodites with this mitotype, thus revealing the presence of restorer genes. This population is in the nuclear phase of the dynamics of gynodioecy. It is strongly differentiated for mitochondrial variability, with two adjacent but very contrasting subpopulations that differ both in female and mitotype frequencies. This result shows that seed migration can be very low, even within populations.

Concluding remarks

The availability of molecular probes allows population studies of mitochondrial variability in relation to male sterility. During the course of this study, the extent of the mitochondrial polymorphism in some populations of *Beta maritima* in the Channel's coastal region has been analyzed. Natural populations are polymorphic for mitochondrial information and there is a high differentiation both between and within estuaries, revealing a low level of gene flow through seeds between populations. Among the plants analyzed, male sterility appears to be highly correlated to a particular mitochondrial type. Some situations predicted by the theoretical models were identified and, in particular, some of the phases of the dynamics of gynodioecy.

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Species relationship in the genus *Beta* as revealed by crossing experiments

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Introduction

The genus *Beta* (beet) comprises several species grouped in four sections: *Beta* (= *Vulgares*), *Corollinae*, *Procumbentes* (=*Patellares*) and *Nanae*. Until recently, extensive taxonomical and phylogenetical investigations were mainly based on morphological similarities and on results of interspecific hybridization. Now, those criteria have been supplemented by different, sophisticated, biochemical research methods.

In the IHAR, collecting and crossing *Beta* species was initiated by the late professor Filutowicz, back in the fifties. This report attempts to recapitulate the main results of IHAR investigations in the field of wild- and cultivated beet taxonomy, relationship and origin, as well as investigations by some foreign research centres.

Hybridization within sections

The section Beta

The section *Beta* comprises highly self-fertile species (*B. atriplicifolia*, *B. orientalis*, *B. macrocarpa*), as well as species that are as a rule self-sterile (*B. maritima*, *B. vulgaris*), both represented by annual and biennial forms. Maritime beet (*B. maritima*) shows very wide variation and within North Atlantic biotypes perennials also occur (Zossimovic, 1940; Jassem, 1985 and others). *B. vulgaris*, instead, comprises different biennial cultivars. Recently, further species have been singled out, namely *B. adanensis*, *B. trojana* and *B. palonga*, which are, however, absent from the IHAR collection.

The earlier named self-fertile species are morphologically very uniform when isolated from each other but they can cross-breed to some extent when they occupy the same neighbourhood, yielding vital and fertile progeny. This confirms their close relationship.

Research on *B. maritima* x *B. vulgaris* hybridization was initiated by Munerati (1932). His work was aimed at developing introgressive cultivars resistant to *Cercospora* leaf-spot and some virus diseases. Recently, breeders have also tried to take advantage of *B. maritima* as a new source of sterile cytoplasm (Coe and Stewart, 1977; Dalke and Szota, 1986). In France and Italy, wild *B. maritima* and *B. macrocarpa* sometimes cross spontaneously with sugar beet grown for seed. Hybrid weed-beets are very difficult to control when they occur in beet fields. They can also be incorrectly identified as wild species.

The species belonging to the section *Beta* easily cross with each other and the resulting hybrids are fertile. Therefore, Zossimovic (1940) included all forms considered in his time as different species of *Vulgares* section, in one species - B. *vulgaris sensu lato*. Some recent authors who consider successful hybridization to be

a crucial taxonomical proof, seem to be of one mind with him (see Frese *et al.*, 1989). Researchers at IHAR are of the opinion that at least some taxa of this section, in particular *B. macrocarpa* and *B. patula*, deserve the rank of species. The distinct character of these two taxa was also confirmed by Abe *et al.* (1986, 1987) using biochemical methods.

The origin of cultivated beets belonging to the species *B. vulgaris* is not clear. Recently, Ford-Lloyd and Williams (1975) reported the finding of plants in Turkey which one could consider to be their common progenitor ("*provulgaris*"). Without doubt, spontaneous hybridization played a substantial role in the evolution and divergence of this cultigen. Such hybridization between leaf beet (Swiss chard) and fodder beet with large storage roots most probably resulted in the origin of the sugar beet. Fischer recently (1989) proved this hypothesis by experimental resynthesis of the sugar beet.

The section Corollinae

Within section *Corollinae*, perennial diploids and polyploids occur; both amphimictic and apomictic. Although their taxonomy has been extensively studied, the results obtained so far are not unambiguous. The majority of authors (Filutowicz and Dalke, 1976; de Bock, 1986 and others) agree with the opinion of Zossimovic (1938, 1940), who considers the two diploid species, *B. lomatogona* (genome L) and *B. macrorhiza* (genome M), as primary in this section. The taxonomical distinctness of the third diploid species, *B. foliosa*, is disputable. Coons (1975) acknowledges it, while Transchel (1927) and others consider it to be an ecotype of *B. macrorhiza*. Buttler (1977) also includes *B. corolliflora* (genome C) in the so called "Basisarten," the basic or initial species. *B. corolliflora* is currently known only as a tetraploid, but one spontaneous dihaploid has been found in the IHAR collection (Filutowicz and Jassem 1975). Zossimovic discovered *B. corolliflora* in the Caucasus and considered it to be autotetraploid. Earlier, it had been considered to be a tetraploid form of *B. trigyna* and this opinion is shared by Coons (1975) and others. Diploid species and *B. corolliflora* are amphimictic, whereas the remaining polyploid species and forms belonging to section *Corollinae* are, in most cases, highly apomictic.

In both diploid species, *B. macrorhiza* and *B. lomatogona*, a slight tendency to produce unreduced gametes was noticed. This may explain the spontaneous occurrence of triploids within both species (Buttler, 1977). Despite different times of blooming, it was possible to cross these species. Scarce hybrids were highly fertile and, as in the case of parental species, sporadically produced unreduced gametes (see Zajkovskaja, 1938; 1965). Consequently, in the following generation (beside diploids) triploids and tetraploids occurred, most of them sterile but some showed different degrees of apomixis and these yielded scarce vital seeds (Jassem *et al.*, 1985). In the F_3 generation, apomictic penta- and hexaploids have also been found. Therefore, there is good reason to regard all apomictic beets of section *Corollinae*, described as *B. trigyna* 4x and 6x, multigerm *B. lomatogona*, *B. intermedia*, *B. penta* and others, as allopolyploid hybrids, in which genomes L, M, and most probably also C, are present in different combinations.

Such hybrids are represented in nature as well as in collections, gene banks and herbarium materials, by numerous forms different with respect to morphology, reproduction biology, and chromosome number. This explains the lack of an unequivocal opinion concerning their taxonomy and origin. The occurrence of intermediate forms makes it difficult to divide the section *Corollinae* into two sub-sections, *Lomatogonae* and *Trigynae*, as was suggested by Barocka (1966) and Fritsche *et al.* (1987).

The appearance of apomixis in polyploid hybrids confirms the opinion that the production of unreduced gametes in primary diploid species may suggest that such species carry elementary factors of apomictic reproduction. As a result of hybridization, these factors may become complete and be activated. Such processes most probably give rise to the occurrence of polyploid *Corollinae* taxa. The majority of such plants surely disappear but all the time new ones may arise, resulting in a dynamic, facultatively apomictic, agamic complex.

Crossing of diploid species with *B. corolliflora* and with multigerm *B. lomatogona* yielded triploid hybrids. In the former case the progeny was amphimictic and sterile, whilst in the latter it was apomictic but hardly fertile. The results of crossings, as well as investigations on meiosis (Kuzdowicz and Szota, 1970) confirmed the hybrid, allopolyploid origin of multigerm apomictic *B. lomatogona* used in these experiments, whilst the monogerm form discovered in the IHAR collection is most probably autotetraploid.

Tetraploid hybrids between diploid species and hexaploid *B. trigyna* were amphimictic or showed different degrees of apomixis and were fertile in a great part. Some higher polyploids, up to 8x, occurred in their progeny (Jassem 1976, 1980). Tetraploid hybrids between *B. corolliflora* and *B. lomatogona* were successfully achieved but they turned out to be sterile.

Among pentaploids which resulted from crossing *B. corolliflora* and *B. trigyna* were amphimicts and apomicts, both partly fertile. In natural habitats, hybrids between *B. lomatogona* and *B. trigyna* probably occur. Surely *B. penta* belongs here, as reported by Walther (1963).

The investigations of hybrids within section *Corollinae* show that the genome of *B. trigyna*, apart from genomes L and C noted by Zossimovic (1940), probably also comprises the genome M from *B. macrorhiza* but the possibility that IHAR hexaploid specimens and those investigated by Zosimovic were of different origin cannot be excluded. As mentioned before, this agamic complex includes a great variety of different allopolyploid forms.

The section Procumbentes

Section *Procumbentes* comprises three species, *B. webbiana*, *B. procumbens* and *B. patellaris*, which are perennial in their native conditions in the Canary Islands. The first and the second species are diploid and self-sterile, whilst *B. patellaris* is tetraploid and self-fertile. In IHAR's collection, forms intermediate between *B. webbiana* and *B. procumbens* arose, which clearly indicates the feasibility of their spontaneous hybridization (Szota, 1964). Experimental crossing supported this assumption (Szota, 1971). The hybrids were fertile, with no disturbances in microsporogenesis, which confirms the hypothesis of Curtis (1968), Buttler (1977), and Wagner *et al.* (1989) that these two taxa should be considered as extreme biotypes of one and the same species.

By means of colchicine treatment, it was possible to obtain tetraploid forms of both species. *B. procumbens* 4x proved to be self-fertile and morphologically very close to *B. patellaris*, which suggests that the latter species is a spontaneous autotetraploid (Szota and Jassem, 1967).

Hybridization of species belonging to different sections

Crossing of species from different sections was, in most cases, aimed at obtaining introgressive cultivars with valuable wild traits, such as monogermness and/or resistance to cold, drought, pests and diseases. Sugar beet was the most commonly used partner. The results have been summarized by Kuzdowicz (1959), Bosemark (1969), Coons (1975) and Filutowic (1979).

Hybrids with Corollinae species

On the diploid level, fertile hybrids between sugar beet and *B. macrorhiza* were successfully obtained. This could suggest that, from among the *Corollinae* species, this one is phylogenetically closest to the section *Beta*. Crossing of sugar beet with diploid *B. lomatogona*, however, was unsuccessful. Introgressive monogerm hybrids of the sugar beet type resulting from such crossings were described by Schreiber (1953, 1956 in Knapp 1958). However, his results were not reliably tested.

When tetraploid sugar beets were crossed with multigerm *B. lomatogona* 4x, the F_1 hybrids were either sterile or else apomictic, yielding very few fertile seeds. Thus, it was not possible to generate further hybrid generations. Better results were obtained by Cleij *et al.* (1968, 1976) who crossed diploid sugar beet with *B. lomatogona* 4x and *B. intermedia*. Triploid hybrids were facultatively apomictic and it was possible to obtain hybrids of further generations.

Tetraploid hybrids of sugar beet and *B. corolliflora* were either sterile or showed different degrees of fertility. Backcrossing to sugar beet resulted in obtaining further hybrid generations. Similar results were earlier obtained by Helen Savitsky (1969) in the USA. As a result of crossing sugar beet with *B. trigyna*, IHAR researchers only obtained a few hybrids of very poor fertility.

To summarize, based on the results of crossing experiments, it can be assumed that *B. macrorhiza* is relatively closest to the section *Beta*, whereas *B. lomatogona* is the most remote. Within section *Beta*, however, *B. macrocarpa* is probably closest to section *Corollinae*, but this has not been directly confirmed.

Hybrids with Procumbentes species

Resistance of the *Procumbentes* species to beet eelworm encouraged numerous breeders to test the feasibility of hybridization with sugar beet. Without doubt, it was Helen Savitsky's contribution in that field that was the most significant. However, the results of Filutowic (1961), Löptien (1984, 1985) and many others contributed considerable progress. IHAR crossing experiments resulted in obtaining di-, tri- and tetraploid hybrids, unable to grow on their own roots. When grafted on sugar beet, only tetraploid hybrids were partly fertile (Szota, Z. and Szota, M., 1971). The almost complete lack of homology between the chromosomes of *B. vulgaris* and those of the *Procumbentes* species, points to their extremely remote relationship and makes the transmission, stabilization and expression of resistance genes in sugar beet extremely difficult (Bosemark, 1969; Kukdowicz and Brzeskwidska, 1970; Brandes *et al.*, 1987 and others).

The species which link sections *Beta* and *Corollinae* with section *Procumbentes* are, respectively, *B. patula* and *B. lomatogona* ? : but this has not been proved directly in IHAR crossing experiments.

B. nana never appears among the parental components of numerous wide hybrids. It was not possible to cross this endemic, self-fertile species with any other representative of the genus *Beta*, which clearly demonstrates its distinct phylogenetical status.

Summary

The results of interspecific hybridization within the genus *Beta* confirm the accuracy of its division into four sections. In the course of the phylogenic development, the earliest separation from the common stem most probably concerned the diploid species *B. procumbens*, *B.webbiana* and *B. nana*. The species belonging to the section *Beta* (= *Vulgares*) are mutually very closely related and give fertile progeny. The species *B. maritima s.l.* and *B. vulgaris s.l.*, in particular, manifest a specially close relationship. *B. macrocarpa* seems to be closest to section *Corollinae* and *B. patula* to section *Procumbentes*. Within section *Corollinae*, *B. macrorhiza* (genome M) seems to be closest to the section *Beta*, and monogerm *B. lomatogona* (genome L) is the most remote. The diploid form of *B. corolliflora* (genome C) is quite unlikely to appear in the natural habitat. All apomictic forms described as *B. trigyna* 4x and 6x, *B. intermedia*, *B. penta* and multigerm *B. lomatogona* constitute an agamic complex, in which genomes M, L, and C are represented in different combinations (Fig. 1).

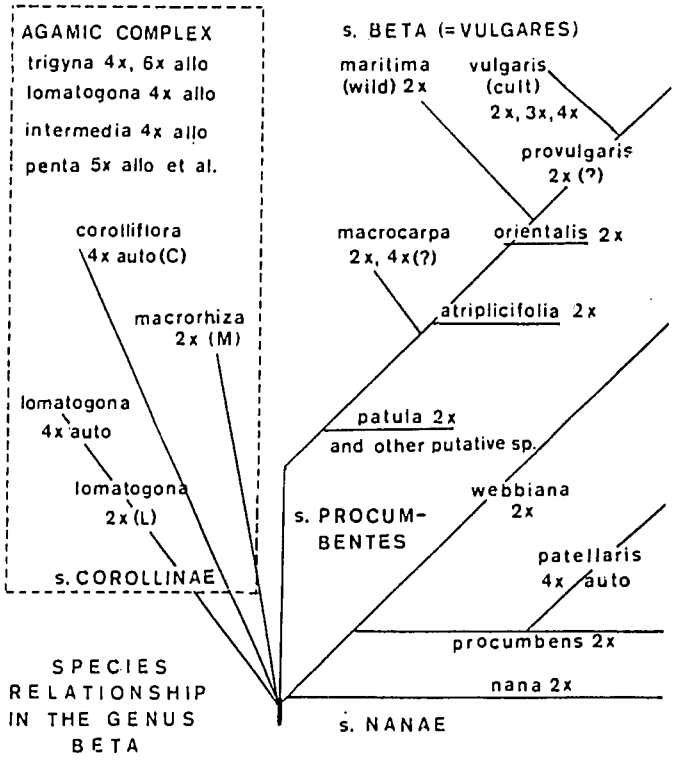

Figure 1: Species relationships in the genus *Beta*

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Abstracts of the Posters

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Conception and results of collaborative activities of evaluation of *Beta* gene resources at Klein Wanzleben 1983 - 1991

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Introduction

Working with *Beta* species has a long tradition at Klein Wanzleben (until 1945 with the Rabbethge and Giesecke Company and since 1952 with the Institute of Plant Breeding and Sugar Beet Research). As early as 1935/36 the first programmes for utilization (crossing) of wild beets were started. Between 1952 and 1963, a large collection of almost all *Beta* species was managed. Since the beginning of the eighties, the evaluation of wild beets of all sections has been extended continuously. Research projects were focussed on the characterization of cytoplasm types and of wild species by means of plastid DNA and, for section *Procumbentes*, nuclear DNA.

In recent years the collection has been tested increasingly for resistance to leaf yellowing viruses (BMYV, BYV and BMV). At the moment, a large seedbank (more than 800 samples), an *in vivo* collection (perennial plants of the section *Corollinae*) and another *in vitro* collection are available for evaluation.

Research basis for the evaluation

Klein Wanzleben has had experience with *Beta* species since the middle of the nineteenth century. The scientists working there have been dealing, in some part, with *Beta* species for 15-20 years. Their knowledge is related to problems of hybrid and heterosis breeding, genetic investigations on the male sterility system and maintainers (O-types), and to cytogenetics of polyploid forms.

In the eighties, a molecular-biology department for the evaluation of *Beta* species was established. Working in cooperation with the genetic research groups of the universities at Halle and Berlin, a relationship and cms analysis was performed on the basis of plastid DNA and, using mitochondrial DNA (mt DNA), cytoplasmic types were determined. To a larger extent, cytoplasm types were also determined for breeding problems. Mitochondrial DNA probes are available for analyzing both N-cytoplasm and S-cytoplasm. Detailed analysis of mt DNA is made by restriction analysis combined with DNA probes.

Since 1987, nuclear DNA of *Beta* species has also been included in the analysis. For that purpose nuclear DNA probes have been developed and tested, in cooperation with the university in Halle. The aim was to identify *Beta procumbens* chromosomes in addition [illegible] verifying systematic experiments with various isozyme systems, both on [illegible] GE, led to the conclusion that lines in breeding processes can be identified [illegible] reliably as *Beta* wild beets or as addition lines with sugar beet. The isozyme [illegible] ICD, MDH and SP are especially suitable.

The in[illegible] abilities and has experience in *in vitro* culture techniques for the p[illegible] propagation of evaluated forms of the sections *Beta*, *Corollinae* [illegible] There are also personnel and technical prerequisites for performing in [illegible] tests for leaf yellowing viruses, BMYV, BYV and BMV. The selected [illegible] resistant plants are preserved *in vitro* and serve to

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establish a "special collection" with virus resistance. Seeds (some kept in deep frozen storage) as well as *in vivo* collections (field and greenhouse) are used for preservation and taxonomical (phenotypical, herbarium) characterization. After unequivocal characterization of genetic resources, genetic analysis of single traits is also possible after crossing with cultivated forms.

In summarizing, it can be stated that conditions for the evaluation programme of genetic resources of *Beta* species at the institute at Klein Wanzleben, are good.

Prospects

The institute could participate in a number of Network activities ranging from the search for new cytoplasmic sources to elucidating the taxonomy of *Beta*. The following three sub-sections outline in more detail Klein Wanzleben's possible contributions.

Establishment of a special collection for cytoplasm types

Both cytoplasm types occurring in sugar beet, namely, S (male sterile plants) and N (pollinators and/or maintainers), can be identified definitely by DNA-probes and restriction analysis. New cytoplasm types, especially those occurring in section *Beta* (*B.maritima*) can be described in detail (after testing with DNA probes and detailed analysis by restriction patterns), established *in vitro*, reproduced, and tested with O-types. A special collection of cytoplasm types can be established and made available for interested breeding and research institutes as an alternative to the common cytoplasm type.

Establishment of a special collection for resistance to the yellowing viruses

This work can be performed with artificial and natural infection by aphids, with special emphasis on species of the section *Corollinae*. Twelve percent of all tested plants showed complete resistance. *B.maritima* accessions were tested first in 1991. Virus infection was controlled by DAS-ELISA. All resistant plants were established *in vitro* (especially those with resistance to BMYV and BYV). This was followed by taxonomic verification, screening for fertility, and determination of self-incompatibility and the ability to hybridize. The ability to produce DH-lines by ovule culture will be examined for all resistant plants. One DH-line of *B. corolliflora* already exists. In future introgression programmes, the genetics of virus resistance and localization of resistance genes will be analyzed. This resistance will be used in the creation of new sugar beet breeding material. The resistant plants (mostly *in vitro*) will be available for interested research groups and breeders.

Application of molecular-biological methods to identify and characterize Beta species

Identification of species of section *Beta* and especially of section *Corollinae* is often problematic. Cytological analysis gives important information. In the Klein Wanzleben Institute of Sugar Beet Research the following molecular-biological markers have been, or are being, tested:

- plastid DNA (evolution by maternal cytoplasm),
- mitochondrial DNA (determination of cytoplasm types,occurrence of plasmids),
- nuclear DNA: application of isozyme analysis, and
- isozyme analysis.

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Germplasm collection of leaf beet (*B. Vulgaris* ssp. *cicla*) in China

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Introduction

In China, leaf beets (*Beta vulgaris* ssp. *cicla*) have a cultivation history of 1500 years. They are grown in some provinces along the Chanziang river. People use the plant as a vegetable or as fodder for livestock. Because of its unimportant economic status, scientists have never studied this germplasm extensively.

In order to get a complete picture of the problem, a collection of some leaf beet samples was made during 1989 and 1990. These were investigated superficially. To date, no species of sections *Procumbentes, Corollinae, Nanae* or other wild beets have been found in China.

Results

The collected samples came from 6 provinces. Their geographical distribution was between approximately 25° N to 40° N and 100° E to 120° E. The simple description of these samples is given in Table 1:

Figure 1 shows the distribution of these samples. They stem from different regions and differ considerably in leaf shape and colour, leaf stalk colour, annuality etc. It is possible, therefore, that they are not the same variety or type. After adaptation and artificial selection over a long time, they have formed many varieties or types.

Figure 1: Collection of leaf beet in China

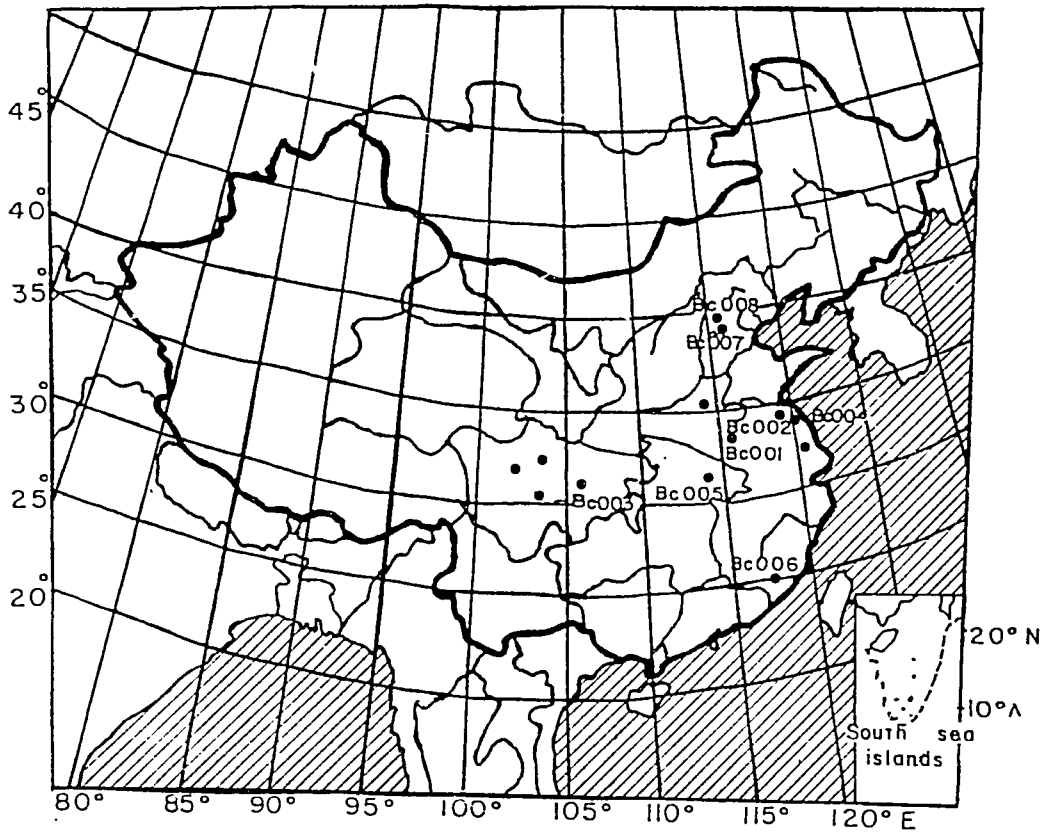

Table 1. Description of leaf beet collection.

Collection number	Location	Province	Rough description
BC001	Liquan	Anhui	multigerm, 2n, light green leaves, more leaf sprouts
Bc002	Huai Ying	Jiangcu	bigerm-multigerm, 2n, light green leaves, annual or biennial
BC003	Fuling	Sighan	bigerm-multigerm, 2n, dark green leaves, fewer leaf sprouts
BC004	Liansui	Jiangcu	bigerm-multigerm, 2n, brown leaf sprouts
BC005	Wuchang	Hubei	mono-bigerm, 2n, 50% red hypocotyl
BC006	Zhang Zhong	Fuijang	bigerm-multigerm, 2n, 65-70% red hypocotyl
BC007	Bao Ding	Hebei	multigerm, 2n, more than 80% red hypocotyl
BC008	Bao Ding	Hebei	multigerm, 2n
BC009	Weiyuan	Sighan	Bai Niupicai, multigerm,2n, white leaf stalk, medium bolting, biennial
BC010	Neijiang	Sighan	Siji Niupicai, multigerm, 2n, white-green leaf stalk
BC011	Yibin	Sighan	Qianjin Niupicai, multigerm, 2n, wide and white leaf stalk, curly spear leaf, late bolting, biennial
BC012	Neijiang	Sighan	Hong Niupicai, multigerm, 2n, purple-red vein and leaf stalk, green leaf, early bolting, biennial

Researchers will continue to collect samples of leaf beets and other wild beets to study the morphology, ecology, and taxonomy of these landraces.

Application of mapping systems for the analysis of the geographical origin of collected material

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In 1987 the International Database for *Beta* (IDBB) was started. It is a central crop database to support the World *Beta* Network activities. Over the years a substantial amount of data was accumulated in the IDBB and it was felt that geographical mapping studies could help in the analysis of the information. The various application fields under consideration were:

- integrity checking of longitude/latitude data
- rapid surveying of the distribution of species
- identifying sparsely and densely sampled areas
- tracing overlapping distribution areas
- illustrating geographical variation patterns

Suitable systems to perform such studies are geographical information systems (GIS) and mapping systems. Although GIS would certainly be able to fullfil the needs and allow even more sophisticated analysis, they require substantial investments in hardware, software and expertise. This did not seem feasible. Alternatively, a mapping system suffices to perform a simple analysis of the information. For this purpose the public domain software package MAPVIEW 2.0 for IBM compatible computers was used. MAPVIEW allows simple plotting with various projection methods and has a "zoom" facility to enlarge portions of a plotted map. It is not able to plot textual information on the maps. This type of information can be added at a later stage, by importing the maps produced by MAPVIEW into a separate drawing program. To convert the data extracted from the IDBB in a suitable format for MAPVIEW, a BASIC program was developed. This program allows the definition of various plotting symbols for:

- integrity checking of longitude/latitude data
- distribution patterns of qualitative characteristics
- distribution patterns of quantitative characteristics

A public domain geographical dataset of the world was also acquired. Although the map data lacks accuracy for the analysis of small areas (e.g. a single island), it certainly provides enough information for an overall impression. If necessary, the map data can be updated relatively easily, as has been done for the Aegean archipelago. In general the lack of geographical datasets combining accuracy with large area coverage is evident and seriously restricts the application of mapping studies.

The poster showed examples of the various application fields that were mentioned above. Even within the limited capabilities of MAPVIEW it attempts to give an impression of the potential of geographical mapping studies in genetic resources.

Breeding and improvement of sugar beet for sub-tropical climates

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Sugar beet (*Beta vulgaris* L.), primarily a crop of temperate climate, has been introduced into sub-tropical countries, such as Egypt, Iran, Iraq, Afghanistan, Pakistan and India, as a supplementary sugar crop to augment cane sugar production. In most of these countries the root crop is grown in the winter months. In Europe and America the sugar beet root crop is harvested from September/October onwards when the temperatures are low. On the other hand, in sub-tropical climate, the sugar beet is sown in October/November and the root crop is harvested in the hot summer months (April/June). Therefore, efforts have been made to develop diploid and polyploid varieties, hybrids, composites and synthetics that can tolerate high temperatures (40°C and above) and that have tolerance to diseases prevalent under such conditions. This, in addition to having high gross sugar yields.

In India, some diploid composites (IISR C-1, LKC-2) a synthetic (LKS-10), a diploid hybrid (LK Hy-1), and a polyploid hybrid (LK Polyhybrid-1) have been developed. In addition, one variety has been developed through modified mass selection (LS-6). These have shown about 10-20% increases in root yield and gross sugar

yield over the open pollinated commercial check, Ramonskaya-06. The above approaches have shown that, under sub-tropical conditions, synthetic, composite and polyploid breeding are easier to handle than the use of male sterility. After five years of multiplication testing, IISR Comp-1 and LS-6 have been recommended for commercial cultivation in India. Some of these genotypes may prove useful for other sub-tropical countries as well. Merits and demerits of the different approaches have been described in this paper.

Application of *in vitro* methods for conservation, multiplication and production of *Beta* DH-Lines

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The development of *in vitro* methods to maintain and multiply *Beta* species started at the Institute of Sugarbeet Research Klein Wanzleben in 1970. During the past 15 years, sugarbeet genotypes of interest to research and breeding were maintained and multiplied *in vitro*. In depots at temperatures of about 5° C, plant material can be stored from 6 to 24 months without additional passages. In the early eighties, *in vitro* cloning was used increasingly to multiply genebank accessions with low seed productivity.

Evaluation work on genetic resources, i.e. establishing a core collection and special collections, became a new application field for methods of *in vitro* culture. Completely resistant plants of the sections *Corollinae* and *Beta* are now maintained and multiplied *in vitro* to establish special collections with resistance to the leaf yellowing viruses, BMYV, BYV and BMV (Melzer *et al.*, 1991). Furthermore DH-lines, suitable for the application of marker-supported selection, are being produced (Lux *et al.*, 1990, 1991). Also, forms with different types of cytoplasm (*B. maritima*) are maintained by *in vitro* culture.

In summary, the following work is performed by the institute:

- Depot storage *in vitro* (core collection, special collection)
- Multiplication *in vitro* (forms with low seed yield)
- Production of DH-lines by *in vitro* ovule culture.

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Some considerations regarding the classification of *Beta*

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Species classification consists of recognizing species, accurately describing them, arranging them in degrees of relationship and placing them in a classification system. It is done in conformity with criteria which rely on different concepts specific for every species. Among several classifications of the genus *Beta*, beginning with Linné's, the most important are the typological species concept (which relies on the morphological features of one individual of a species) and the biological species concept (which relies on biological criteria). The typological species concept is based on the theory of species fixation and preservation, which asserts that every species, once it has appeared, keeps its morphological characteristics, in time and space. Species diagnosis based only on morphological features (often observed in a small number of individuals) frequently leads to unconvincing results, since morphological features represent just a part of species variability.

Therefore, the authors think that classification of the genus *Beta* should be considered from the viewpoint of the new species theory (especially in the case of section *Vulgares*, in which all taxa may be considered varieties of the species *Beta vulgaris*) as has already been suggested, in 1990, at the IIRB Symposium at Dijon.

In contrast with the typological species concept, is the contemporary concept, a modern one which determines species according to biological criteria. As a consequence of Mayr (1940, 1957a, 1957b, 1959, 1963 and 1969), Dobzhanski (1950 and 1951), Simpson (1961) and other researchers' contributions, the biological species concept relies on two essential elements, namely: crossing barriers and a common genepool. In accordance with that concept, a species is a group of populations which possess a common genetic background and which are able to cross but are isolated from populations of other species as far as reproduction is concerned. The mechanism which protects the genetic integrity of species is sexual isolation.

Considering the above, it follows that, from the biological point of view, a species is a taxonomic unit, a reproductive unit , an ecological unit and a genetic unit, since it possesses its own gene background which provides for species existence and continuity.

Until recently, all sugar beet classifications suggested (such as those of Transchel, Zossimovic, Coons and Knapp) were based on the typological species concept. Perhaps that is one reason why researchers have not come to the same conclusions about the classification of sugar beet. Another reason for disagreement may be that only some of the populations of each species were studied and, therefore, the limit zones of species could not be determined. Unless all populations of species are studied, it is not possible to determine whether these species have stable preferences for the pedoclimatic conditions in the geographical or ecological zones they populate.

Moreover, the discovery, gathering and use of those species and populations were not achieved in an organized way, sufficient to obtain thoroughly studied material. Possibly, more-or-less stable populations, being a different age and thus showing reproductive isolation, should have been used. To have studied hybrids among the populations of some recent species may have revealed a high degree of segregation. In order to support this assertion it is enough to mention the work of Proskovetz, who after

order to support this assertion it is enough to mention the work of Proskovetz, who after just a few years of experiments (1986-1990) could obtain biennial plants of *B. patula* with a root weight ten times higher and with a sugar content eight to nine times higher than those of annual plants of the same species. Such a spectacular leap could not be achieved by individual selection over one or two generations or by superior agrotechnical methods. It could be explained by the hypothesis that the plants used in these experiments were natural hybrids between *B. patula* and *B. vulgaris*. This hybridization could have taken place before the plants reached Moravia. Under conditions of backcrossing to *B. vulgaris*, the large roots and high sugar content of such hybrids could have become more prominent.

From the habitat point of view, some *Beta* species, such as *B. vulgaris*, *B. trigyna* and *B. patellaris* could be considered multidimensional species, characterized by a large habitat in which every population holds a certain ecological niche. According to that concept, multidimensional species represent a population system which is able to cross "actually or potentially", in which case it is necessary to elaborate a very careful study of populations, especially those populations which hold the outlying zones of the habitat. Outlying populations can interfere with populations of other species and they occupy positions where the pressure of natural mutations is higher.

Analysis of the reports on the species of *Beta* suggests debate on the question of how to clear up the mechanisms of species development in the genus.

On the problem of phylogenesis of the genus *Beta* L.

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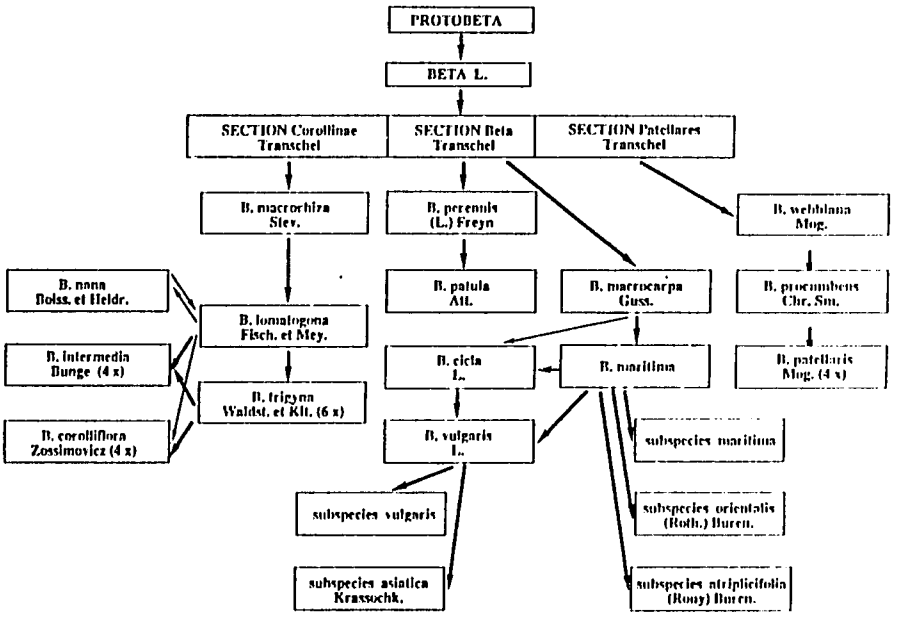
It is assumed that the genus *Beta* descends from *Protobeta*, an extinct ancestor, which was presumably distributed in the region of the Tethys sea. As a result of geological processes and the Tethys formation, the surviving forms of *Protobeta* found themselves in different habitats. Almost all researchers regard section *Patellares* as the most ancient. It might be a relict part of *Protobeta*, preserved until the present time with insignificant changes. The section *Patellares* is also characterized by the most primitive root structure (Zossimovic, 1940). Plants of these species are marked for their longer growth period, which is immanent to a series of tropical forms. Remoteness of section *Patellares* from the other sections is proved by the data of immunochemical seed protein analysis. Within this section, the species are also rather isolated. *B. patellaris* obviously evolved through polyploidization of *B. procumbens*, as described in Figure 1 .

Two other sections, *Beta* and *Corollinae*, are younger. They show much smaller differences in proteins than do section *Patellares* species. Presumably, they originated from a common "plain land" ancestor, which may have resembled the still existing *B. patula*. Zossimovic (1968) regards this species as a relict remnant of the Tertiary Period area of distribution. Krasochkin (1971) assumes that, judging by the general habit of the plant, number of flower stigmata, shorter chromosomes and ecological peculiarities of growth, *B. patula* is close to section *Patellares*. However, by its type of compound fruits (highly multigerm) and other characters, or by its area of distribution, it more closely resembles section *Beta* species. Similar findings have been made by

Section *Corollinae* species evolved in mountainous regions. They are perennial. The occurrences of hybrid types in areas where the distributions of *Corollinae* forms overlap and changes in chromosome numbers (polyploidization) indicate that the process of species formation is still in progress. *B. intermedia,* for instance, presumably originated by hybridization between forms differing in their chromosome numbers, such as *B. lomatogona* (3n, 4n and 5n) or *B. lomatogona* (2n), with *B. trigyna* (6n) (Zosimovich, 1940 and 1968). Most researchers trace the origin of *B. corolliflora* (4n) to *B. trigyna* (6n). According to Filutovich and Trebinski (1969), *B. corolliflora* is closer to *B. lomutogona* than to *B. trigyna.* A connection can also be traced between *B. lomatogona* and *B. nana,* especially by the habit of fruitlets. Immunochemical parameters within section *Corollinae* are rather similar, but the crossing ability of its species is low.

Phylogenetic links between species within section *Beta* appear to be much more complex. Most of the discrepancies between researchers concentrate upon this section. On the basis of the data available in publications and gained from the author's experiments, an infrageneric system is suggested in Figure 1. *B. perennis* is the initial species of section *Beta. B. macrocarpa* and *B. maritima* are regarded as ancestors of *B. cicla* and *B. vulgaris,* the cultivated beets. Their close relationship is sufficiently proved by the data of morpho-anatomical and immunochemical analysis.