

International *Beta* Genetic Resources Network

*A report on the 4th International Beta Genetic Resources
Workshop and World Beta Network Conference
held at the Aegean Agricultural Research Institute, Izmir, Turkey
28 February – 3 March 1996*

L. Frese, L. Panella, H.M. Srivastava and W. Lange, editors



IPGRI is an institute of the Consultative Group on International Agricultural Research (CGIAR)

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The European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR) is a collaborative programme among most European countries aimed at ensuring the long-term conservation and facilitating the increased utilization of plant genetic resources in Europe. The Programme, which is entirely financed by the participating countries and is coordinated by IPGRI, is overseen by a Steering Committee (previously Technical Consultative Committee, TCC) composed of National Coordinators nominated by the participating countries and a number of relevant international bodies. The Programme operates through ten broadly focused networks in which activities are carried out through a number of permanent working groups or through ad hoc actions. The ECP/GR networks deal with either groups of crops (cereals, forages, vegetables, grain legumes, fruit, minor crops, industrial crops and potato) or general themes related to plant genetic resources (documentation and information, *in situ* and on-farm conservation, technical cooperation). Members of the working groups and other scientists from participating countries carry out an agreed workplan with their own resources as inputs in kind to the Programme.

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Preface

The World *Beta* Network (WBN) was founded in 1989 on the initiative of IPGRI. The goal of all crop-specific networks is to improve international collaboration between curators of collections and researchers and users of germplasm, and, through task-sharing, to enable maximum use of the often limited funds for conservation and utilization. This international collaboration actually began in The Netherlands in 1987 with a workshop of curators of collections in Europe and the USA. Subsequent meetings have been held in Wageningen (The Netherlands, 1989), Braunschweig (Germany, 1991) and Fargo (USA, 1993). The WBN is a voluntary association with no membership fee or any other formal obligations of the members. Scientific input and financial support come from various partners of the public and commercial sector. It is the common interest of researchers and plant breeders working with this crop that provides the impetus for the activities of the WBN, which benefit the entire user community of *Beta* collections. This report once again gives evidence of the fruitfulness of cooperation between partners in the USA, Asia, North Africa and Europe.

World Beta Network Coordinating Committee (1993-96)

W. Lange, Wageningen, The Netherlands

L. Panella, Ft. Collins, USA

L. Frese, WBN secretary, Braunschweig, Germany

H.M. Srivastava, Lucknow, India

Local Organizing Committee of the AARI

A. Ertug Firat

A. Tan

S. Tan

S. Kostak

Contributing Organizations

Agra (Italy)

Dieckmann-Heimbürg (Germany)

International Plant Genetic Resources Institute (Italy)

Kleinwanzlebener Saatzucht AG (Germany)

van der Have (The Netherlands)

Südzucker AG (Germany)

Programme

4th International *Beta* Genetic Resources Workshop

29 February 1996, Thursday

08.30-09.00	Registration and payments	
09.00-09.30	Welcome addresses	Director A. Ertug Firat (AARI) Dr L. Frese (WBN secretary)
09.30-10.00	Dr S. Padulosi (IPGRI, Rome) Possible impacts of the Convention on Biodiversity on the World <i>Beta</i> network	
10.00-10.30	Coffee break	

Scientific session: Biosystematics and taxonomy

10.30-11.00	Dr A. Tan Characterization of wild beets in Turkey	
11.00-11.30	Dr W. Lange, Dr W. Brandenburg and Ir. Th. de Bock Proposal for a new taxonomy of the cultivated beet, <i>Beta vulgaris</i> L.	
11.30-12.00	Prof. H. van Dijk Variation for developmental characters in <i>Beta vulgaris</i> subsp. <i>maritima</i> in relation to latitude: the importance of <i>in situ</i> conservation	
12.00-12.30	Dr A. Tan Prospects for <i>in situ</i> conservation of beet populations in Turkey	
12.30-14.00	Lunch	

Scientific session: Genetic diversity

14.00-14.30	Prof. B. Michalik and Dr D. Grzebelus Genetic diversity of red table beets	
14.30-15.00	Prof. N.O. Bosemark Genetic diversity for male sterility in wild and cultivated beets	
15.00-15.30	Dr A.V. Mglinets and Dr S.G. Veprev Collection of S-cytoplasm from various sources of sugar beets	
15.30-16.00	Coffee break	
16.00-17.30	Poster presentations	
20.00	Joint dinner (restaurant)	

1 March 1996, Friday

Scientific session: Genetic diversity and pre-breeding

09.00-09.30	Dr H.M. Srivastava Genetic diversity for high temperature tolerance in sugar beet	
09.30-10.00	Mr M. Nasser Arjmand et al. Pre-breeding for major disease resistances in sugar beets in Iran	
10.00-10.15	Short coffee break	
10.15-10.45	Dr L. Panella Screening and utilizing <i>Beta</i> genetic resources with resistance to <i>Rhizoctonia</i> root rot and <i>Cercospora</i> leaf spot in a sugar beet breeding programme	
10.45-11.15	Dr D. Doney	

Beta evaluation and sugar beet enhancement from wild sources

World *Beta* Network Conference

1 March 1996, Friday

- 11.15-12.30 Report of the WBN secretary
 Discussion
12.30-13.30 Lunch
13.30-17.00 Reports on national genebank activities
 Discussion on future WBN activities
 Recommendations and commitments
 Election of the BCC
 Other matters

2 March 1996, Saturday

Visit of the AARI facilities and/or excursion

3 March 1996, Sunday

Departure of participants

Poster session

Dr M. Asher and Dr H.G. Smith: Evaluation of *Beta* germplasm for disease resistance and stress tolerance

Dr D. Bartsch, Mrs U. Brand and Mrs M. Schmidt: Influence of sugar beet breeding on a wild beet population in Italy

Dr V.I. Burenin: Adaptive potential in the VIR *Beta* collection

Prof. L. Dalke: Variation of morphological and agricultural traits in fodder beets

Dr B. Desprez: Towards a French network for beet genetic resources

Dr L. Frese: Variation patterns in Swiss chards

Dr G. Kikindonov: Development of differentiated dihaploid sugar beet lines

Dr M.A. El Manhaly, D.M.A. Badawy and D.L. Doney: Evaluation of some Egyptian wild types of beet (*B. vulgaris* subsp. *maritima*)

Dr H.M. Srivastava: Evaluation of sugar beet varieties for salt tolerance in India

Sangeeta Srivastava and Dr H.M. Srivastava: Preliminary studies on cytomorpho-logical variations in diploid and polyploid beets

Prof. Z. Stanescu and Eng. L. Pop: *Beta* genetic resources in Romania

Prof. Sun Yichu: Breeding of sugar beets in China

Report on the scientific meeting and workshop

Welcoming addresses and introductions

The WBN conference was opened on 29 February 1996. Dr A. Ertug Firat, the director of AARI, welcomed the participants to Turkey and gave a brief outline about activities of the AARI and its association with the World *Beta* Network. Dr L. Frese, WBN secretary, welcomed the participants of the 4th WBN meeting and reviewed the history of the WBN. On behalf of the participants Dr L. Frese warmly thanked the local organizing committee and the *Beta* Coordinating Committee (BCC) for their dedicated support and excellent work. Special thanks were directed to the financial sponsors. Without their help it would not have been possible to convene 26 scientists from 15 different countries of the northern hemisphere at Izmir.

A presentation by Dr S. Padulosi (IPGRI) on the Convention on Biological Diversity (CBD) and the possible role of networks for defining policies followed these opening remarks. He reminded us that nature is full of surprises and no breeder can anticipate the amount and type of genetic diversity required for crop improvement today and tomorrow, and therefore, we need each other. He also made the point that no country can do without the help of others and emphasized that the WBN should make a distinction between material evaluation and use of germplasm in breeding programmes. The WBN needs to ensure the safest and most effective conservation methods of *Beta* germplasm and remember that the plant genetic resources should be accessible to those engaged in research, evaluation, crop improvement and use. In discussions at the international level, all countries have expressed the importance of crop networks. The WBN reflects the desire of almost all countries in the world that are striving for collaboration and a joint sharing of the resulting benefits. According to Padulosi, sharing of benefits would include joint research, access to new germplasm, access to technology, and access to information and training facilities, as well as sharing of financial profits. The network should continue to be instrumental in ensuring a share in the benefits arising from making plant genetic resources available to all.

Tours

Wild beets of *Beta* section *Beta* occur naturally in the area surrounding Izmir. For many of the WBN participants, the meeting in Turkey was a unique opportunity to visit growing sites of *Beta vulgaris* subsp. *maritima*. On 2 March the AARI organized a bus tour guided by the *Beta* expert, Dr A. Tan. This additional offer was readily accepted by most of the participants.

Paper and poster session: a summary

During the meeting 12 papers and 11 posters were presented. The session **Biosystematic and taxonomy** was chaired by Dr D. Doney (USA), the session **Genetic diversity** by Dr L. Panella, and the session **Genetic diversity and pre-breeding** by Prof. N.O. Bolemark.

Dr A. Tan described variation in Turkish wild beets and discussed taxonomic aspects. More evidence was presented that *Beta vulgaris* subsp. *maritima* and *B. vulgaris* subsp. *adanensis* are distinct taxa. Within the species *B. vulgaris* the trojana-type forms a particular group of populations. In a second contribution she explained the objectives and organization of an *in situ* conservation

programme in Turkey which also includes areas for *in situ* conservation of wild beet populations. The Secaria valley in the area of the town Eskisehir is particularly well suited for *in situ* conservation of *Corollinae* species because all of them occur in that valley.

Following the recommendations of the WBN meeting at Fargo, Drs W. Lange, W. Brandenburg and Th. de Bock provided new information on the state of discussion on the proposed new International Code of Nomenclature. A new taxonomy for cultivated beets had been introduced during the WBN meeting 1993 and accepted by the participants, provided that the suggested system be in agreement with the International Code of Nomenclature. The nomenclature for cultivated beets first suggested in 1993 is indeed in accordance with the new rules of the Cultivated Plant Code. Within the cultivated beets, four cultivar groups – Leaf Beet, Garden Beet, Fodder Beet and Sugar Beet – can be distinguished. Because this system has been accepted officially, the taxonomy and nomenclature of the whole *Beta* section *Beta* has now been consolidated. Prof. H. van Dijk explained that natural selection favours different evolutionary programmes in different environments which can be described by the geographic latitude of the natural growing site. He concluded that such variation is best maintained *in situ*.

At the last network meeting, it was suggested that more attention be given to leaf and garden beet research activities. Dr D. Grezebelus, for the first time since the establishment of the WBN, presented a paper describing genetic diversity of garden beets and crop-specific breeding objectives. Prof. N.O. Bosemark contributed a very comprehensive review on the type and occurrence of male sterility in wild and cultivated beets. This paper was followed by a presentation of Dr A.V. Mglinets, who described a Russian sugar beet collection of S-cytoplasm sources.

Dr H.M. Srivastava gave an overview on sugar beet breeding progress in India, and, in more detail, the use of genetic diversity for the improvement of high-temperature tolerance. Following this presentation, the paper of Dr M. Nasser Arjmand, which described the breeding of disease-resistant sugar beet varieties for Iranian production areas, was presented by H.M. Srivastava. Dr L. Panella introduced the *Rhizoctonia* root rot and *Cercospora beticola* screening programme and specific inoculation techniques used at Ft. Collins. The scientific session was ended by a contribution of Dr D. Doney, who explained the long-term conservation and utilization strategy underlying the American beet genetic resources programme. The programme has now reached a stage where every 2-4 years germplasm releases can be expected. This paper was a very impressive summary of the progress achieved by this group. It demonstrated the value of a well-structured collecting, conservation, evaluation and utilization programme.

WBN secretary report

The participants of the WBN meeting at Fargo agreed upon a number of recommendations which were published in the 1993 workshop report. To implement those recommendations, three conference calls were organized by Dr L. Panella and funded by the Beet Sugar Development Foundation (BSDF). At Fargo, it was suggested to convene the 4th WBN meeting at the AARI at Izmir, Turkey in 1995. Because of funding problems, which were partly solved during the ECP/GR *Brassica* workshop held at Lisbon in November, 1994, the meeting was postponed to 1996. The 1993 meeting participants also recommended that more efforts should be undertaken by the new BCC and the organizer to include

under-represented countries such as Pakistan, Iraq, Syria, Lebanon, Algeria, Albania, Italy and Spain. It was also recommended that the dates of the conference be scheduled immediately before or after the summer meetings of the IIRB. Dr Srivastava tried to contact an expert in Pakistan. No specific initiatives with respect to Iraq, Syria, Lebanon, Algeria and Albania were taken because WBN funds did not allow support of so many scientists. During the preparatory phase, new contacts have been established with Dr Carravedo at Zaragoza, Spain. The organization of the 4th meeting was the main activity during the past 3 years.

It has been recommended that future **collecting** efforts should include, in addition to wild types, old landraces, which are not very well represented in the world *Beta* holding. The following areas of naturally occurring *Beta* germplasm were identified as being deficient in *ex situ* collections: Spain, Iran, India, Morocco, Egypt, Red Sea, Turkey, Syria, Lebanon, Iraq, Romania. New *Beta* material was collected in Italy in 1994 during a joint German-Russian plant exploration in Italy, 1994. Also, Iranian collecting activities have been continued and the passport data of the new material have been sent to the IDBB. Dr R. Boguslavski from the genebank at Charjkov, Ukraine has undertaken a collecting mission in the Crimean area. He sent a *Beta trigyna* accession to Braunschweig along with the information that wild beets are distributed in the Crimean area.

In 1993, the BCC was asked to persuade genebanks to fill the gap between **seed increase and evaluation** capacity by sending surplus seed lots from their yearly routine seed regeneration programmes to Braunschweig from where they can be forwarded to various plant breeders. There was only a weak response to the request. Only curators specifically requested to send material to Braunschweig did so as in the case of Dr Kleijer (Nyon, Switzerland). This material was then evaluated in Italy. Mainly BGRC seed samples were sent to breeders during the past 3 years. In Europe, the following companies or institutes have cooperated very well in the field of evaluation and/or seed increase: KWS at Einbeck, Germany, Società Produttori Sementi, Bologna (Italy) and Istituto Sperimentale per le Colture Industriali, Rovigo (Italy). Thanks to their assistance, sources of rhizomania as well as *C. beticola* resistance were identified.

The 1993 meeting asked W. Lange to follow the discussion on the **new taxonomic system** for cultivated material. It was recommended that the new taxonomic system for *Beta* section *Beta* be adopted as soon as the prerequisites of the International Botanical Society are fulfilled. Lange maintained contact with the botanical experts, and as soon as the new taxonomy became official, the nomenclature used by the IDBB was altered accordingly.

The participants noted at the 3rd meeting that they would prefer detailed evaluation data to be added the **International Database for *Beta* (IDBB)** and that a dBASE version of the IDBB should be developed and distributed. It was also noted that because of limited staff the secretary would need support to implement this work. The new BCC was asked to consult IPGRI in that matter. Contacts with IPGRI clarified that funds to fulfil this work are not specifically reserved. An attempt to apply for project funding was not deemed worthwhile because the necessary work is routine work, not innovative and, therefore, probably not competitive with other project proposals. Finally the easiest part of the work, i.e. building a dBASE IV version of the IDBB passport data set (D_IDBB), was done by the permanent BGRC staff. Five copies of the D_IDBB developed in March 1994 were sent to plant breeders. Addition of evaluation data coming from the

various WBN members is more time-consuming because the different data sets have to be harmonized before they can be entered into the IDBB. Currently, the IDBB standard version running under ORACLE is being updated. New passport data have been provided by the Ukrainian genebank, by Karaj (I.R. of Iran), and from Zaragoza (Spanish Swiss chards holding). Via internet the newest pcGRIN *Beta* version from the USA has been acquired with the objective to update passport data and to make a first step toward an expanded IDBB containing evaluation data.

Distribution of information among WBN members repeatedly has been an issue of discussion. The BCC has discussed the WBN recommendation to develop a **WBN Newsletter**. Dr L. Panella has suggested publishing this newsletter on the Internet. Recently, the BGRC has developed its own Plant Genetic Resources home page (<http://www.fal.de/german/bgrc/bgrc-e.html>). This World Wide Web (WWW) site could be used for brief information on WBN activities. Not all of us have access to the WWW, and, therefore, some would be excluded from accessing the information if no printed version were being disseminated. From the ECP/GR *Brassica* group it is known that lack of staff can be a bottleneck to publishing a newsletter. This aspect needs further discussion before a WBN Newsletter will be established.

Publication of technical WBN reports was suggested by the 1993 meeting. A pamphlet on seed-increase procedures and others, as applied by the WBN members should be published. It is still a valuable idea to write a booklet dealing with handling of germplasm in seed multiplication and screening projects. No actions were taken because of shortage of people to do the work.

Financial problems will remain since the WBN group has to rely on sponsors. According to the recommendations, the organizers of the 4th WBN meeting have increased the registration fee. More than 30 begging letters asking for financial support have been sent to major companies of the sugar beet sector. No other alternatives seemed feasible.

National reports, recommendation and commitments

The last working session was chaired by Dr H.M. Srivastava. The chairman noted in his introduction that the WBN is a partly self-sustaining organization, which has to rely on the willingness of participants to contribute to the overall aims of the network. Participants were asked to briefly present highlights of their *Beta* programmes and to describe their possible contribution to the network activities between 1996 and 1999. Participants were asked to take only those responsibilities which they very likely could fulfil. Representatives from China, Germany, India, Egypt, Iran, Turkey, Poland, Romania, Russia and the USA presented a brief account of the current national *Beta* activities and future programmes. The following recommendations and commitments were noted during discussion.

Collecting missions

Several participants (Drs H.M. Srivastava for M. Nasser Arjamand, L. Frese, V.I. Burenin) felt that there is now a good chance to organize multicrop collecting missions in Iran and in the adjacent areas like the Talysch mountains in the Republic of Azerbaijan. Frese noted that it would be highly interesting to also collect *Cichorium* and *Lactuca* species in that region since such material is missing in collections and he agreed to approach the German-Dutch board in that matter

and to try to find funding sources. Dr Tan noted that collecting in the province of Thrakya will be done by the PGRRI. Dr Z. Stanescu said that collecting of *Beta* would be continued in Romania. *Beta* germplasm from North African countries was mentioned as being under-represented in the WBN holding. It was recommended that the WBN secretary should find out whether West Asia and North Africa (WANA) countries could support the WBN collecting activities. A joint Indian/USA mission had been planned. Although funds have been made available by the USA, the implementation of the mission was not supported by the Indian government. Dr Srivastava promised to find out how to arrange the exploration of Indian native *Beta* material.

Seed increase

The following commitments were noted (Dr H.M. Srivastava, 20 accessions; Prof. Sun Yichu, 10 accessions; Dr Doney, 100-120 accessions, German-Dutch cooperation, 60-70 accessions). Dr V.I. Burenin said that the VIR currently has problems with multiplication of *Beta* accessions because of the loss of research stations in the southern regions of the former Soviet Union. He agreed to attempt to make new arrangements. Dr A. Tan said that the WBN would be informed later about the contribution of the Turkish partner in the field of seed multiplication. Dr Stanescu also noted that he would require discussion on the national level before making any commitments.

Evaluation

Dr Srivastava explained that high-temperature tolerant sugar beet germplasm is becoming more important to Asian countries, and said that 50 accessions in 3 years would be evaluated for that character. Furthermore, the Indian counterpart is committed to evaluating this material for morphological and agronomic traits as well as salt tolerance. It was also noted that Iranian partners would be willing to screen 30-40 accessions for salt tolerance. Prof. Sun Yichu agreed to describe morphological characters of 30 accessions per year. Dr Grzebelus explained that he would recommend to the University of Kraków to strengthen the evaluation and pre-breeding programme with particular emphasis on agronomic and morphological traits and virus resistance. Dr M. El Manhaly agreed to send recently gathered evaluation data to the IDBB as well as yield and isozyme data. He informed the group that there are plans to screen for *C. beticola* resistance in Egypt. A. Hodgdon noted that 20-30 accessions could be evaluated for horticultural characters as well as for disease resistance in the USA. Dr Tan informed the group of a planned cooperation with the Turkish sugar industry aiming at the detection of new male-sterile forms in *B. vulgaris*. She noted that characterization and isozyme studies would also be carried out, and that they intend to develop a taxonomic determination software programme which would also include photos of various *Beta* species and populations. The chairman stressed that all evaluation data should be sent to the WBN secretary for inclusion in the IDBB so that they become available to a broader user community.

WBN newsletter

Dr H.M. Srivastava raised again the issue of a WBN Newsletter. He suggested that, because WBN meetings now will take place every 3 instead of 2 years, there is a necessity to publish the activities of the *Beta* network to keep all members and member countries informed about the progress of WBN activities. It was decided

that a WBN newsletter will be distributed once a year by the permanent secretary of the WBN as its Chief Editor. The secretary can coopt any member onto the editorial committee. For the time being, the BCC was asked to collect contributions which could be compiled by the WBN secretary and distributed through regional focal points to keep the WBN secretary costs low. This was unanimously approved by the house. The meeting also recommended that different participating countries establish Internet connections as early as possible which would allow access to a WBN notice board on the BGRC home page (Braunschweig, Germany).

Election of the new *Beta* Coordinating Committee (BCC)

The plenum re-elected Dr Lee Panella (USA) as BCC member. As new BCC members, Dr M. Asher (U.K.) and Prof. Sun Yichu (China) were suggested and elected by the participants. Dr Asher agreed to find out whether Broom's Barn would be prepared to host the next WBN meeting in 1999. (They will.) After this very intense discussion had ended Dr Doney took over the chair and thanked all participants for their enthusiastic discussion and valuable contributions. The WBN belongs to everybody. As partners, we all make use of the facilities the WBN offers, and should, therefore, contribute our share, because this voluntary association of crop experts can only be continued and filled with life if everybody contributes to the activities. Dr Doney warmly thanked the director of the AARI, the head of the genetic resources department, and all members of the local organizing committee for their hospitality and the excellent organization.

National reports

Peoples Republic of China

Sun Yichu

The national plant germplasm resources programme receives financial support from the government. The local institutes carry out the collecting and evaluation of crop germplasm. The germplasm resources programme is managed by the Institute of Sugar Beet of the Chinese Academy of Agricultural Sciences (CAAS). By the end of 1995, 1200 *Beta* germplasm accessions, including 1120 sugar beet and 80 leaf beet or garden beet, will have been stored in the national genebank in Beijing. All of these accessions are also stored in the northwestern part of China for safety-duplication. Storage conditions at the national genebank are excellent. Currently, 570 accessions also are stored at the Institute of Sugar Beet, CAAS, located in the Hulan county, Heilongjiang province.

Regeneration is performed by the institutes or local genebanks which offered the germplasms to national genebank. Often the storage conditions are not satisfactory there. In some local genebanks or institutes, the temperature at which the seed is stored can reach 20-25°C in summer, with a relative humidity of 55-65% in the rainy season. After the accessions have been stored for 4-5 years, the percent germination is reduced by 30-40%. They need to regenerate 20-25% of the accessions each year.

All of the accessions have been evaluated before being handed over to the national genebank. Thirty-five descriptors, including 24 botanical characters (germination, fertility, flower, leaf, root, seed, stalk) and 11 biological characters (susceptibility to disease, bolting, sugar and harmful material content, root yield, etc.) have been evaluated.

The local genebank has not used isoenzyme or RFLP markers for identifying varieties of sugar beet, leaf beet or wild beet. Breeding for disease resistance is the major objective, especially for root rots and rhizomania. Breeding lines or hybrids, which combine tolerance to root rots, have been selected through screening in heavily infested fields and with ELISA.

The planned expansion of the *Beta* germplasm collection has the following objectives:

- acquisition of sugar beet material
- acquisition of newly registered diploid varieties and tetraploid material
- collection of leaf beet and garden beet germplasm, especially in the highlands and hilly land in the southwestern part of China.

During 1994-95, we have collected 10 leaf beet or garden beet samples from the countryside, farmer's vegetable gardens and the suburbs. These samples will be evaluated and duplicated in 1996-97.

Germany

L. Frese

Germplasm acquisition and collection

Our collection located at Braunschweig ('the BGRC collection') now consists of more than 50 000 accessions of about 940 species. We have been offered a garden

beet collection by the Dutch Nickerson breeding company. This collection is being increased now and will be added to the holding stepwise. The number of samples received was about 140. Germplasm collecting in Italy in 1994 yielded 32 new accessions of *B. vulgaris* subsp. *maritima* and *B. vulgaris* subsp. *vulgaris* Leaf Beet, respectively.

Seed multiplication and safety-duplication

Each year, 60-70 accessions are multiplied at Braunschweig. The breeding companies KWS (Germany), Maribo (Denmark), Dieckmann-Heimbürg (Germany), and SES Seeds (Belgium) support the BGRC in the field of *Beta* seed multiplication. Currently 260 subsamples of *Beta* accessions are being prepared for safety-duplication at the CPRO-DLO CGN (Netherlands).

Evaluation and research

Within the framework of the Chinese-German cooperation a replicated field test on resistance to *Cercospora beticola* of 101 *Beta* sp. accessions was carried out at Braunschweig in 1995. Project partners were Assist. Prof. Ma Yahuai from the Sugar Beet Research Institute (CAAS), Hulan county, Heilongjiang province, P.R. of China and Dipl. Agr. Ing Holger Adams, Research assistant, Institute of Sugar Beet Research (IfZ), Göttingen (Germany). Strikingly, the most promising material has been collected in the mountainous areas in Turkey and Transcaucasia region (11 accessions of *B. corolliflora*) and in England (1 accession of *B. vulgaris* subsp. *maritima* from Severn Estuary) where the leaf spot disease seldom occurs.

Within the framework of Council Regulation 1467/94 of the European Commission (EC), a project proposal was submitted last year. The project was selected for funding and will receive 550 000 ECU over a period of 5 years. Project partners are the Hillesjö breeding company in Sweden, the University of Birmingham and Broom's Barn in England, the Greek Gene Bank, CPRO-DLO in the Netherlands, the breeding companies Dieckmann-Heimbürg and KWS, IPK at Gatersleben in Germany, the breeding company SPSB at Bologna and the ISCI at Rovigo, both in Italy. The BGRC is project coordinator. The project is titled 'Evaluation and enhancement of *Beta* collections for extensification of agricultural production'. Priority is given to evaluation of germplasm, and the rationalization of the European *Beta* collection. With the European *Beta* project a synthetic *Beta* core collection (similar to barley, see Knüpffer and van Hintum 1995) will be developed. The underlying principle of a synthetic core collection is that each genebank should take responsibility for a part of the core collection, as with seed multiplication and distribution. The further improvement of a synthetic core collection could become a task of all curators collaborating within the WBN organization. This issue will be discussed during the 5th WBN meeting.

Planned collecting missions

Because of the pending decisions with respect to the reorganization of the BGRC, no further efforts have been undertaken in this area. It has been planned to resume activities in the Transcaucasia region jointly with VIR. There are good contacts with China. But the specific situation at the BGRC requires us to give a higher priority to the maintenance of the whole BGRC collection for the time being.

Reference

Knüpfper, H. and Th.J.L. van Hintum. 1995. The Barley Core Collection: An international effort. Pp. 171-178 *in* Core Collections of Plant Genetic Resources (T. Hodgkin, A.H.D. Brown, Th.J.L. van Hintum and E.A.V. Morales, eds.). John Wiley & Sons, Chichester, UK/IPGRI, Rome, Italy/Sayce Publishing, Devon, UK.

Islamic Republic of Iran

M. Nasser Arjmand

Introduction

The world's population now stands at over five billion people. Of these, more than 75% are in the developing countries. By the end of the next century, conservative estimates predict that this figure will have doubled. Population growth rates are significantly higher in developing countries than in the developed states and the available land and water resources for food production are not increasing as quickly as the population. Population control may be one way of tackling an immense world problem, but agriculture will still be called upon to feed, and to some extent, to house and clothe the world's growing population. To increase crop productivity, agriculture will need to employ the most appropriate technology available, whether this be in the form of mechanization, fertilizers, agrochemicals, or improved crop varieties.

To meet an increasing demand for food and fibre, crop varieties must be improved. Plant genetic resources (in comparison with other national resources such as ore and oil) are the most valuable renewable resources to improve crop varieties. The availability of diverse germplasm, its characterization, and knowledge of the different species and forms of any crop, are essential for a successful breeding programme, because wild relatives of crop plants are being destroyed in their natural habitat through overgrazing, accidental fire, war, industrialization and development. It is essential that this germplasm be conserved for future use. IPGRI therefore initiated a crop network to conserve valuable genepools at the global level, and also to study them systematically for future use.

Geographical situation and agricultural features of Iran

The Islamic Republic of Iran has an area of 164 million hectares located between 25 and 40 degrees North. Elevation ranges from sea level to 5600 m, and temperature varies from -30 to 50°C. Annual precipitation in different regions of the country varies from zero to 2000 mm. The total land area is 164 million ha (1/25 of the total land area of Asia). Approximately 62% of the land is mountainous with steep slopes and the rest is covered by plains, deserts, rivers, lakes, etc. In general, with due consideration to its geographical and natural situation, Iran has dry climatic conditions. Therefore, Iran has a very good potential to grow different agricultural crops.

According to the latest official statistics, approximately 18.5 of 51 million ha of land which has been estimated to possess agricultural capability is in the crop production chain. This takes into consideration the land under perennial irrigated crops (orchards), fallow irrigated lands, rain-fed fallow lands, irrigated and non-irrigated lands of the country. Other statistics show that if second crops are included, the area of the country under cultivation in 1991-92 was approximately 17.8 million ha, of which nearly 7.3 million ha was irrigated and about 10.5 million ha was rain-fed.

In addition to the 51 million ha of cultivable land, Iran has 90 million ha of reclaimable land and about 10.2 million ha of land worthy of transformation into commercial forests, 2700 km of water frontier, nearly 120 billion m³ of water capable of control and exploitation, and 14 different climates linked with abundant solar energy. These important national resources should be exploited

for the development of economic social welfare, and guarantee the independence of our country.

Sugar crops

The major crops in this group are sugar cane and sugar beet. The total annual sugar production in Iran is about 850 000 t, of which 200 000 t is obtained from sugar cane and the rest comes from sugar beet.

Sugar cane

Cultivated sugar canes (*Saccharum* spp.) probably are derived from indigenous wild species on the islands of Melanesia. It is postulated that they arose by selection from wild canes in New Guinea. They were carried by man as stem cuttings to various centres where they were further modified by natural hybridization with other wild grasses. Sugar cane of economic usefulness probably has been transported by man for thousands of years. Sugar cane was cultivated in India as early as 1000 BC, while in China crude sugar was being made from sugar cane by 760 BC. The first refined white sugar was made in Iran about 600 AD.

Cultivation of sugar cane in Iran has a history of several thousands years. In old Persia, sugar cane cultivation was common in Khouzestan in the southwest and in Mazandaran in the north around the Caspian seashore. The cultivation of sugar cane was abandoned owing to historical events.

In the years 1937 through 1939, some seed (stem cuttings) was planted in Ahoo Dasht, Ahwaz, Hamidieh, and other areas of Khouzestan but the former Anglo Iranian oil company found the operation against its own interest and opposed the project in 1940. In a few short years the seedlings died and disappeared. During 1951 to 1957, the planning organization invited groups of experts from the United Nations FAO division to conduct feasibility studies on the development of sugar cane cultivation. Thus in 1960, 2400 ha were planted. In December 1961 the factory at Haft Tappeh was inaugurated, and so after many long years, sugar cane culture in Iran began anew. Since the sugar cane cultivation was successful in Khouzestan, the second factory named Karoon was established in 1978. These two factories belong to the Agriculture ministry. The capacity is 10 000 and 25 000 t cane per day for Haft Tappeh and Karoon, respectively.

From the historical point of view, sugar cane cultivation in Mazandaran has a long story. According to the historical literature, stem cuttings were imported from the Eastern states (Sand and Mokran) to Mazandaran and Gorgan through Khorasan about 10 to 15 centuries ago. At present, this old sugar crop is a native in the Eastern Caspian seashore and covers 1200 ha, which is cultivated traditionally. To improve production, stem cuttings of cp48, cp57 and Nco varieties were transferred to Mazandaran. A modern sugar cane factory recently was established in Mazandaran by the private sector and may be inaugurated this year.

Sugar beet

Sugar beet (*Beta vulgaris*) is a man-made crop that originated about 200 years ago from a limited range of beet types. All the cultivated beets and a wild species belong to section *Vulgares* in the family Chenopodiaceae. It is believed that a wild type of *B. vulgaris* referred to as *B. vulgaris* subsp. *maritima* was the progenitor of the sugar beet.

The first factory for the extraction of sugar from sugar beet, established in 1894 in Kahrizak near Tehran, was a failure. The second sugar factory was built in 1933 in Karaj, and, at present, there are 35 sugar factories with a total capacity of

6200 t of beet per day. The largest has a capacity of 5000 t per day and the smallest 450 t. Sugar beet is grown in all regions of Iran with the exception of Sistan-Balouchestan, where water is insufficient, and Gilan and Mazandaran because of the priority of other main crops. The country's sugar beet seed, which is about 5000 t/year, is supplied by the sugar beet seed institute (SBSI) and distributed to sugar factories.

Beta germplasm in Iran

It is generally believed that the different forms of beets (leaf beets, fodder beets, garden beet and wild beets) originated in Northeastern Europe and the Mediterranean. Wild forms of *Beta* show a wide distribution, and are found in Northeastern Europe, along the Mediterranean coasts of Europe and Africa, and extend eastward to Iran, India, China and other Asian countries. Exploration for collecting wild species of *Beta* has been conducted by staff of the *Beta* Gene Bank, which was established in 1989. During the past 5 years, the *Beta* Gene Bank has succeeded in collecting wild *Beta* species and finding them in their natural habitats. Collected accessions include *Beta* species from section *Beta* (formerly section *Vulgares*) and *Corollinae* (Table 1).

Evaluation of wild accessions and landraces is being done by the *Beta* Gene Bank in the field and greenhouses, for biotic and abiotic stresses. A map of distribution of wild species and landraces has been prepared. Sugar beet germplasm has been used in Iran since 1935 for developing varieties suitable for its 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 that have been developed are being cultivated in different areas of Iran, and have made a useful contribution in terms of production and productivity.

Table 1. Sections and species of *Beta* L. accessions

Section	Species	2n =
<i>Beta</i> (syn.: <i>Vulgares</i>)	<i>B. vulgaris</i> L.	18 (27, 36)
	<i>B. vulgaris</i> subsp. <i>maritima</i> L.	18
	<i>B. macrocarpa</i> Guss.	18, 36
	<i>B. patula</i> Ait.	18
<i>Corollinae</i>	<i>B. corolliflora</i> Zoss.	36
	<i>B. lomatogona</i> Fisch. et Mey.	18, 36
	<i>B. macrorhiza</i> Stev.	18
	<i>B. trigyna</i> Wald. et Kit.	36, 54
	<i>B. intermedia</i> Bunge.	36
<i>Nanae</i>	<i>B. nana</i> Boiss. et Held.	18
<i>Procumbentes</i>	<i>B. patellaris</i> Moq.	36
	<i>B. procumbens</i> Chr. Sm.	18
	<i>B. webbiana</i> Moq.	18

SBSI Beta Gene Bank activities

Collecting

Through the *Beta* Gene Bank programme, during the past 4 years we have collected wild accessions and landraces in regions where SBSI has research

departments. Our colleagues visited areas and determined the sites containing wild species and landraces. We have continued our exploration in Khuzestan and Ardabil to collect new samples of *B. vulgaris* subsp. *maritima* and *B. lomatogona*. In Hormozgan province, at the research station of Minab, we have collected *B. vulgaris* subsp. *maritima*. In Ardabil new sites of *B. lomatogona* have been found by the SBSI *Beta* Gene Bank group and seed samples were collected in bulk and from single plants. *Beta lomatogona* has been growing in the different field crops in Ardabil. When the farmers harvested their crops, the seed from the *B. lomatogona* plants also was harvested by farmers and could not be collected. For future collecting, we have encouraged the farmers to inform our colleagues in Ardabil when they will harvest their crops. It is hoped that we can collect new seed samples of *B. lomatogona* this year.

In Khuzestan, we collected four samples of *B. vulgaris* subsp. *maritima* at new sites. In Moghan, in June 1993, we found a *Beta* species in the sugar beet farms with immature seed balls that may be *B. vulgaris* subsp. *maritima*. Exploration for new samples will be continued in this region. We have continued collecting landraces in the same provinces: Fars, Tehran Khorasan, Semnan, and Khuzestan.

Evaluation

In addition to evaluating beet germplasm for disease resistance, searching for germplasm with tolerance to abiotic stress has been started in regions where the sugar beet has been exposed to stress factors such as drought and salinity.

Multiplication

Multiplication of 30 landrace accessions is carried out in isolation plots at different experiment stations. The technique applied is the traditional 2-year cycle system.

Karyotype determination and C-banding

Study of the wild species in the genus *Beta* in Iran was merely limited to identification and determination of their distribution, which has resulted in collecting of their seed from different parts of the country since 1993. We are beginning cytological studies on these species and, to obtain information on morphological structure of their chromosomes, mitotic studies have been carried out; karyotyping of the mentioned species, along with a cultivar of sugar beet and red beet, has been prepared.

Owing to the small size of chromosomes in the *Beta* genera and similarity of their size and situation of centromere, determination of differences between chromosomes is very difficult; therefore it is not possible to easily distinguish homologues and study chromosomes individually. For the sake of more precise chromosome studies on sugar beet, a banding technique has been used. Application of the C-banding method revealed heterochromatic bands in centromeric regions. In general, distal regions of arms lacked bands; however, we have not seen bands in other parts of chromosomes. This technique did not help us to study chromosomes individually.

To examine the possibility of hybridization between the wild species *B. lomatogona* and sugar beet and the ability to use the desirable features in the mentioned species, a few crosses were made. Different stages of meiosis in hybrids and rate of homology between chromosomes of the two above-mentioned species were investigated. The studies carried out are as follows: (1) study on the

karyotype of species of *Beta* in Iran, (2) assessment of sugar beet chromosomes applying C-banding method, and (3) study on meiosis among the hybrids *B. lomatogona* \times *B. vulgaris*.

Poland

L. Dalke

The *Beta* collection in the Department in Bydgoszcz is a unit of the Plant Genetic Resources Gene Bank at the Plant Breeding and Acclimatization Institute in Radzików. It was established to coordinate, finance and provide storage facilities for genetic resources of crop plants in Poland. The activity for the genus *Beta* collection is concentrated on collecting, maintenance, evaluation and documentation of wild species and cultivated forms of beets (sugar and fodder beets). The Polish collection contains wild species of sections *Beta*, *Corollinae*, *Nanae* and *Procumbentes*. Species of the *Corollinae* section are maintained in the field (perennial species). *Beta nana* and male-sterile *B. vulgaris* subsp. *maritima* ecotypes are kept as *in vitro* cultures. Other wild species and cultivated forms are stored as seed samples. Seed samples are kept in glass jars at -20°C and 5-8% moisture content in the Gene Bank of the Institute in Radzików, near Warsaw. A part of the material is stored in Bydgoszcz under medium-term storage (0 - 4°C) as an active collection.

New accessions have been obtained from national sources (breeding institutions) and also on an exchange basis from foreign collections. Neither wild species nor landraces occur in Poland. The total number of accessions stored in February 1996 amounted to eight accessions of wild species and 193 accessions of cultivated beets. All stored samples (as determined by the last germination test) had good germination. Therefore regeneration is not necessary for the moment. The safety-duplication storage is still an unsolved problem in our genebank.

For evaluation, the *Beta* descriptor list of IPGRI was chosen. Germplasm evaluation and seed multiplication are done at Bydgoszcz (wild species) and at the Experimental Station in Kończewice (cultivated beets). Evaluation of cultivated beets is done on 10-m² plots in two replications with standard check varieties in a 2-year cycle. Evaluation is performed continuously as part of our germplasm programme. Each year, about 25 accessions are evaluated. The evaluation is of both morphological and agronomic features. The collected and evaluated germplasm is being utilized in sugar and fodder beet breeding and in several research programmes.

No collecting mission abroad is planned by us. From our point of view, it would be of great advantage to organize an expedition to collect different ecotypes of male-sterile *B. vulgaris* subsp. *maritima*. Such exploration could be implemented as an international activity.

United States of America

A. Hodgdon

The Plant Introduction *Beta* collection was transferred to the Western Regional Plant Introduction Station (WRPIS) Horticulture Program at Pullman, WA, USA in late 1994. We are now in the process of completing the inventory of the collection and getting the seed increase programme operational. The composition of our collection is described in Table 2.

In the field each accession is isolated in a pollen-proof cloth cage. We have two field sites. One site is at low elevation (200 m), where we transplant in the autumn for natural vernalization; one at a higher elevation (800 m), where we spring-transplant artificially vernalized plants. We also have a greenhouse increase programme, in which each accession is isolated in a greenhouse section. The greenhouse increases are of either annual accessions or artificially vernalized biennials. We are utilizing greenhouse space from all of the WRPIS programmes, as well as space in the Washington State University greenhouse system. Artificial vernalization is in cold growth chambers (4°C) for 4 months. In the 1996 crop year, we anticipate increasing 93 *Beta* accessions, and, in 1997 and beyond, we hope to increase at least 110 accessions per year. Forty of these will be done in greenhouses each year. The focus for the next few years will be to increase *Beta* germplasm which has poor germination and low seed supply. Also, we will concentrate on growing hard-seeded species of *Beta*.

In 1996, we hope to write a *Beta* Operations Manual which will outline our growing, storage and inventory procedures. We will complete the back-up of all the *Beta* accessions with sufficient seed at the National Seed Storage Laboratory. We also will back up our seed at a separate -20°C degree facility at WRPIS, where we will store original and regeneration samples of as many accessions of the *Beta* collection as possible.

Many of the *Beta* accessions have good evaluation data. The curator works with the *Beta* Crop Germplasm Advisory Committee to choose approximately 30 accessions per year for evaluation by researchers. Periodic collecting trips for *Beta* accessions are funded by the USDA, ARS, National Plant Germplasm System. The information on the *Beta* accessions in the PI system is on the Genetic Resources Information Network (GRIN). The address for GRIN on the World Wide Web is <http://www.ars-grin.gov/npgs/>. The Internet address is: [gopher://gopher.ars-grin.gov/](http://gopher.ars-grin.gov/). For reference use on desk-top computers, pcGRIN is available from the address listed below or can be directly downloaded from the gopher address or website.

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Table 2. Status of the *Beta* collection maintained at the USDA, ARS, NPGS Western Regional Plant Introduction Station, Pullman, Washington

[illegible]

Presented papers

Proposal for a new taxonomical classification of the cultivated forms of beet, *Beta vulgaris* L.

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Abstract

This paper puts forward a proposal for a new taxonomical classification for the cultivated forms of beet. Part one includes a short description of concept and rules of the new classification system for cultivated plants, as published in the International Code of Nomenclature for Cultivated Plants 1995 – the so-called Cultivated Plant Code 1995. The classification has an open character, and is formed around several basic elements, which are called taxon (plural: taxa) of cultivated plants, or culton (plural: culta). Culta are not grouped in the hierarchic way as used in the botanic nomenclature system, and their names (epithets) have to be written in a modern language. The relevant culta for beet are the named cultivars and the newly formed cultivar-groups. Part two of the paper includes a summary of the latest revision of the taxonomy of *Beta* section *Beta*. The section has three species: *B. vulgaris* L., *B. macrocarpa* Guss. and *B. patula* Ait., and *B. vulgaris* has three subspecies: subsp. *vulgaris* (all cultivated materials), subsp. *maritima* (L.) Arcang. (a large and variable group of wild forms) and subsp. *adanensis* (Pamuk.) Ford-Lloyd & Will. (a small and distinct taxon). Finally the new classification system for cultivated plants was applied to beet, which led to the proposal to distinguish four cultivar-groups: the Leaf Beet Group, the Garden Beet Group, the Fodder Beet Group and the Sugar Beet Group. The four groups are briefly described. For the time being it should be stressed that this proposal has to be discussed to become accepted. Suggestions are very welcome. On the basis of the comments a more formal cultivar-group classification for beet will be written and published.

Introduction

During the preceding meeting of the World *Beta* Network at Fargo, ND, USA, Wouter Lange and colleagues presented a paper on the Taxonomy of *Beta* Section *Beta* (Letschert *et al.* 1994), which was written on the basis of the PhD study of Letschert (1993). That paper mainly dealt with the wild forms of section *Beta*. The cultivated materials of beet were left out, because at that time the international discussion on the concept of nomenclature of cultivated plants was not yet finished. This situation has changed now, so that it is time to review the taxonomy of cultivated beet, according to the new concepts and rules.

Thus the present paper mainly will deal with a proposal for a new taxonomical classification for the cultivated forms of beet, *Beta vulgaris* subsp. *vulgaris*. First we will explain the need for the revision of the taxonomical classification of the cultivated forms of beet, and present details of the relevant parts of the new rules for the nomenclature of cultivated plants. Next we will briefly review the taxonomy of Section *Beta* of the genus *Beta*. And finally we will show how the

new rules could be applied in *Beta vulgaris*, and we will formulate the proposal for the new taxonomical classification of this group of cultivated plants.

The Cultivated Plant Code

Since 1953 the nomenclature of cultivated plants has been governed by the International Code of Nomenclature for Cultivated Plants, the ICNCP or the Cultivated Plant Code. At the end of last year the 6th and fully revised edition of this Code was published (Trehane *et al.* 1995), which here will be called The Cultivated Plant Code. Drafts of this new code were discussed in August 1994 at the 2nd ISHS Symposium on the Taxonomy of Cultivated Plants at Seattle, Washington, USA. Then a final version was prepared, which was adopted by the International Commission for the Nomenclature of Cultivated Plants, and was published.

The Cultivated Plant Code has been set up according to the successful format of the International Code of Botanical Nomenclature (ICBN, Greuter *et al.* 1994), which is being used by botanical taxonomists, and deals with the formation and use of botanical names in Latin form of both wild and cultivated plants. Such names are based on a hierarchic classification system. The Cultivated Plant Code aims at presenting principles and rules for the nomenclature of distinguishable groups of cultivated plants, whose origin or selection is primarily due to intentional actions of humans. Thus the Cultivated Plant Code is meant to promote uniformity, accuracy and stability in the naming of plants in agriculture, horticulture and forestry.

The open classification system

The basic idea behind the proposed classification system is the concept of open classification (Brandenburg *et al.* 1982; Brandenburg 1984, 1986; Brandenburg and Schneider 1988). In this concept the descriptions of both cultivar-groups and cultivars are demanded. Since open classification of cultivated plants deviates from the usual botanical classification with its known hierarchy, a new term has been proposed: the culton. A culton is a taxonomic group of cultivated plants, which is described under the new open classification system. The culton is a parallel to the word taxon in the usual botanical classification (Hettterscheid and Brandenburg 1995).

Regarding the nomenclature rules and the classificatory guidelines the Cultivated Plant Code (1995) is based on the concept of the culton. As in other Codes, the order of the nomenclatural process for fixing the names of cultons is (1) publication, (2) establishment and (3) acceptance. The Code is meant to be used by plant taxonomists, plant registration authorities, the seed and plant trade, and all others who use plant names in agriculture, horticulture and forestry. Thus, the Cultivated Plant Code also is meant for those who work with genetic resources of beet. The rules of the Code mainly deal with the final part of the name of cultivated plants, the so-called cultivar epithet. This epithet is used to identify the man-made products, and according to the rules it has to be formed in a modern language, and not in Latin. The Cultivated Plant Code deals with three taxonomic groups of cultivated plants: the cultivar, the cultivar-group and the graft-chimera. For the purpose of the present discussion on the taxonomy of cultivated beet, we will focus on the nomenclature of cultivars and cultivar-groups.

The cultivar

A cultivar is a taxon of cultivated plants, or a culton, that is clearly distinct, uniform and stable in its characteristics and which, when propagated by appropriate means, retains those characteristics. A cultivar is not equivalent to the botanical categories *varietas* and *forma*. Thus the word variety, and translations of it in other languages, may no longer be used as a synonym for the word cultivar. The full name of a cultivar will consist of the botanical name of the genus, and optionally the species, as well as a unique epithet in a modern language. The Code gives many rules and recommendations for the formation of such epithets, and also the Registration Authorities can be asked for assistance. The name must be registered at the appropriate Registration Office, and must be published to be fixed. The cultivar epithet must start with an initial capital, and should be enclosed by single quotation marks. The use of the abbreviation cv. is no longer permitted.

The cultivar-group

A cultivar-group is a taxon of cultivated plants, or a culton, that denotes an assemblage of similar and named cultivars. The cultivar-group is described by the similarity between its cultivars. As for cultivar names, the Code gives rules and recommendations for the names of cultivar-groups. Thus the names should be written with capital initials. When used in conjunction with a cultivar epithet the name of the group is written in parentheses, next to the cultivar name. When used as a group name, without the conjunction to a certain cultivar, the parentheses are not to be used. Sometimes a cultivar can be assigned to more than one cultivar-group.

Taxonomy of *Beta*

The first valid description of the binomial name *Beta vulgaris* was given by Linnaeus, in the first edition of *Species Plantarum* of 1753. The description contained eight varieties with Latin names. In the second edition of *Species Plantarum* (1762), the species was split up into wild and cultivated materials. The wild taxon was named *Beta maritima*, and the cultivated material remained to be split up into varieties carrying Latin names. A few years later Linnaeus even proposed to split the cultivated species into two species: *Beta vulgaris* and *Beta cicla* (Linnaeus 1767).

The sections of *Beta*

As a result of the studies of Transhel (1927) and Ulbrich (1934) the genus *Beta* was subdivided into four sections (Table 1). All cultivated materials and a part of the wild taxa were brought together in Section *Beta*, which in earlier publications also was known under the synonym Section *Vulgares*.

Table 1. Sections of the genus *Beta* L.

Section <i>Beta</i> (syn. <i>Vulgares</i> Ulbrich)
Section <i>Corollinae</i> Ulbrich
Section <i>Nanae</i> Ulbrich
Section <i>Procumbentes</i> Ulbrich (syn. <i>Patellares</i> Transhel)

In the earlier studies on the taxonomy of Section *Beta* there was still insufficient knowledge on the wild materials and a strong overestimation of the importance of the man-made variation in the cultivated stocks. The wild and cultivated taxa were treated as different species or at the infraspecific level. Various systems for the taxonomic treatment of this section have been proposed, with different levels of infraspecific categories, or with different interpretations of such categories by both plant breeders and taxonomists. Recent examples of such systems are those by Helm (1957), who used the categories *convar* and *provar*, or the two treatments by Ford-Lloyd (Ford-Lloyd and Williams 1975; Ford-Lloyd 1986), who in fact made the section monotypic, with one species and various numbers of subspecies.

Taxonomic revision of Beta Section Beta

Recently Letschert of the Agricultural University Wageningen, the Netherlands, carried out extensive studies to be able to make a revision of the taxonomic classification of the wild taxa of Section *Beta*, according to formal taxonomic criteria, including the rules of the ICBN. First a stable nomenclature was created through typification of the Linnaean names (Letschert 1993; Letschert *et al.* 1994). The stabilization of these names proved to be necessary for further classification of the wild and cultivated materials. Thus, herbarium sheets were designated or made as lectotype or neotype for *Beta vulgaris*, its varieties *cicla* and *rubra*, and for *Beta maritima*. Next an extensive morphometrical study and cluster analyses were carried out, including herbarium specimens and a specially composed collection of wild accessions. Patterns of variation of 10 allozymes were studied, and were also put together in a cluster analysis. Finally the taxonomic revision was formulated (Table 2) and completed with more detailed descriptions of the taxa as well as a key to the wild taxa of *Beta* Section *Beta*.

In this new classification three groups are ranked as species: *Beta vulgaris*, *Beta macrocarpa* and *Beta patula*. *Beta vulgaris* contains three subspecies. All cultivated materials were brought together in subspecies *vulgaris*. The subspecies *maritima* contains a large complex of various morphological types, which occur over a large geographical area. However, the separation between types was insufficient to allow for further subdivision. The subspecies *adanensis* is a distinct group of semi-annual plants, with specific morphological characteristics, and also with a high degree of self-fertilization. *Beta macrocarpa* also has a distinct morphology, and in addition has four unique allozymes. The species shows a relatively high degree of self-fertilization, and is able to maintain its identity when growing in sympatry with *B. vulgaris*. The last species, *Beta patula*, a geographical isolate from the islands of Madeira, shows a substantial morphological differentiation from the other taxa.

It could well be that the full consequence of the adoption of the culton concept as a separate dimension of classification of cultivated plants might lead to a need for merging subspecies *vulgaris* and *maritima*. In that case the name *maritima* will be lost, which does not seem appropriate. Such a change should therefore be considered very carefully, and should include a serious study on the systematic position of weed beets. Thus for the present discussion we propose to use the taxonomic treatment by Letschert (1993) as a starting point for the classification of *B. vulgaris*.

Table 2. Revision of the taxonomy of *Beta* Section *Beta*

Beta vulgaris L.

subsp. *vulgaris* (cultivated materials)

subsp. *maritima* (L.) Arcang.

subsp. *adanensis* (Pamuk.) Ford-Lloyd & Will.

Beta macrocarpa Guss.

Beta patula Ait.

Taxonomic classification of cultivated beet

About 2500 years ago, the first cultivated beet was domesticated. This material possibly was eaten as a vegetable. In later developments the types with swollen parts were selected, which parts consist of root and hypocotyl or of hypocotyl only. The route of domestication has been the subject of many studies and speculations, and will not be discussed in this paper. The development of cultivated beet is characterized by the selection of material for various applications, and for selection of materials with a wide variety of forms and colours, especially in the swollen parts. Modern plant breeding added three important steps to the breeding procedure: polyploidy, monogerm seed and the making of commercial hybrids.

For a long time the cultivated beet was diploid, like its wild progenitor, with 18 chromosomes. Since the discovery of the activity of colchicine, about 60 years ago, polyploid materials were made, especially tetraploids and triploids. Also much attention has been paid to the development of genetically monogerm materials, in which the seed ball contains only one seed. Furthermore a system for the use of CMS, the cytoplasmic male sterility, was introduced, which made it possible to produce commercial hybrid seed. Thus the image of modern cultivated beets is different from that of the crop at the beginning of this century.

The taxonomic criteria

For the subdivision of subspecies *vulgaris* we had to look for useful criteria. We first looked into the man-made characteristics and decided that they are of no use. It does not seem practical to divide the cultivated genepool in diploids, triploids and tetraploids, or in monogerm versus multigerms, or in open-pollinated or hybrid stocks. Next we studied characteristics like form or colour. Frandsen (1958) described some of the variation of the swollen parts of cultivated beets, as used by plant breeders. The major differences concern root length, root width, head length and plumpness, but many more characteristics could be described. Part of the cultivated material can even be characterized by the absence of a swollen root, or by a root which is not thicker than a thumb. Such material is grown for its leaves. Also the colour of the skin, the flesh of the swollen roots and the midribs of the leaves can show considerable variation. Helm (1957) tried to use the variation in form and colour as a basis for a taxonomic system of subspecies *vulgaris*. Helm proposed to distinguish between leaf beets and root beets at the level of the category convariety, and within these two groups two plus four provarieties were described. A further subdivision was proposed at the level of the forma. Thus finally 19 different types could be distinguished. All types got Latin names, according to the rules at that time. Although the system of Helm (1957) gives the impression of being well-founded, it now is being considered as a typical example of a system with too many groups and too many details. We also know that most of the variation should be considered as being continuous, making it not a sound basis for a detailed classification system. Thus our search for taxonomic criteria ended up with one single criterion: the application of the cultivated material in agricultural practice.

The cultivar-groups

With this criterion in mind we propose four cultivar-groups: Leaf Beet Group, Garden Beet Group, Fodder Beet Group and Sugar Beet Group. Note that all group names should be written with initial capitals. In the following short

descriptions of each of the four cultivar-groups the difference between the conventional hierarchic botanical system and the new open classification system will be marked by not making any cross-reference to the Latin names of the old systems. We also would like to stress that in the open classification system it is only necessary to describe the similarities between the cultivars within a group.

The Leaf Beet Group

The Leaf Beet Group contains cultivars of which the leaves and petioles are used as a vegetable. There might be variation for size and form of the leaf blade, and also for thickening of midrib and petiole. Cultivars which are mentioned under names such as spinach beet, chard or Swiss chard are all to be included in the Leaf Beet Group. Sometimes cultivars of the Fodder Beet Group are being used as a leafy vegetable. Such cultivars therefore can be placed in two cultivar-groups.

The Garden Beet Group

The Garden Beet Group consists of cultivars with swollen hypocotyls, which mostly are used as a vegetable, also in soups or in salads. The beets are used in the canning industry, and also to make pickles. The majority of the group consists of cultivars with a dense red colouration. However, also yellow and pinkish cultivars are available, so that the colour is not used as a describing criterion. This explains the name Garden Beet Group instead of Red Garden Beet Group. The beets sometimes may be used to produce a red natural dye. We do not know if there are special cultivars made for that purpose, and if so, if this group would be distinct enough to be handled as a separate group. Another English name for the whole group could have been Beetroot Group. However, a beetroot written as one word means the vegetable, and a beet root written as two words means a root of any beet. We therefore have chosen for the name Garden Beet Group.

The Fodder Beet Group

The Fodder Beet Group is a large group of cultivars that are used as fodder for cattle. The plants have a large swollen part, which consists of hypocotyl and root. This group includes a large variation in form and colour of the swollen parts and their skin colour. However, as said before, we do not use this variation to distinguish subgroups. A synonym name of the group could be Forage Beet Group, but because of present use in the literature we have chosen Fodder Beet Group. In older literature the name Mangold was used. However, such usage proved to be ambiguous so that we propose to discontinue the use of that name.

The Sugar Beet Group

The last cultivar-group concerns the Sugar Beet Group. The cultivars of this group are used for the production of sucrose in special sugar factories. These cultivars are bred for a high sucrose content and good extraction qualities. This cultivar-group has less variation in form and colour than the Fodder Beet Group.

Finally the various ways in which the name of the sugar beet cultivar 'Evita' should properly be written are as follows: *Beta* L. 'Evita'; *Beta vulgaris* L. 'Evita'; *Beta vulgaris* L. subsp. *vulgaris* 'Evita'; *Beta* L. 'Evita' (Sugar Beet Group); *Beta vulgaris* L. 'Evita' (Sugar Beet Group); *Beta vulgaris* L. subsp. *vulgaris* 'Evita' (Sugar Beet Group).

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Variation for developmental characters in *Beta vulgaris* subsp. *maritima* in relation to latitude: The importance of *in situ* conservation

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Abstract

Plants grown from seed originating from more than 100 wild populations were studied under greenhouse and semi-natural conditions. The origin varied from the French Mediterranean to the North Sea coasts (between 43°E and 52°E latitude). The characters studied were flowering behaviour and lifespan, both related to physiological processes like allocation of biomass and reproductive effort, which are partially regulated by environmental stimuli such as photoperiod and temperature. Variation was found for the age of first flowering, flowering phenology and lifespan. An analysis of this variation showed that the genetic component was considerable and that a significant correlation existed with latitude. Apparently, natural selection favours different developmental programmes at different latitudes. Such variation is best conserved under the original environmental conditions.

Introduction

The wild relatives of crop plants may contain interesting Mendelian genes for all sorts of economically important characters. Such genes are easy to conserve once they have been found and characterized. The conservation of quantitative genetic variation is more difficult. The genes are less evident because the effect of each of the underlying genes is small. The genes involved are not recognizable individually, unless they have been localized by markers. This quantitative variation may concern a developmental programme, e.g. the timing and the relative importance of the developmental phases, the allocation of resources to the various organs and functions, etc. Wild material can be highly variable for such developmental programmes. Multiplication under standard conditions is in principle unfavourable for the conservation of such variation, because natural selection will favour those programmes that give the largest seed or pollen production under these artificial and uniform conditions. In the case of *in situ* conservation, natural selection will have different outcomes in different environmental situations. This provides the best guarantee for the protection of this type of genetic resource.

In the present study we describe the timing of flowering and the lifespan of *Beta vulgaris* subsp. *maritima* under identical (greenhouse) conditions, with the goal of estimating the genetic variation that exists in nature for such characters. For this purpose, about 100 wild populations were sampled, originating from the French Mediterranean coast to the North Sea (between 43°E and 52°E latitude). Differences found, if any, point to genetic variation in the timing and intensity of physiological processes that determine the allocation of biomass to the different functions.

We already know that the frequency of the *B*-allele, which cancels vernalization requirement completely, is high in the south and zero in the north

(Van Dijk and Boudry 1992; Boudry *et al.* 1994). Plants that do have a vernalization requirement are known to need more cold when their origin is more to the north (Van Dijk and Boudry 1992). Vernalization is one of the important characters which influence the timing of flowering. The other major factor is photoperiod, which is probably the key factor for flowering date. Lifespan on an individual plant is negatively correlated to the annual reproductive effort.

Materials and methods

Plant material

Beta vulgaris subsp. *maritima* is a perennial, self-incompatible, wind-pollinated species. It is salt-tolerant, and mainly found on the Mediterranean and European Atlantic coasts (see Letschert 1993 for more details). In Europe, only a few truly natural populations exist in inland regions.

In August 1989, seeds were sampled in 93 populations around the French coast and in adjacent regions (see Van Dijk and Boudry 1992). Seeds from individual plants were collected separately; the number of plants sampled varied between 1 and 30 per population. In small populations, all plants with a minimum of about 20 ripe seeds were sampled, and, in large populations, a random sample of 20-30 plants was taken. What we call seeds are in fact seed "balls", which can contain up to 6 seeds from different flowers.

Culture conditions

Unless otherwise indicated, seeds were sown and plants were initially grown in 750-ml pots of soil in the 'warm greenhouse' at 20°C with a photoperiod of at least 16 hours per day. Additional light was provided by sodium high pressure lamps. Afterwards, plants stayed in the 'cold greenhouse' where no additional light was given and temperatures were kept between 5 and 12°C during winter and 15 and 25°C during summer.

Experimental design

In 1990, a large greenhouse collection was built up by sowing 718 plants from all 93 populations. In each subsequent year, one or a few plants of each population were added to the collection by sowing some of the original seeds. These plants were used for all of the following three experiments.

Complete absence of vernalization requirement

Complete absence of vernalization requirement can be easily identified by growing plants from seed in the greenhouse at 20°C, with a photoperiod of 16 hours, and with sufficient nutrients and light intensity. Seeds of plants from the 93 populations were sown in the greenhouse during the time of the year which most promotes flowering (end of May, early June), and checked for flowering induction without any vernalization. The total number of plants observed was 1439 (mean number of plants per population = 15.5). Any plant which flowered in these conditions does not require vernalization. Plants were observed for 120 days from germination.

Flowering date under uniform conditions

The plants from the previous experiment were used here, except those sown in 1996. The total number of plants is 1156 (mean number of plants per population

= 12.4). After the initial check for bolting without vernalization, the plants were kept in the cold greenhouse and repotted each winter (3-L pots with soil). Flowering date (date of first anthesis) was recorded each year (from 1991 to 1996), but flowering of the plants without vernalization requirement was not considered in their first year. In order to compare data from different years directly, all data were transformed into standard normal deviates. This was done by calculating the mean and standard deviation over all 93 population means for each year, subtracting all individual flowering dates from the mean value for that year and dividing these deviations by the corresponding standard deviation.

Lifespan under uniform conditions

The plants from the previous experiments were scored for survival. The mean lifespan of a population was calculated as the moment after germination at which 50% of the plants were still alive. For a few populations, over 50% of the plants sown in 1990 were alive in 1996. The calculation was done as if all those plants were dead in 1997 (age 7), which is obviously wrong, because a considerable part of them are still in good health. For those populations, mean lifespan will therefore be underestimated.

Results

Complete absence of vernalization requirement: occurrence and frequencies

The frequencies of flowering and non-flowering phenotypes in a non-vernalizing environment show a marked geographical pattern. Within the sampled area, different regions can be distinguished. Inland populations consisted almost exclusively of plants without any vernalization requirement and therefore flowered readily in the conditions provided. On the Mediterranean coast their frequency is also very high, but on the Atlantic coast, between the Spanish border and the southwest point of Brittany, only a small percentage of plants can flower without vernalization. Further north all plants have a vernalization requirement which may vary in strength (Van Dijk and Boudry 1992). Figure 1 shows the relationship between population latitude and flowering percentage without vernalization.

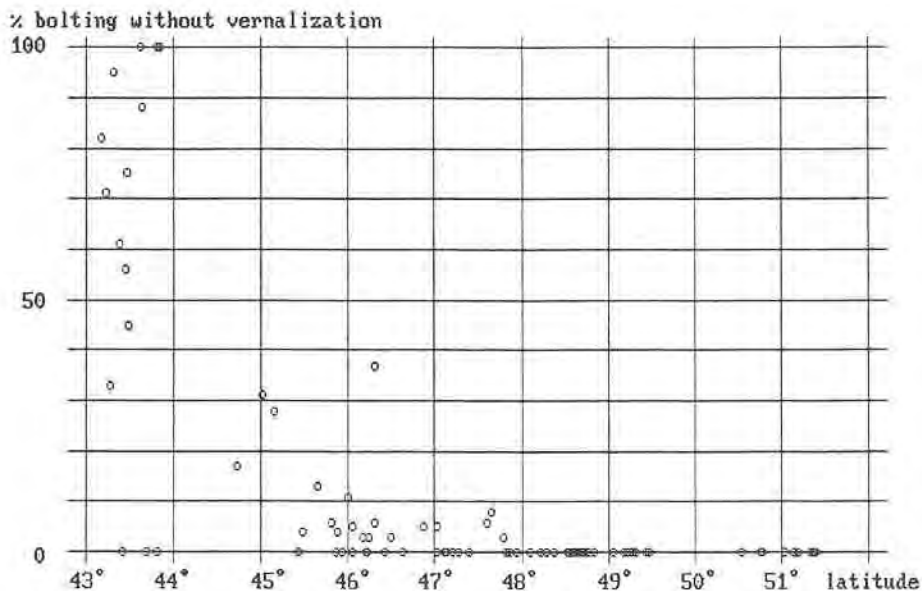


Fig. 1. The relationship between latitude and the percentage of plants without any vernalization requirement in each of the populations.

Flowering time under uniform conditions

In the cold greenhouse with natural daylength, the first signs of bolting in some of the plants were evident at the end of February, when days are still short (less than 12 hours). This clearly shows that the influences of vernalization and photoperiod are interdependent, in the sense that a strong vernalization can, to a certain extent, replace long days.

Flowering date (date of first anthesis) took place between mid-April and late June under our cold greenhouse conditions. The annual means of all population means were 20 May in 1991, 25 May in 1992, 15 May in 1993, 21 May in 1994, 23 May in 1995 and 28 May in 1996. The respective standard deviations were 9.4 days in 1991, 6.3 days in 1992, 7.9 days in 1993, 8.3 days in 1994, 7.2 days in 1995 and 7.0 days in 1996. For the statistical treatment, these differences were eliminated by transforming all individual values into standard normal deviates.

Geographical variation was observed, but a systematic south-north cline such as that observed for vernalization requirement did not appear. Figure 2 shows the relationship between flowering date and latitude for all populations sampled, which seems to have the shape of a V. For analysis of the regression of flowering date on latitude, two groups of populations had to be considered: those with a negative and those with a positive linear regression. From the Mediterranean northwards to the western part of Brittany, plants flowered gradually earlier. The correlation for this part is highly significant ($r=-0.777$ for the 51 populations involved; $P<0.001$). The slope is -0.47°F per degree of latitude. Going from the western part of Brittany to the northeast, the situation is reversed: flowering is later at higher latitudes with a slope of $+0.57^\circ\text{F}$ per degree of latitude. This correlation is also highly significant ($r=0.736$ for the 42 populations involved; $P<0.001$).

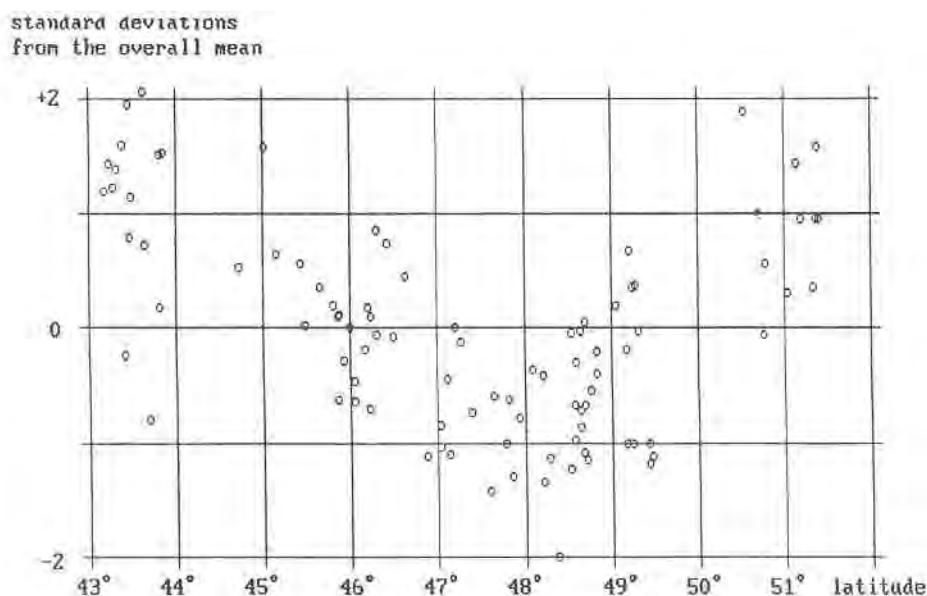


Fig. 2. The relationship between latitude and mean flowering date for each of the populations. Flowering date is expressed in standard deviations from the unweighted mean over all populations.

Lifespan under uniform conditions

Large differences were observed for lifespan, related to the geographical origin of the populations (Fig. 3). The shortest-living plants were those from inland populations in the southwest of France. Their mean lifetime was 2 years ($SD=0.7$), which means that, on the average, they flowered three times, because they also flowered in the year of germination (see the first experiment). Mediterranean plants were also rather short-lived: 3.4 ± 1.3 years. Among the longest-living plants were those from Northern Brittany (5.5 ± 0.6 years), and especially those from the Channel Islands of Jersey and Guernsey: 5.9 ± 0.5 years. As explained in **Materials and methods**, the latter two estimations will be somewhat underestimated. In the northern populations (between $50^\circ E$ and $52^\circ E$), lifespan was found to be somewhat shorter: 4.7 ± 1.2 years.

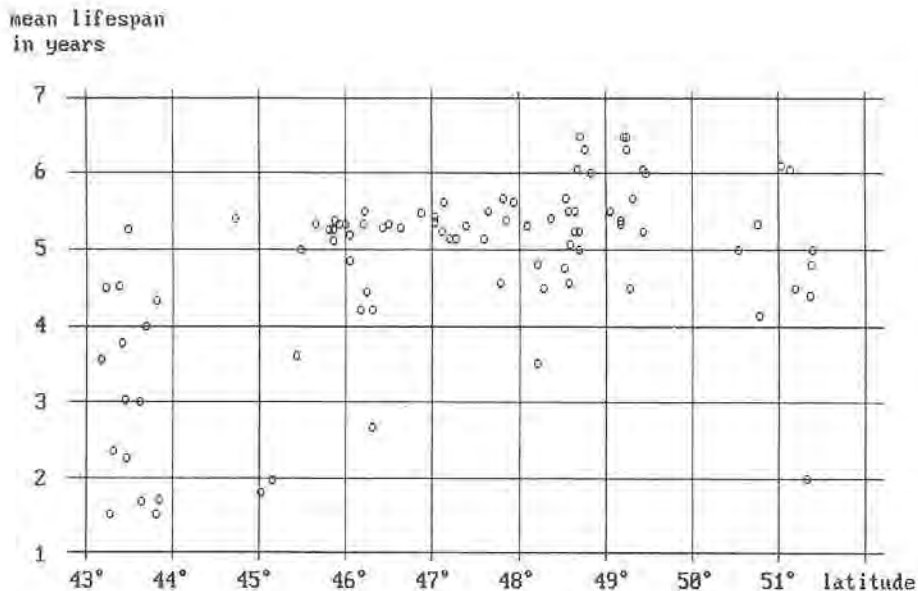


Fig. 3. The relationship between latitude and mean lifespan for each of the populations. Mean lifespan is estimated as the moment after germination at which 50% of the plants are still alive.

Discussion

In the study material, we observed considerable geographical contrasts together with gradual changes related to latitude. A strikingly different position is occupied by the inland populations. Plants from these populations flower immediately but are short-lived, phenomena that are typical for ruderal plants. Indeed, these plants are found in environments disturbed by humans, and can be called 'weedy'. Nevertheless, they are able to flower several times if disturbance is absent. Interestingly, the weedy traits of these inland plants are at the origin of the 'weed beet problem' (Boudry *et al.* 1993; Boudry 1994). They are situated in one of the largest sugar beet seed production areas, and through pollination of the male-sterile sugar beet plants they form hybrids which contain, apart from the *B*-allele, genes for 'weedy characters'. In the sugar-production areas, these hybrids lead to a

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genes for 'weedy characters'. In the sugar-production areas, these hybrids lead to a contamination of several bolting plants per hectare. If they are not (manually) removed, weed beet populations will rapidly develop.

The Mediterranean coastal plants occur in less disturbed environments but, also here, we see a relatively high frequency of the *B*-allele in combination with a relatively short lifespan, although considerably less extreme than found for inland material. Along the Atlantic coasts, from the Spanish border to the North Sea, we find a gradient. Towards the north, the possibility to flower in the year of germination disappears. Lifetime increases along this gradient but seems to have a maximum around the Channel; further to the north there was some decrease in lifespan.

For all plants in our study area, we can state that in general there exists a correlation between latitude and the relative investment in reproduction or survival. As one moves to the north, the emphasis lies on the survival of the individual, whereas in the south (and in particular in the inland populations but for other special reasons already discussed), investment in reproduction is more pronounced.

The reported variation in flowering date points to differences in sensitivity to flowering-inducing factors. Rathcke and Lacey (1985) have found that, in general, plants from northern origins flower earlier than plants from southern origins, when grown under identical conditions. Northern plants receive less strong environmental signals in the form of warmth and radiation, and therefore have to be more sensitive to those signals to flower at the most favourable moment in the year. Under identical circumstances, this is expressed by flowering earlier than less sensitive plants. Indeed, this pattern is found in our results, but only in our material from the Mediterranean to the southwest point of Brittany. Further north, flowering is gradually later again. The most plausible interpretation of the V-shape in Figure 2 is that plants in the southern part of our study area are not vernalization-limited in our cold greenhouse. Their flowering date is entirely determined by the combination of temperature and daylength, and we saw that sensitivity to those factors increases with latitude. However, it is known that the vernalization requirement is higher for plants with northern origins (Van Dijk and Boudry 1992). We state that, from the southwest of Brittany on, plants flower gradually later, because they are gradually more restricted by the degree of vernalization. It is known (Smit 1983; personal observations) that less cold than necessary for reaching the saturation level leads to later flowering.

The variation found in the frequency of the *B*-allele is by definition heritable. Also the variation in flowering date appears to have a considerable genetic component (Van Dijk *et al.* 1998). For lifespan, no genetic analysis has been carried out so far but the clear relationship with geographical origin also points to a substantial heritability. It is important to conserve all such types of genetic variation as genetic resources. The wild beet is not directly a threatened species in its present distribution area, but there is a clear and increasing human influence. Recreation activities along the European coasts lead to an increasing level of disturbance. Therefore, it is possible that, in the absence of protection measures, the species will gradually evolve to more weediness, and will lose characters that are interesting for the cultivation of the related crop species, which is exploited for its root biomass instead of weedy characters!

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Genetic diversity of red table beets

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Abstract

The diversity of morphological characteristics of the root and foliage of red table beets (*Beta vulgaris* subsp. *vulgaris*, Garden Beet) is discussed in this paper. Differences in chemical composition of roots of different red beet cultivars are presented.

Introduction

Although red table beet is not a very popular vegetable on Western markets, it is a crop of great importance in Central and East European countries and traditionally used as one of the main components for a range of dishes, including soups and salads. It shows excellent storability and is present on the fresh market throughout the year. Red table beet is also a valuable crop for the food industry, which uses it in canning and for juice production. It is a main source of natural, non-toxic, red pigments widely used for food colouration.

In Poland in 1994, red table beets were grown on 25 700 hectares (ranked sixth in area after cabbage, onion, carrots, cucumbers and tomatoes), and 517 000 tonnes of red table beets were harvested (ranked fourth after cabbage, onions and carrots) with the average yield over 200 dt/ha. Traditionally, open-pollinated red table beet cultivars have been bred by the pedigree method. Currently, the majority of new cultivars introduced on the market are F_1 hybrids, created with the use of cytoplasmic male sterility. Efficient breeding programmes require good knowledge of the available genetic resources.

Morphology

Cultivars of red table beet vary in their root shape and colour, as well as for leaf characteristics. The root of red table beet is developed mainly from the hypocotyl part of the seedling. Its shape depends on the cultivar. Holland (1956) divided red table beet varieties into four distinct groups, each including one or more classes: Flat (class A - 'Flat Egyptian' types), Globe (class B - 'Egyptian Globe' types, class C - 'Detroit Globe' types), Intermediate (class D - 'Intermediate' types) and Long (class E - 'Cheltenham Greentop' types, class F - 'Non-bleeding' types, class G - 'Dwarf Black' types, class H - 'Covent Garden' types, class I - 'Rouge Crapaudine' types). The groups were divided on the basis of the typical root shape. The subdivision was based on differences concerning other features, like root colour and foliage characteristics.

Root shape can be assessed from the longitudinal section of the root or be expressed by the shape index, i.e. length/diameter ratio. According to the UPOV (1976) guidelines for red table beets the root shape can be **transverse narrow elliptic** ($L/D = 1/3$) - 'Flat Egyptian', **transverse elliptic** ($L/D = 1/2$) - 'Egyptian Globe', **circular** ($L/D = 1/1$) - 'Detroit Globe', **broad elliptic** ($L/D = 1.5/1$) - 'Intermediate', **narrow oblong** ($L/D = 2/1$) or **narrow obtriangular** ($L/D = 3/1$) - classes E - I. The shape of transverse section can be **regular, intermediate or irregular**.

The root section preferably should show evenly coloured tissue and possess no differences in the pigmentation of rings. Flesh colour and presence of rings, as well as root tip and crown shape, are cultivar-dependent characteristics. The root surface can differ in roughness and be covered by the cork tissue to differing extent. Roots can vary in their skin colour (white, yellow or red) and root tissue colour (white, yellow, orange, red or purple). Most of the cultivars known on the European market are red. However, there are a few commercial yellow cultivars (like 'Burpee's Golden'), mainly in the USA, which are used for food processing as a component of vegetable salads. The lack of red pigments allows retention of the natural colours of other vegetables in the product.

Several characters of red table beet foliage vary among cultivars, including foliage height, and leaf size, thickness and shape. Banga (1962) grouped red table beets into three categories: forming short, medium and tall foliage. Leaves could be lobed and bullate. All these characteristics, though cultivar-dependent, can be strongly modified by environmental conditions. Foliage colour can vary from light green to dark green and deep purple. Green leaves can have purple midribs.

The length of the vegetative period for red table beet cultivars ranges from 80 to 120 days. Sowing date and the length of the vegetative period can strongly influence the morphology of cultivars, and change the shape and pigmentation of leaves and the shape of roots. They also have an effect on the chemical composition of the root tissue.

Nutritional quality

Dry matter content lower than 8% is considered to be very low. The average dry matter content ranges from 12 to 17%, but some accessions can reach over 22% dry matter in root tissue. Michalik *et al.* (1995) reported that dry matter content varied from 14.7% for breeding line 279 A to 16.9% for cv. Okragly Ciemnoczerwony. Sugar content in 11 genotypes examined in the same experiment ranged between 8.7% for lines 279 A and 391 A and 10.5% for cv. Okragly Ciemnoczerwony.

Genetic analysis of percent solids made by Watson and Gabelman (1984) showed that this trait was quantitatively inherited and general combining ability greatly affected percent solids in the roots of F_1 hybrids. The content of soluble solids measured by the refractometric index varied from 12.8 for breeding line 279 A to 14.2 for cv. Okragly Ciemnoczerwony. However, the examinations carried out on individual roots showed that soluble solids content can vary from around 5 to 20%, even within one cultivar.

Red table beets can produce red (betacyanin, BC) and yellow (betaxanthine, BX) pigments, mainly in the form of betanin and vulgaxanthin, respectively. Betanin content ranged between 48 and 97 mg/100 g fresh tissue, while vulgaxanthin content varied from 26 to 68.5 mg/100 g fresh tissue, for cv. Egyptian and Okragly Ciemnoczerwony, respectively (Michalik *et al.* 1995).

Wolyn and Gabelman (1989) showed that BC/BX ratio was governed by the three alleles at the R locus, *rr* plants being yellow, *R'r* had low BC/BX ratio, *R'r* - medium, *R'R'* - high, *R'R'* and *R'R'* - very high BC/BX ratio. The genetic analysis revealed that the amount of pigments in root tissue was quantitatively inherited, and the pigment contents in F_1 hybrids strongly depended on specific combining ability. There was no interaction between genes governing pigments content and those responsible for solids content. Wolyn and Gabelman (1990) also showed that the selection towards the increased pigment content combined

with selection towards increased or decreased solids content can be efficient,
leading to the

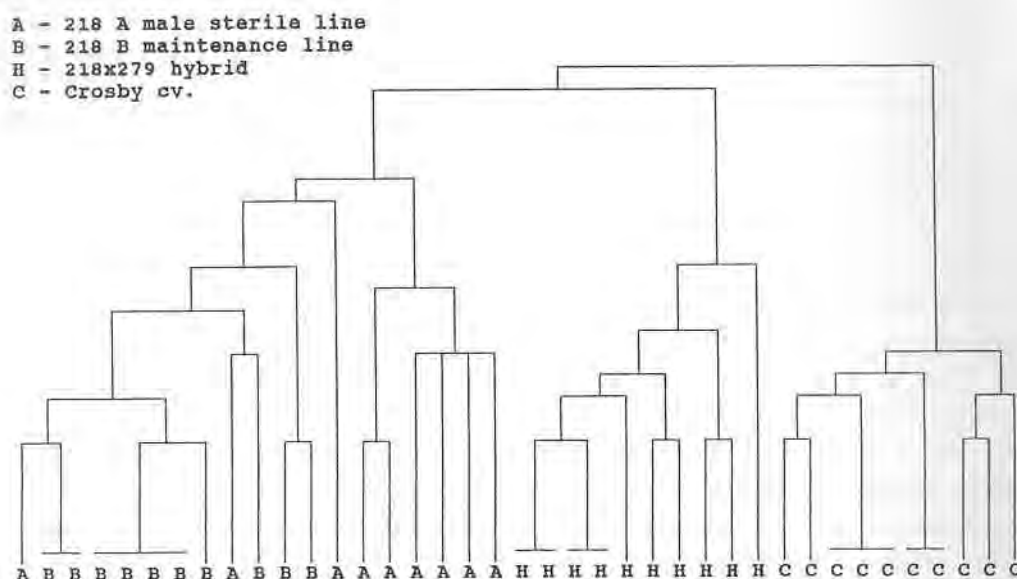


Fig. 1. Dendrogram generated by cluster analysis showing relationship between individual plants belonging to four beet root genotypes. A = 218 A male-sterile line; B = 218 B maintenance line; H = 218 × 279 hybrid; C = cultivar Crosby.

development of high pigment/high solids (HPHS) and high pigment/low solids (HPLS) half-sib families.

The content of nitrates, compounds dangerous for human health, can be very high in red beets. This trait is quantitatively inherited. The level of nitrates in the roots of F_1 hybrids of red table beet mainly is affected by specific combining ability. The maximum limit for nitrates in red beet roots, introduced in Poland in 1993, is 2000 mg NaNO_3/kg fresh tissue (equals 2930 mg KNO_3/kg fresh tissue). The average amount of nitrates of 11 accessions grown for 3 years ranged from 1276 mg KNO_3/kg fresh tissue for cv. Okragly Ciemnoczerwony to 2203 mg KNO_3/kg fresh tissue for line 391 A (Grzebelus 1995). Great differences were observed for individual roots belonging to the same genotype.

The chemical composition of red table beets depends upon genotype but can also be strongly modified by a range of environmental factors. Low light intensity can decrease sugar and pigment content and increase the level of nitrates in the roots. Drought and high temperatures cause increases of dry matter and pigment content but yield is significantly lower. The level of nitrates and betanin decreases with the age of plants, while vulgaxanthin content increases. Higher nitrogen fertilization limits dry matter, sugars and betanin content, has no effect on vulgaxanthin and causes a significant increase of nitrates in the roots.

The assessment of genetic diversity can be made by means of molecular biology. The use of RAPD markers for this purpose revealed great differences between the open-pollinated cultivar Egyptian Crosby and a pair of breeding lines (male sterile and maintenance) of American origin. The F_1 hybrid obtained on the basis of this line differed from cv. Crosby, but also was clearly different from the parental line (Fig. 1) (Baranski *et al.* 1995). These preliminary studies show that molecular markers can be a very efficient tool for the evaluation of genetic diversity of red table beet accessions.

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Genetic diversity for male sterility in wild and cultivated beets

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Abstract

Nuclear male sterility (NMS) has been found in both sugar beet and wild *Beta* beets. However, in spite of a likely high rate of mutations to *ms* genes, there are few reports of NMS in beets. This is probably due to (1) the mostly recessive nature of such genes in combination with predominantly outpollinated beet populations, and (2) the limited incentive to search for alternatives to the readily available *a₁* gene. Cytoplasmic male sterility (CMS), on the other hand, has been found to be a surprisingly common and widely distributed phenomenon in *Beta* beets. However, despite quite extensive surveys of natural populations and genebank accessions, only five main types of S cytoplasms, designated S₁ - S₅, have been found so far. Together with the finding that CMS is associated with drastic structural rearrangements of the mitochondrial genome, which manifest themselves in distinctly different mtDNA restriction profiles – both when different origins of S cytoplasm are compared with each other and when compared with fertile cytoplasm – this suggests that the mutational changes required to produce CMS occur rather seldom. As for the merits of the S₂ - S₅ cytoplasms as possible alternatives to the Owen (S₁) source of CMS in sugar beet hybrid breeding, not enough is known to express a view. The answer to this question can be obtained only through appropriate molecular and genetic studies, preferably in a sugar beet breeding context. Until then such work should take priority over a search for additional sources of CMS in *Beta*.

Introduction

Male sterility in plants implies an inability to produce or release functional pollen, and is the result of failure of formation or development of functional stamens, microspores or gametes. However, only "pollen sterility", in which male-sterile plants differ from normal only in the absence of functional pollen grains, plays a major role in plant breeding and is thus the only kind of male sterility dealt with here.

On the basis of its inheritance and origin, male sterility is divided into: (1) Nuclear male sterility (NMS), also called "genic", "genetic" or "Mendelian", in which the male sterility is governed solely by one or more nuclear genes; (2) Cytoplasmic male sterility (CMS) in which male sterility comes about as a result of the combined action of nuclear genes and changes in the cytoplasmic organellar genomes resulting in what is often referred to as "sterile cytoplasm" (S) as opposed to normal "fertile cytoplasm" (F).

Nuclear male sterility

Owen (1952) reported on two cases of male sterility caused by single non-allelic recessive nuclear genes designated *a₁* and *a₂*. The *a₁* gene was originally discovered in Munerati's annual beet but the same gene was subsequently found in a wide variety of populations and inbreds and Owen (1952) even suggested that "by a consistent search in populations of a few hundred plants" it might be found in most inbred lines.

The a_2 gene was obtained from the open-pollinated progeny of a male-sterile beet found in England. It was not as extensively studied as the a_1 gene and appears never to have been used in sugar beet breeding. A few other cases of nuclear male sterility in section *Beta* have been reported (see Kaul 1988), but only one of these appears to have been studied in more detail. This type, which was found in the progeny of an annual *B. vulgaris* subsp. *maritima*, was due to a single recessive gene designated *ms* and shown to be different from the a_1 gene of Owen (Kinoshita and Takahashi 1972).

Similarly, in 1965, I found nuclear male sterility in a seed sample of a primitive leaf beet population collected in Yugoslavia in the 1950s. The male sterility was due to a single recessive gene and shown by the appropriate test crosses to be non-allelic to the a_1 gene (Bosemark, unpublished). Because the new gene source did not appear to have any advantages over the a_1 gene, which was already in use at Hilleshög, no further studies of this male sterility were conducted.

Cytoplasmic male sterility

The Owen source of CMS

Cytoplasmic male sterility was first discovered and studied in sugar beet by Owen (1945) who found a low frequency of male-sterile plants in the curly-top resistant sugar beet variety US-1. Owen proposed that completely male-sterile plants have the genotype (S)xxzz, with the remaining genotypes [(S)XXZZ through (S)Xxzz] usually showing a varying degree of pollen fertility. However, Owen (1945) emphasized that "occasional results were not fully explained by the two-factor hypothesis, indicating that more than two Mendelian factors may influence the degree of semi-male-sterility".

Several researchers have since tried to interpret their own testcross results according to the Owen hypothesis or proposed modified or alternative models of inheritance. However, as emphasized by Knapp (1969), with a more or less continuous variation in pollen fertility, a pronounced influence of environmental factors on the expression of sterility in some genotypes, and a system of phenotypic classification, which frequently presents difficult decisions; a more detailed traditional genetic analysis is not possible. However, strict selection for maintainer genotype permits the production of stable CMS lines (Nielson and Nemazi 1967; Bosemark 1972).

Bliss and Gabelman (1965) and Theurer (1970) found monogenic restorers in table beets when they were crossed with CMS sugar beets. Similarly, Theurer and Ryser (1969) and Bosemark (unpublished) found a strong, dominant restorer gene, not allelic to the X and Z genes, in different sugar beet materials.

New sources of CMS found in wild *Beta* beets

The first to report on new sources of CMS in *Beta* was Dr R.K. Oldemeyer of the Great Western Sugar Company, who had searched the USDA wild beet collection and found two new sources of male-sterile cytoplasm, one in *B. macrocarpa* and one in a wild leaf beet from Turkey (Oldemeyer 1957). Preliminary studies indicated little difference in action between these sources and the Owen CMS. However, the search continued in the mid-1960s, which resulted in several new sources of CMS in populations from Turkey, India, Pakistan and Manchuria. To facilitate the assessment of these new sources of sterile cytoplasm they were

subsequently backcrossed to a highly inbred sugar beet maintainer for more than six generations (Oldemeyer, pers. comm.).

Similarly to Oldemeyer, I discovered five new sources of male sterility when, in 1965, I increased a number of wild beet seed samples which had been collected in Asia Minor and some Mediterranean countries in the 1950s. Of these male steriles, one turned out to be strictly nuclear and has been referred to earlier in this paper, one was lost before having been properly characterized, while the remaining three, originating from Morocco, Yugoslavia and Turkey, were found to be cytoplasmic in nature. Subsequent test-crossing indicated that the cytoplasms from Morocco and Yugoslavia require quite different maintainer genotypes from the Owen CMS, while in this respect the type from Turkey appeared to be very similar to the latter (Bosemark 1979).

Also Coe and Stewart (1977) reported on a new source of CMS found in an accession of *B. vulgaris* subsp. *maritima* collected near Plymouth, England in 1955. They suggested that the components of CMS, as well as other desirable characters, are available in *B. vulgaris* subsp. *maritima* and can be used for enrichment of the sugar beet germplasm should the need arise.

Molecular characterization and classification of different sources of CMS

Although the requirement of different maintainer genotypes strongly suggests also differences in the cytoplasmic genotypes, it was only with the advent of the restriction endonuclease analysis that it became possible to characterize these new CMS sources based on differences in their mitochondrial mtDNA.

The first to apply this technique to sugar beet was Powling (Powling 1981, 1982; Powling and Ellis 1983) who analyzed mtDNA of normal fertile (F) cytoplasm and the Owen sterile (S) cytoplasm and found that the two cytoplasms differed both with respect to number and type of small, circular DNA molecules and mtDNA restriction patterns. The original suggestion that the presence or absence of specific minicircles may be associated with the expression of male sterility (Powling 1981) has since been proven wrong (Duchenne *et al.* 1989; Halldén *et al.* 1989). However, the finding that the F and S type mtDNAs show widely different restriction profiles (Powling 1982), while the chloroplast ctDNAs show only little variation (Powling and Ellis 1983), was soon confirmed (Mikami *et al.* 1984a, 1984b) and provided strong evidence that CMS in sugar beet, similarly to the situation in some other crop species, has a mitochondrial origin.

These reports on CMS in sugar beet were soon followed by studies of new sources of male-sterile cytoplasms extracted from various wild beets. Thus, Mikami *et al.* (1985, 1986) compared the restriction profiles of mt and ctDNAs from sugar beet breeding lines with the F and the Owen S type cytoplasms with those from six S cytoplasms extracted from different wild beet sources, supplied by Dr R.K. Oldemeyer. Of these new S cytoplasms, three had mt as well as ctDNA restriction profiles indistinguishable from those of the Owen S cytoplasm, while for the remaining three the mtDNA restriction profiles were clearly different. When compared with each other these latter S types showed somewhat different mtDNA restriction profiles with one of the three restriction enzymes used, while the two others did not reveal any heterogeneity. Also with respect to the ctDNA the three deviant S cytoplasms differed from the others and the Owen cytoplasm. However, the difference was limited to one of the four restriction enzymes used and appeared to be due to a difference in a single restriction site only.

Similarly, restriction analysis of mt and ctDNAs from the three sources of CMS found by myself in wild *Beta* beets from the Mediterranean area, and referred to earlier, showed that two of the CMS types, C7051 from Morocco and C8640 from Yugoslavia, differed greatly from both the Owen and the fertile cytoplasm in mtDNA fragment patterns, while the third source, C8640 from Turkey, showed the same restriction pattern as the Owen CMS (Halldén *et al.* 1988). In contrast to the large differences between cytoplasm in mtDNA restriction profiles, the analysis of ctDNA detected only little variation between cytoplasm. The result of the molecular characterization thus supported earlier test cross results (Bosemark 1979). Finally, Mann *et al.* (1989) analyzed the sterile cytoplasm derived from male-sterile plants found in a population of *B. vulgaris* subsp. *maritima* collected in 1955 near Plymouth, England (Coe and Stewart 1977) and found that its mtDNA restriction profile clearly differed from that of the Owen cytoplasm.

Another series of studies of particular interest concerns the origin and nature of male sterility in gynodioecious populations of *B. vulgaris* subsp. *maritima* along the French Atlantic Coast (Boutin *et al.* 1987; Boutin-Stadler *et al.* 1989; Saumitou-Laprade *et al.* 1991, 1993). In these populations, which have been shown to consist of a mixture of non-segregating hermaphrodites with F cytoplasm, and male-sterile females and segregating hermaphrodites and intermediates with S cytoplasm, the percentage of male steriles may vary, depending on either the frequency of plants with S cytoplasm or the frequency of nuclear restorer genes or both (Boutin-Stadler *et al.* 1989). Besides an S cytoplasm with an mtDNA profile distinctly different from that of the F cytoplasm in the non-segregating hermaphrodites, found in the first population examined (Boutin *et al.* 1987), later studies have revealed additional variant mt as well as ctDNAs. As a consequence, Saumitou-Laprade *et al.* (1993) subdivided the F mitochondrial type into three subtypes and distinguished an additional S cytoplasm which they called R. In a study of ctDNA variation with *Hind* III restriction analysis, also three somewhat different ctDNA types were found (Saumitou-Laprade *et al.* 1991). However, because one type was associated with N type mitochondria, one with S type mitochondria and the third was found in both fertile N and sterile S plants, it was concluded that this ctDNA polymorphism is not involved in the expression of male sterility.

From extensive comparisons of the mtDNA restriction profiles of a wide range of cultivated and wild *Beta* beets Halldén *et al.* (1988, 1989, 1990) concluded that the S cytoplasm discovered so far in *Beta* belong to one or another of four major types designated S₁, S₂, S₃ and S₄. The S₁ type includes the Owen cytoplasm, the S₂ and S₃ types are the S cytoplasm originating from Morocco and Yugoslavia, respectively, and the S₄ includes the new S cytoplasm described by Mikami *et al.* (1985) and the S cytoplasm from *B. vulgaris* subsp. *maritima* populations along the French Atlantic coast (Boutin *et al.* 1987). While the S₂ and S₃ cytoplasm have as yet been found only in two and one population, respectively, the S₁ and S₄ cytoplasm have been found in widely different populations and are represented by more or less deviating subtypes (Mikami *et al.* 1985; Weihe *et al.* 1991; Saumitou-Laprade *et al.* 1993).

That gynodioecy and variability in mtDNA type both between and within populations may be a rule rather than an exception was demonstrated in an extensive study of natural populations of *B. vulgaris* subsp. *maritima* along the entire French coast (Cuguen *et al.* 1994). Of the 93 populations sampled, 42% were gynodioecious, ranging from 23% along the coast of the Channel to 71%

along the Mediterranean coast. Studies of the polymorphism of the mtDNA revealed a total of eleven restriction patterns, including the normal F pattern. Five of the variant DNA types correspond to the three variant F types and the two S types described by Saumitou-Laprade *et al.* (1993). Although male-sterile individuals were found among all but three of the eleven mtDNA types, the normal F and the deviant F types mentioned were rarely associated with male sterility while male-sterile plants were often found among the plants with the two S type mtDNAs. There were also differences between regions. Thus the F mitotypes associated with male steriles were almost entirely confined to the Mediterranean area. The small sample size did not permit analysis of the relationship between mtDNA type and male sterility in the six remaining rare mitotypes, three of which were not represented by male-sterile plants. We may thus conclude that with the possible exception of the Mediterranean area, cytoplasmic male sterility in populations of *B. vulgaris* subsp. *maritima* along the French coast is associated with either the S₄ type of cytoplasm or, more rarely, the R type.

Additional information on the occurrence of gynodioecy and the association of mtDNA types with male sterility was obtained in a study of mtDNA restriction pattern variation among fertile and male-sterile plants from 17 wild populations of different *Beta* beets known to contain male-sterile plant and provided by Drs Frese and Ford-Lloyd (Lind-Halldén and Halldén 1995). Of these 17 populations six originated from Spain, three from Italy, four from Greece, two from Turkey and one each from Tunisia and India. With the exception of the population from India, where both male-fertile and male-sterile plants had the S₂ cytoplasm, all other populations had either the F or the S₄ type cytoplasm or contained a mixture of plants with these two cytoplasm. Thus, in two populations from Italy and one from Greece both fertile and male-sterile plants had the S₄ cytoplasm, in two populations, one from each of Greece and Tunisia, the male steriles had the S₄ cytoplasm and the male fertiles the F cytoplasm, while in one population from Turkey with an S₄ male-sterile plant the male-fertile plants were not characterized. In the remaining 10 populations, which included all six Spanish populations, two populations from Greece and one each from Italy and Turkey, both male-fertile and male-sterile plants had the F type cytoplasm. Besides demonstrating that the apparent absence of S₄ cytoplasm along the French Mediterranean coast is not a characteristic of other Mediterranean areas, this study suggests a frequent association of F cytoplasm and presence of male-sterile plants in populations of wild beets from these areas. However, the nature of this sterility is unclear. Saumitou-Laprade *et al.* (1993), Cuguen *et al.* (1994) and Lind-Halldén and Halldén (1995) discuss the possibility that the concept of a "completely fertile" type of mtDNA in *Beta* is an oversimplification, and also that F cytoplasm may be "old" sterile types having almost completely fixed their restorer genotypes. Although all authors emphasize that this question needs further investigation, only Saumitou-Laprade *et al.* (1993) and Lind-Halldén and Halldén (1995) mention the possibility that the male steriles associated with the different F type cytoplasm may be strictly nuclear. However, the importance of this question for the understanding of CMS in *Beta* – as indeed the whole concept of nuclear versus cytoplasmic male sterility – merits a few comments.

The nature of male steriles associated with F cytoplasm

If male fertility is achieved only through a well-functioning cooperation between nuclear and organellar genomes, the fertility determining cytoplasms and nuclear genes which occur most frequently in nature, should be considered normal (Horner and Palmer 1995). Nuclear male sterility, due to a recessive mutation which disturbs the normal function of a dominant gene involved in fertility, or a dominant mutation that interferes with fertility, is primarily a nuclear genetic defect. Similarly, structural rearrangement of the mitochondrial genome resulting in male sterility is primarily a cytoplasmic defect. That this is a valid distinction, and that there are indeed nuclear *ms* genes which are not dependent on a specific cytoplasmic genotype for their manifestation, is demonstrated by the fact that several non-allelic *ms* mutants are known to have arisen in one and the same variety in self-pollinated crops like barley (Hockett and Eslick 1968), and that these genes function equally well in the cytoplasms of other varieties. Thus, nuclear male sterility is not likely to be the result of an *ms* mutation in an S cytoplasm as was once hypothesized by Hermesen (1965, 1968), nor is it likely that the "normal" F cytoplasm is an S cytoplasm predominantly restored and the male-sterile plants, occasionally arising, are thus rare non-restored segregants. Also providing a strong argument against the latter interpretation are the rather dramatic differences in mtDNA restriction patterns among the five undisputed S type cytoplasms and all F types studied.

The alternative explanation to the male-sterile plants found to be associated with F type cytoplasms – that this male sterility is not cytoplasmic but strictly nuclear in nature – is supported by the very common occurrence of nuclear male sterility in both self-pollinated and cross-pollinated plants, monocots as well as dicots. Thus Kaul (1988) lists 60 known non-allelic *ms* genes in maize, 55 in tomato, 48 in barley and 24 in garden pea, to mention only a few examples. The occurrence of a large number of non-allelic *ms* genes in these species indicates a high frequency of *ms* mutations. Since these genes are almost always recessive, the elimination of the *ms* genes from normally outpollinated populations is slow, while at the same time the male sterility is rarely manifested. However, with increasing degree of inbreeding, due to restriction of population size or self-compatibility, homozygous recessive male steriles will appear in varying frequencies.

Providing an argument in favour of nuclear male sterility, in at least some of the populations where male sterility was found to be associated with F cytoplasm, is the fact that, of the ten such populations encountered by Lind-Halldén and Halldén (1995), two belonged to *B. macrocarpa* and one to *B. vulgaris* subsp. *adanensis*, both species that are known to be predominantly selfing also under natural conditions (Letschert 1993). Because, in my experience, Mediterranean *B. vulgaris* subsp. *maritima* populations often show a varying degree of self-fertility and frequently produce plenty of seed under isolation (Bosemark, unpubl.), I believe that many, if not all, of the cases of male sterility associated with F cytoplasm may, in fact, be strictly nuclear in nature. To settle this important question, a representative sample of the populations in question should be further investigated by appropriate genetic and molecular methods.

The origin of the different CMS types

CMS may arise either within a species as a result of a spontaneous mutational change in the cytoplasm or in intra- or interspecific crosses that result in poor

cooperation between nuclear genes and organellar genes that are foreign to each other.

Although restriction mapping of the mitochondrial genome of the Owen male-sterile cytoplasm (type S_1) has revealed a complex multicircular organization, generated by intraspecific homologous recombination over repeated DNA sequences (Brears and Lonsdale 1988), this does not preclude some of the other spontaneous sterile cytoplasms in *Beta* being the result of species crosses introducing a foreign fertile cytoplasm into a new nuclear genetic background. To resolve this question, Halldén *et al.* (1990) compared ct and mtDNAs from the four sterile cytoplasms S_1 - S_4 and 22 normal fertile sugar beet lines and accessions covering all the main species of the genus *Beta*. Restriction analyses of the ctDNAs showed only little variation in fragment profiles between species within sections, whereas fragment profiles for species from different sections were clearly different, suggesting that all four S cytoplasms belong to the section *Beta*.

Comparison of mtDNAs of the fertile accessions showed the same pattern as for the ctDNAs, i.e. the variation between species belonging to different sections was much greater than that within sections. However, the representatives of the four S cytoplasms showed very different restriction profiles when compared with each other as well as when compared with all fertile accessions within the genus *Beta*. Thus, there were no indications of cytoplasmic introgression in any of the four S cytoplasms investigated.

To study the organizational changes in the mitochondrial genomes of the F and S_1 - S_4 cytoplasms, Southern hybridizations of nine clones containing mitochondrial genes were used. This study confirmed that the F and the CMS cytoplasms are highly rearranged relative to each other and indicated that rearrangement of the mitochondrial genome is a common property of spontaneously occurring CMS systems in *Beta*.

As emphasized by Halldén *et al.* (1990) the results of these studies indicate that none of the four CMS types studied are the direct result of alloplasmic introgression and thus a reflection of an evolutionary divergence. Instead they strengthen the suggestion that there is a direct association between the mtDNA rearrangements and the occurrence of CMS, and that the rearrangements in the four S types are the outcome of rare but drastic mutational events rather than an accumulation of minor events over time. This conclusion is supported both by the pattern of variability in mtDNA restriction profiles found within the S_1 and S_4 types (Mikami *et al.* 1985; Weihe *et al.* 1991; Saumitou-Laprade *et al.* 1993), which is very similar to that found between similar accessions with fertile cytoplasm, and the absence of a sterile cytoplasm with an intermediary degree of reorganization (Halldén *et al.* 1990; Lind-Halldén and Halldén 1995).

Recently Michaelis *et al.* (1995) described two regions in sugar beet mtDNA, which are specific to the Owen (S_1) sterile cytoplasm, and which hybridize to different regions of a linear 10.4 kb mitochondrial plasmid commonly occurring in French *B. vulgaris* subsp. *maritima* populations (Saumitou-Laprade *et al.* 1991; Michaelis *et al.* 1995). Although the presence of the plasmid in plants with S as well as such with F cytoplasm rules out a direct influence of this element on CMS in sugar beet (Saumitou-Laprade *et al.* 1991), Michaelis *et al.* (1995) suggest that the 10.4 kb mitochondrial plasmid may cause insertions and rearrangements in the mtDNA genome and, thereby, might be responsible for the CMS. However, it remains to be shown that the two S_1 -specific regions are present also in the

mtDNA from other S cytoplasms identified in *Beta* and to demonstrate a functional role in the development of the CMS phenotype.

Mitochondrial protein synthesis

Since it has been shown that the extreme sensitivity of maize lines with the Texas (T) CMS cytoplasm to a specific race of *Helminthosporium maydis* is strongly associated with the expression of a 13 kDa polypeptide synthesized by the T type mitochondria (Newton and Walbot 1985), it is of great interest to know to which extent CMS in sugar beet may be associated with expression of novel polypeptides.

Lind *et al.* (1991) conducted a study of protein synthesis in purified mitochondria from roots, leaves and flowers of fertile and male-sterile sugar beet lines. Although they found a number of organ-specific differences in polypeptide patterns and in organello protein synthesis patterns, in contrast to Boutry *et al.* (1984), no unique polypeptides were found to be synthesized by male-sterile plants with the S₁ cytoplasm as compared with their male-fertile counterparts.

In a later study, Halldén *et al.* (1992) compared mitochondrial protein synthesis from roots and leaves within and between three fertile and ten male-sterile lines representing the S₁ - S₄ cytoplasms. The patterns of polypeptides synthesized were very similar for the F and the S₁ and S₂ cytoplasms, whereas leaf mitochondria from plants containing the S₃ cytoplasm were found to synthesize a unique 6 kDa polypeptide and those from S₄ cytoplasm a 10 kDa polypeptide. Although the S₄ cytoplasm was represented by three variants, known to show small differences in their mtDNA restriction profiles (Mikami *et al.* 1985), they all showed identical polypeptide patterns, including the presence of the 10 kDa polypeptide. Even if it is not known to which extent these variant polypeptides are synthesized in the flowers of male-sterile plants, the authors conclude that it cannot be excluded that they are associated with the expression of CMS.

Conclusions

If we leave out the as yet unidentified male steriles found to be associated with F cytoplasms, our present knowledge of the genetic diversity of male sterility in wild and cultivated beets may be summarized as follows.

Nuclear male sterility has been found in both sugar beet and wild *Beta* beets. However, in spite of a likely high rate of mutations to *ms* genes, there are few reports of NMS in beets. This is probably due to (1) the mostly recessive nature of such genes in combination with predominantly outpollinated beet populations and (2) the limited incentive to search for alternatives to the readily available *a₁* gene.

Cytoplasmic male sterility, on the other hand, has been found to be a surprisingly common and widely distributed phenomenon in *Beta* beets. However, in spite of quite extensive surveys of natural populations and genebank accessions, only five main types of S cytoplasms have been found so far. Together with the finding that CMS is associated with drastic structural rearrangements of the mitochondrial genome, which manifest themselves in distinctly different mtDNA restriction profiles – both when different origins of S cytoplasm are compared with each other and when compared with fertile cytoplasms – this suggests that the mutational changes required to produce CMS occur rather seldom. This conclusion is likely to hold true even if the structural rearrangements are caused by the insertion of specific DNA sequences as recently suggested.

As may be seen in Table 1, which summarizes our present knowledge of the origin and geographical distribution of the five main types of CMS in *Beta*, the S_2 and S_3 types so far have been found only in two and one populations, respectively. Both S_1 and S_4 types, on the other hand, have been found in a wide range of populations and are represented by more or less deviating subtypes. The S_5 type, finally, has been found in a restricted number of populations, mainly in Brittany.

The classification of variants of the S_1 and S_4 types as subtypes of these major mitochondrial types is supported by the pattern of variability in mtDNA restriction profiles found within the S_1 and S_4 types, which is very similar to that found between similar accessions with F cytoplasm. Also the identical pattern of polypeptide synthesis shown by mitochondria from different variants of the S_4 cytoplasm provides strong evidence of the above classification.

Table 1. Origin and geographic distribution of the five main types of CMS in *Beta*.

Main type of S cytoplasm	Origin of cytoplasm	Designation	References	Designation	Molecular characterization references
S ₁	Sugar beet (US-1)	Owen (S)	Owen 1945	Owen (S)	Powling 1982; Powling and Ellis 1983; Mikami <i>et al.</i> 1984a, 1984b
	<i>B. vulgaris</i> sp., Turkey; PI 120704	I-12 CMS(4)	Oldemeyer, pers. comm.	S ₁	Halldén <i>et al.</i> 1988, 1989; Weihe <i>et al.</i> 1991
	<i>B. vulgaris</i> sp., Turkey; PI 120705	I-12 CMS(5)	Oldemeyer, pers. comm.		Mikami <i>et al.</i> 1985, 1986
	<i>B. vulgaris</i> sp., Turkey; PI 169027	I-12 CMS(8)	Oldemeyer, pers. comm.		Mikami <i>et al.</i> 1985, 1986
	<i>B. vulgaris</i> sp., Turkey; PI 164747	I-12 CMS(1)		S ₁	Mikami <i>et al.</i> 1985, 1986
S ₂	<i>B. vulgaris</i> subsp. <i>maritima</i> , Turkey	C8684	Bosemark 1979	S ₁	Halldén <i>et al.</i> 1992
	<i>B. vulgaris</i> subsp. <i>maritima</i> , Morocco	C7051	Bosemark 1979	S ₁	Halldén <i>et al.</i> 1989
	<i>B. vulgaris</i> subsp., India	IDBB6960	Dutch-German Coop. Programme	S ₂	Halldén <i>et al.</i> 1992
	<i>B. vulgaris</i> subsp. <i>vulgaris</i> Leaf Beet, Yugoslavia	C8640	Bosemark 1979	S ₂	Lind-Halldén and Halldén 1995; Beta Genetic Resources
S ₃	<i>B. vulgaris</i> subsp. <i>vulgaris</i> Leaf Beet, Yugoslavia	C8640	Bosemark 1979	S ₃	Halldén <i>et al.</i> 1988, 1989
S ₄	<i>B. vulgaris</i> sp., Turkey; PI 177272	I-12 CMS(2)	Oldemeyer, pers. comm.		Mikami <i>et al.</i> 1985, 1986
	<i>B. vulgaris</i> sp., Pakistan; PI 218063	I-12 CMS(3)	Oldemeyer, pers. comm.		Mikami <i>et al.</i> 1985, 1986
	<i>B. vulgaris</i> sp., Manchuria; PI 141919	I-12 CMS(7)	Oldemeyer, pers. comm.	S ₄	Mikami <i>et al.</i> 1985, 1986; Halldén <i>et al.</i> 1989
	<i>B. vulgaris</i> subsp. <i>maritima</i> , England		Coe and Stewart 1977		Mann <i>et al.</i> 1989
	<i>B. vulgaris</i> subsp. <i>maritima</i> , France		Boutin <i>et al.</i> 1987; Saumitou-Laprade <i>et al.</i> 1993; Cuguen <i>et al.</i> 1994	S	Halldén <i>et al.</i> 1988, 1989; Weihe <i>et al.</i> 1991
S ₅	<i>B. vulgaris</i> sp., Italy	IDBB 2254	Dutch-German Coop. Programme	S ₄	Lind-Halldén and Halldén 1995
	<i>B. vulgaris</i> sp., Italy	IDBB 2231	Beta Genetic Resources	S ₄	Lind-Halldén and Halldén 1995
	<i>B. vulgaris</i> subsp. <i>maritima</i> , Greece	IDBB 3356	Beta Genetic Resources	S ₄	Lind-Halldén and Halldén 1995
	<i>B. vulgaris</i> subsp. <i>maritima</i> , Greece	IDBB 3117	Beta Genetic Resources	S ₄	Lind-Halldén and Halldén 1995
	<i>B. vulgaris</i> subsp. <i>maritima</i> , Turkey	IDBB 3546	Beta Genetic Resources	S ₄	Lind-Halldén and Halldén 1995
	<i>B. vulgaris</i> subsp. <i>maritima</i> , Tunisia	IDBB 3196	Beta Genetic Resources	S ₄	Lind-Halldén and Halldén 1995
	<i>B. vulgaris</i> subsp. <i>maritima</i> , France		Saumitou-Laprade <i>et al.</i> 1993; Cuguen <i>et al.</i> 1994	R	Saumitou-Laprade <i>et al.</i> 1993; Cuguen <i>et al.</i> 1994
	<i>B. vulgaris</i> subsp. <i>maritima</i> , France		1993; Cuguen <i>et al.</i> 1994		

As for the merits of the S_2 - S_5 cytoplasm as possible alternatives to the Owen (S_1) source of CMS in sugar beet hybrid breeding, not enough is known to express an informed view. Nor is it known if the differences in mtDNA restriction pattern between variants of the S_1 and S_4 cytoplasm are manifested in differences in maintainer genotype requirement or in some other critical character. The answer to these questions can be obtained only through appropriate molecular and genetic studies, preferably in a sugar beet breeding context. Until these questions have been answered such work should have priority over a search for additional sources of CMS in *Beta*.

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Creation and study of S-cytoplasm collection from varied sources of sugar beet

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Abstract

The purpose of the present investigation was to create and study cytoplasmic male sterility (CMS) germplasm from different origins. The following sources of S-cytoplasm were used: (1) hybrids and varieties created with the use of CMS from the former Deutsche Demokratische Republik (FDDR), (2) CMS germplasm from Uladovskaja, L'govskaja, Ivanovskaja, Umanskaja, Ramonskaja - selection centres of the former Soviet Union (FSU), (3) CMS germplasm that appeared within fertile germplasm during the inbreeding process. Depending on the expression of the male-sterile phenotype in the F_1 generation, all hybrids can be divided into two groups: (1) progenies of CMS forms that have no fertile plants, and (2) CMS forms that have complete male sterile, semi-male sterile and normal plants. It should be noted that CMS forms of one origin may fall in different groups. A different classification was obtained when the F_1B_1 generation was examined. Progenies of CMS forms from the first and second groups above had no normal fertile plants. On the basis of these results we conclude that the differences between CMS forms in the F_1 generation are conditioned by various nuclear genes.

Introduction

Pollen sterility (or pollen lethality) of plants can be determined by nuclear or cytoplasmic genes, or can be the result of nucleus-cytoplasm interaction (Frankel and Galun 1977). If the factor of lethality is located in the nucleus, the male sterility is called nuclear, genetic or Mendelian male sterility. If the factor of sterility is located in the cytoplasmic genome, male sterility is called cytoplasmic male sterility. If expression of character is the result of an interaction of nuclear and cytoplasmic genes, the male sterility is called genetic-cytoplasmic male sterility. The cytoplasm containing the pollen male-sterility factor usually is designated as sterile and represented by the letter S. There is a sexual specificity of described factors – the abnormal pollen development does not influence the development of female parts of the flower – which demonstrates that the male sterility character is tissue specific. Only genetic and genetic-cytoplasmic (frequently called cytoplasmic male sterility, CMS) male sterility has been found in sugar beet. Genetic-cytoplasmic male sterility (g-CMS) occurred spontaneously in sugar beet. In 1942, Owen published the first information about g-CMS in sugar beet (Owen 1942). According to Owen, expression of this character is caused by interaction of sterile (S) cytoplasm and two genes in homozygous recessive condition (Owen 1945). The genotype of completely male-sterile forms may be recorded as *S fr1fr1 fr2fr2* (the designations *Fr1* and *Fr2* correspond to Owen's *X* and *Z* genes). Plants having normal cytoplasm and recessive nuclear restorer genes (genotype - *N fr1fr1 fr2fr2*) are used to reproduce male-sterile forms and are named as male sterility maintainers or O-types. Such a scheme of g-CMS inheritance now is generally accepted.

Progenies of g-CMS forms found in the cultivar US-1 have become the basis for hybrid cultivars in the USA, Western Europe and Japan. Today about 90% of hybrids are developed with the use of Owen's S-cytoplasm (Kaul 1988). The first publications about the inheritance of genetic-cytoplasmic male sterility stimulated the search for new types of S-cytoplasm. On the basis of the published literature, it is possible to offer some potential search vectors for new types of S-cytoplasm: (1) through mutagenesis, (2) among wild *Beta* species, and (3) among fertile sugar beet material from various sources.

New CMS forms were created from plants with N-cytoplasm by means of ionizing radiation or chemical mutagenesis. The γ -radiation treatment was used on varieties H-19 and H2002. The resulting g-CMS forms were divided into four groups – Si-1, Si-2, Si-3 and Si-4 (Kinoshita 1976) – a division made on the basis of deviations from Owen's scheme of inheritance. The application of chemical mutagenesis also has been used to create g-CMS forms (Balkov 1976; Kinoshita 1980).

New sources of S-cytoplasm have been found among wild *Beta* species (Oldemeyer 1957; Coe and Stewart 1977; Halldén *et al.* 1988; Saumitou-Laprade *et al.* 1993). However, only for a few S-cytoplasm donors have the data about inheritance of cytoplasmic male sterility been presented. The main criterion in allocation of new types of sterile cytoplasms has been the deviations from the generally accepted inheritance for S-cytoplasm described in this body of research.

The largest programme to search for new sources of S-cytoplasm among normal fertile materials was conducted in the 1960s in the FSU, where work with CMS began after Owen's original publication. In each breeding station, Owen's work was repeated with various fertile germplasms. Male-sterile plants were found in cultivars and populations of varied origin, including: Ramonskaja 06, Ramonskaja 032, Ramonskaja 036, Ramonskaja 991 (all obtained at VNISS), L'govskaja 925 (obtained at L'govskaja breeding station), Verhniachskaja 121, Verhniachskaja 511 (both obtained at Verhniachskaja breeding station), Uladovskaja 752 (obtained at Uladovskaja breeding station), Mezotnenskaja 080, Mezotnenskaja 085 (both obtained at the Mezotnenskaja breeding station), and Pervomaiskaja 028 (obtained at Pervomaiskaja breeding station) (Balkov 1978). Cytoplasmic male-sterile forms also were revealed in material of East-European selection, including: Janash 1, Janash 3, Buszczynski (Balkov 1978), and Bijskaja 032 (Iordanski and Lutkov 1966). Sources of male-sterile cytoplasm were isolated in practically each large breeding station, and the search for sterility maintainers began. Therefore, we undertook to create and study a collection of S-cytoplasm of various origins by using the breeding process. The g-CMS forms from the FDDR and FSU have been used, as have other sources of CMS germplasm, which have appeared within normal fertile germplasm in the course of the inbreeding process.

Material and methods

The sources of S-cytoplasm

Only one g-CMS line received from FDDR was used as a source of S-cytoplasm. It was the male-sterile component of 'Denok Mono' hybrid. Hybrids created using male-sterile lines of varied origin were used as other sources of S-cytoplasm (Table 1), including g-CMS material from Russia and the Ukraine. From the Ukraine g-CMS forms from such breeding stations as Ivanovskaja, Uladovskaja and

Umanskaja were used as was the material from the L'govskaja and VNIISS breeding stations in Russia.

Table 1. The geographical origin of CMS forms

Origin of CMS forms		
Country	Breeding station	CMS forms
FDDR	Kleinwanzleben	CMS-50
FDDR	Kleinwanzleben	CMS-52
FDDR	Kleinwanzleben	CMS-53
FDDR	Kleinwanzleben	CMS-58
FDDR	Kleinwanzleben	CMS-63
FDDR	Kleinwanzleben	CMS-DENOK MONO
FSU	L'govskaja	CMS-62
FSU	VNISS	CMS-48
FSU	VNISS	CMS-51
FSU	VNISS	CMS-56
FSU	VNISS	CMS-57
FSU	VNISS	CMS-59
FSU	VNISS	CMS-60
FSU	VNISS	CMS-61
FSU	Umanskaia	CMS-64
FSU	Umanskaia	CMS-65
FSU	Umanskaia	CMS-66
FSU	Umanskaia	CMS-67
FSU	Ivanovskaja	CMS-55
FSU	Uladovskaja	CMS-54

Other sources of S-cytoplasm are male-sterile forms that appeared within normal fertile germplasm in the course of the inbreeding process. Male-sterile plants were discovered in the course of inbreeding in practically all FSU breeding station material. In each case, the nature of male sterility was studied. Only g-CMS forms were used in the present investigation. The g-CMS forms discovered are listed in Table 2. As can be seen, CMS forms were found in material from Polish, FDDR and FSU breeding stations. VNISS and the Institute of Cytology and Genetics, Russian Academy of Science (IC&G) now are located in Russia. Nemerchanskaja and Uladovskaja breeding stations are located in the Ukraine. More g-CMS forms were revealed in selection from cultivars Janash-1 and Janash-3. We have used two male-sterile plants from Janash-1 (Janash-1¹⁷ and Janash-1¹⁶) and two plants from another cultivar (Janash-3¹³ and Janash-3¹⁸). There also were g-CMS forms found in Polish inbred material (DD45 MF, KJ 235-1) received from HBC.

Table 2. The origin of CMS forms appearing within fertile material in the course of inbreeding

Origin of CMS forms		
Country	Breeding station	Line or Cultivar
Poland	HBC	DD45 MF
Poland	HBC	KJ 235-1
Poland		Janash 1
Poland		Janash 3
FDDR	Kleinwanzleben	SOAN-237
FSU	Nemerchanskaja	SOAN-28
FSU	Uladovskaja	SOAN-31-39
FSU	VNISS	SOAN-104
FSU	VNISS	SOAN-105-10
FSU	VNISS	SOAN-116
FSU	VNISS	SOAN-117

FSU

IC & G

SOAN-118

As a rule, only one male-sterile plant found in an inbred line was used as a source of S-cytoplasm. Exceptions were made in lines SOAN-31-39 and SOAN-104. Five male-sterile plants from line SOAN-31-39 (SOAN-31-39¹, SOAN-31-39²¹, SOAN-31-39³⁶, SOAN-31-39⁴⁰ and SOAN-31-39⁴¹) and three from SOAN-104 (SOAN-104³, SOAN-104⁷ and SOAN-104¹⁰) were used. Otherwise, only one was used as donor of sterile cytoplasm. Thus, 20 g-CMS forms received from 6 breeding stations and 21 g-CMS forms obtained from normal fertile material were investigated.

Sterility maintainer

An inbred line, SOAN-98, was used as the sterility maintainer. It was isolated from the population Cp/20 (VNISS) by means of two generations of selfing and sib-crossing.

Test crosses

The F₁ generation was obtained from only completely or semi-sterile plants. Only completely sterile plants were used to obtain the first generation of backcrossing (F₁B₁). Branches of the plants were isolated before flowering by means of paper bags. Over 5 to 7 days, a brush was used to apply freshly collected pollen to branches of male-sterile plants covered with paper bags.

Phenotype description

All plants in F₁ and F₁B₁ generations were classified into three types: completely sterile (c.s.), semi-sterile (s.s.) and normal. The completely sterile type was characterized by light green or white shrunken anthers. The semi-sterile type possessed non-dehiscent yellow or reddish-yellow anthers, in which all of the pollen grains were aborted. The normal type could possess non-dehiscent or dehiscent anthers with yellow or reddish-yellow color but it always had normal fertile grains among the aborted ones.

Observations

Observation of plant phenotype was conducted 2 to 3 times during the flowering period. A plant was determined to be a specific male-sterile type on the basis of maximal displayed pollen fertility. Observations were conducted over several years in Novosibirsk (Russia) and in highland Kyrgyzstan.

Results and discussion

Completely male sterile and semi-male sterile plants were used to obtain the F₁ generation. Semi-male sterile forms can be divided into two groups. Progenies from the first group contain no fertile plants, while progenies from the second group have completely male sterile, semi-male sterile and normal fertile plants. Progenies of the completely male-sterile plants can be divided into the same two groups. It should be noted that CMS forms from one origin may fall in different groups. These differences among progenies were observed early and described in the literature Owen 1945; Oldemeyer 1957; Kinoshita 1976; Balkov 1978). Similar sorts of deviations from Owen's mode of inheritance are used to distinguish new types of S-cytoplasm in sugar beet. However, segregation ratios of male sterility in the second generation are not presented. If new types of S-cytoplasm cause deviations from the expected segregation ratio, the same nuclear genotype in different generations of backcrossing should give identical segregations. If various

nuclear gene interactions are causing the deviations, differences between progenies in the F_1 and F_1B_1 will be observed.

Table 3. Expression of the male-sterile phenotype in F_1 and F_1B_1 generations

Group	g-CMS form numbers	Origin of g-CMS forms	No. of plants in F_1			No. of plants in F_1B_1		
			c.s	s.s	norm.	c.s	s.s	norm.
I	CMS-48	VNISS	30	8		29	13	
I	CMS-51	VNISS	5	6			1	
I	CMS-59	VNISS	10	1		25	11	
I	CMS-57	VNISS	18	9		47	23	
I	CMS-60	VNISS	17	8				
I	CMS-64	Umanskaja B.S	8			14	3	
I	CMS-65	Umanskaja B.S	9			9	5	
I	CMS-66	Umanskaja B.S	15	2				
I	CMS-67	Umanskaja B.S	16	3		28	31	
I	CMS-50	FDDR	1			45	22	
I	CMS-52	FDDR	16	5		37	25	
I	CMS-63	FDDR	20	9		34	61	
I	CMS-DENOK MONO	FDDR	9			5	2	
I	CMS-54	Uladvorskaja B.S	3	7		10	12	
I	CMS-55	Ivanovskaja B.S.	8	1		10	5	
I	CMS-62	L'govskaja B.S	10	1		32	11	
I	SOAN-104 ¹⁷ †	conversion in SOAN-104	3	1		14	6	3
I	SOAN-104 ¹³ †	conversion in SOAN-104	9	11				
I	SOAN-116	conversion in SOAN-116	13	2				
I	SOAN-117	conversion in SOAN-116	30	3				
I	KJ 235-1	conversion in KJ 235-1	1					
I	DD45 MF	conversion in DD45 MF	11	4				
I	SOAN-31-39 ¹²¹	conversion in SOAN-31-39	8					
I	SOAN-31-39 ¹⁴¹	conversion in SOAN-31-39	1			7	2	
I	SOAN-31-39 ¹³⁶	conversion in SOAN-31-39	6			3	1	
I	SOAN-31-39 ¹¹	conversion in SOAN-31-39	4			3		
I	SOAN-105-10	conversion in SOAN-105-10	22	10				
I	SOAN-118	conversion in SOAN-118	4			34	24	
I	Janash-3 ¹³ †	conversion in Janash-3		5		11	9	1
I	SOAN-237	conversion in SOAN-237	1					
I	SOAN-28-5	conversion in SOAN-28-5	12	18				
II	CMS-56	VNISS	2	10	34	53	8	
II	CMS-61	VNISS	11	5	5	30	26	
II	CMS-58	FDDR	10	8	1	38	48	
II	CMS-53	FDDR	10	22	2	6	16	1
II	SOAN-104 ¹¹⁰ †	conversion in SOAN-104	4	10	1	12	40	1
II	SOAN-31-39 ¹⁴⁰	conversion in SOAN-31-39	1		1	1	3	
II	Janash-1 ¹⁷ †	conversion in Janash-1	6	4	5	2	18	6
II	Janash-1 ¹⁶ †	conversion in Janash-1		6	1	1	1	
II	Janash-3 ¹⁸	conversion in Janash-3	4		2	16	10	
II	SOAN-28	conversion in SOAN-28	1	5	2			
Total			356	184	54	556	437	12

† Semi-sterile plants were used to obtain F_1 generation.

An F_1B_1 was generated to investigate the appearance of fertile plants in the F_1 . Only completely sterile plants were selected to obtain the following backcross generation. Results obtained for the F_1B_1 generation differed from the F_1 . Progenies of c.s. and s.s. forms from the first and second groups produced no normal fertile plants (Table 3). On the basis of these results, we conclude that the

differences among CMS forms in F_1 generation are caused by various conditions of nuclear genes. Exceptions were observed in the male sterile SOAN-104¹⁰, SOAN-104¹⁷, CMS-53 and Janash 3¹³. Additional research will be required to conclusively determine the reasons for deviation in F_1 and F_1B_1 from Owen's mode of inheritance.

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Genetic diversity for high-temperature tolerance in sugar beet

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Abstract

Eighteen diploid sugar beet varieties were studied for genetic diversity for high-temperature tolerance under subtropical Indian conditions at Lucknow. Two sets of experiments were sown in a randomized block design; harvesting of experiments and recording of observation were done in May and June. Five economic parameters – root yield, percent sucrose, percent recovery, recoverable sugar and percent root survival – were measured to study genetic divergence. In general, there was a decline in root yield, sucrose and other parameters from May to June. The impurity index increased in June. Reduction in percent from May to June for these parameters was calculated and, on this basis, D^2 was calculated by the method of Mahalanobis. The D^2 values grouped varieties into eight clusters. Clusters I and III had five varieties each, cluster II had three varieties, and the rest of the clusters had one variety each. Maximum divergence was observed between clusters IV and VII. Use of this information for breeding purposes is discussed briefly in this paper.

Introduction

Commercial cultivation of sugar beet (*Beta vulgaris* L.) has extended to subtropical climates of Afghanistan, Egypt, Iraq, Iran, India, Pakistan and Morocco in the last 25 years. In India, sugar beet is cultivated as a supplementary sugar crop to augment sugar production from sugar cane in hot summer months of April and May when sugar recovery from sugar cane starts declining. In temperate climates, sugar beet is generally sown in March/April and harvested from September/October onwards when the temperatures are low. However, in India, Pakistan, Egypt, Morocco and other countries having subtropical climates, sugar beet is sown in October/November and harvested from April into mid-June when temperatures are high (35-45°C) and there are hot dry winds, which result in high transpiration and subsequent mortality of roots at late harvest. Therefore, at the time of harvest, the climatic conditions of subtropical climates are quite different. To calculate the losses due to high temperatures and also to study genetic variability and genetic diversity in diploid sugar beet varieties, two sets of experiments were conducted to measure some economically important attributes at normal (May) and late (June) harvest under Indian conditions. From this study, criteria to assess high-temperature tolerance have been devised.

The study of genetic divergence is very important to identify diverse genotypes for use in breeding programmes. Therefore, clustering of genotypes by D^2 statistics is useful in the selection of appropriate genotypes for hybridization purposes. The present study was conducted to measure: (1) the genetic divergence in this set of genotypes, (2) the relationship of genetic diversity to geographical distribution, and (3) the parental groups that are likely to result in superior segregants after hybridization, in reference to high-temperature tolerance in subtropical India. The results obtained are presented and discussed in this paper.

Materials and methods

Seed of 18 diploid varieties of sugar beet from different countries was sown in a randomized block design with three replications at the experimental farm of IISR, Lucknow. The recommended fertilizer (120 kg N, 80 kg P_2O_5 and 80 kg K_2O per ha) was applied to this crop. Row-to-row and plant-to-plant distance was kept at 50/20 cm. The experimental plots were kept weed-free for the first 2 months. This experiment was planted twice. The first experiment was harvested in the first 2 weeks of May and observations on root yield, percent sucrose and root survival were taken. The second experiment was harvested in June. Ten roots per variety were taken randomly on each date for sucrose determination by Sachs method. The Na, K and α -amino nitrogen contents in beet roots were obtained by the method of Carruthers and Oldfield (1961) to estimate purity and percent recovery. Recoverable sugar was calculated by multiplying root yield with recovery. Reduction in root yield, sucrose content, recovery, recoverable sugar and survival of roots was obtained for each parameter replication-wise by deducting the data obtained in June from that of May. This was then converted into percent reduction for each character. Mahalanobis (1928) D^2 estimates were obtained. Tochers method was used for grouping of varieties into distinct clusters as elucidated by Singh and Choudhary (1985).

Results and discussion

The country of origin and genetic constitution of 18 diploid varieties studied in the present case (Table 1) shows wide variation both with respect to geographic area and genetic constitution. The analysis of variance for five characters shows significant differences among varieties for all the five economic parameters, i.e. root yield, sucrose content, recovery (includes impurities), recoverable sugar and survival of roots at normal date of harvest (May) and also at late harvest (June) as shown in Tables 2 to 5. Data presented in Tables 3 and 4 clearly indicate that, in general, there is a reduction in root yield and sucrose content from May to June. The reduction in root yield was highest in Sharpes Klein E and lowest in Brasov, followed by IISR-2. Percent sucrose was reduced least in the variety RK(GH) and most in Debrovicka-C (Table 3). With the increase in mean temperatures from May to June, accompanied by hot dry winds and higher transpiration rate (Srivastava 1995), there was increase in impurities that resulted in lower recoveries and recoverable sugar in the June harvest (Table 4). On the basis of the reduction from May to June for all the five economic parameters, percent reduction was calculated (Table 5).

Based on D^2 values, the 18 varieties of sugar beet were grouped in 8 clusters (Table 6). The calculated D^2 values ranged from 0.3 to 42.45. There was no correlation between the geographic origin of varieties and the cluster groups of varieties. Accordingly, clusters I and III contain five varieties each, and cluster II contains three varieties. The rest of the clusters have one variety each. This very clearly shows that these varieties are quite divergent from each other and also from varieties in clusters I, II and III (Kapur *et al.* 1987). Cluster means for percent reduction of the five economic parameters (Table 7) show that for root yield the lowest reduction (5.96) was obtained in cluster V which is represented by variety Brasov and the highest reduction in root yield was obtained in cluster VI represented by genotype 2FXX77. Lowest reduction in percent sucrose was observed in cluster VII (Dobrovicka-c) and highest reduction was shown by cluster II represented by three varieties (AJ-3, LS-7 and Stupinizm). Recoverable

sugar, which takes into account the rest of the four parameters, had the lowest percent

Table 1. Diploid sugar beet varieties evaluated for high-temperature tolerance

ID no.	Name of variety	Genetic constitution	Country of origin
1	IISR Comp-1	Composite	India
2	LKC-2	Composite	India
3	IISR-2	Open-pollinated	India
4	LS-6	Open-pollinated	India
5	LS-7	Open-pollinated	India
6	AJ-3	Open-pollinated	Poland
7	AJ-4	Open-pollinated	Poland
8	Brasov	Diploid hybrid	Romania
9	Stupinizm	Open-pollinated	UK
10	2 F xx 77	Open-pollinated	UK
11	Sharpes Klein-E	Open-pollinated	UK
12	OPH	Open-pollinated	Sweden
13	Dobrovicka-C	Open-pollinated	Czechoslovakia
14	V-25	Open-pollinated	USSR
15	US-75	Open-pollinated	USA
16	RK (RH)	Open-pollinated	Indian Sel.
17	RK (GH)	Open-pollinated	Indian Sel.
18	Ramonskaya-06 (check)		USSR

Table 2. Mean data for some economic parameters and their percent reduction at late harvest

Parameter	Month of harvest		Decrease	Percent decrease
	May	June		
Root yield (t/ha)	63.21	34.89	28.32	44.80
Sucrose (%)	34.89	14.23	0.78	5.35
Recovery (%)	28.32	12.58	0.93	6.88
Recoverable sugar (t/ha)	15.00	4.43	4.17	48.57
Survival (%) of roots	14.23	53.00	22.15	29.44

Table 3. Differences in root yield and percent sucrose at two harvest times

Variety	Root yield (t/ha)			Sucrose (%)		
	May	June	Decrease	May	June	Decrease
IISR Comp-1	86.65	48.14	38.5	15.66	15.43	0.29
LKC-2	87.76	53.00	34.76	14.73	14.06	0.67
IISR-2	59.99	45.55	14.44	14.63	14.13	0.50
LS-6	58.88	25.33	33.55	14.66	14.00	0.66
LS-7	63.32	24.08	39.24	15.26	13.53	1.73
AJ-3	59.99	31.55	28.44	16.33	14.53	1.80
AJ-4	63.32	26.66	36.66	15.00	14.60	0.40
Brasov	58.88	55.38	3.50	15.00	14.00	1.00
Stupinizm	56.66	19.20	37.46	15.26	13.66	1.60
2 F xx 77	72.21	28.30	43.91	13.33	12.86	0.47
Sharpes Klein-E	68.88	22.97	45.91	14.50	14.20	0.30
OPH	55.55	34.41	21.14	15.16	14.20	0.96
Dobrovicka-C	58.88	38.16	20.72	15.60	13.66	1.94
V-25	57.77	22.22	35.55	15.33	15.00	0.33
US-75	42.77	23.83	18.94	14.93	14.33	0.60
RK (RH)	56.66	33.52	23.14	14.66	14.46	0.20
RK (GH)	64.43	48.21	16.22	14.66	14.53	0.13
Ramonskaya-06 (check)	78.88	47.55	31.33	15.33	14.33	0.40
Overall mean	63.97	34.89	29.08	15.00	14.23	0.78
CD at 5%	17.80	12.29	—	2.55	1.12	—

Table 4. Differences in percent recovery and recoverable sugar at two harvest times

Variety	Percent recovery			Recoverable sugar (t/ha)		
	May	June	Decrease	May	June	Decrease
IISR Comp-1	14.48	13.64	0.84	12.53	6.44	6.09
LKC-2	13.65	12.48	0.17	11.93	6.64	5.29
IISR-2	13.51	13.13	0.38	7.78	5.98	1.79
LS-6	13.62	12.91	0.71	7.38	3.27	4.11
LS-7	14.12	12.18	1.94	8.76	3.64	5.12
AJ-3	15.70	13.35	2.35	9.18	4.20	4.97
AJ-4	12.83	12.19	0.64	8.19	3.39	4.80
Brasov	13.63	12.45	1.18	7.99	6.90	1.09
Stupinizm	13.90	11.65	2.35	7.87	2.29	5.57
2Fxx77	12.17	11.65	0.52	8.80	3.18	5.62
Sharpes Klein-E	12.61	12.23	0.37	8.66	2.86	5.80
OPH	13.70	12.40	1.30	7.62	4.38	3.23
Dobrovicka-C	14.13	11.90	2.23	8.35	4.51	3.83
V-25	13.18	13.29	0.08	7.64	3.00	4.64
US-75	13.20	12.77	0.43	5.65	2.40	3.25
RK(RH)	13.62	12.38	1.24	7.74	4.15	3.50
RK(GH)	13.88	12.84	1.04	8.78	6.25	2.52
Ramonskaya-06 (check)	12.70	12.98	0.82	10.01	6.18	3.82
Overall mean	13.51	12.58	1.09	8.60	4.43	4.40
CD at 5%	17.81	1.80		0.66	1.66	

Table 5. Differences in percent reduction of root yield, percent sucrose, percent recovery, recoverable sugar and root survival at two harvest times

Variety	Percent reduction from May to June harvest				
	Root yield	Sucrose (%)	Recovery (%)	Recoverable sugar	Root survival
IISR Comp-1	44.44	1.85	5.80	48.63	24.33
LKC-2	39.60	4.55	1.25	44.36	24.02
IISR-2	24.07	3.42	2.79	23.08	25.98
LS-6	56.98	4.50	5.21	55.74	39.56
LS-7	61.97	11.34	13.75	58.52	33.77
AJ-3	47.40	11.02	14.97	54.21	35.31
AJ-4	57.89	2.73	4.99	58.59	27.36
Brasov	5.94	7.14	8.66	13.67	25.27
Stupinizm	66.11	11.71	16.90	70.90	24.93
2Fxx77	60.80	3.65	4.27	63.88	33.12
Sharpes Klein-E	66.65	2.11	2.93	67.00	41.61
OPH	38.05	6.76	9.49	42.42	24.55
Dobrovicka-C	35.19	14.20	15.78	45.97	22.95
V-25	61.53	2.20	0.60	60.70	39.48
US-75	44.28	4.19	3.25	57.53	37.83
RK (RH)	40.84	1.38	9.10	46.39	23.57
RK (GH)	25.17	0.89	7.49	28.78	23.45
Ramonskaya-06 (check)	39.71	2.68	5.98	38.24	22.19
Mean	45.37	5.35	7.40	47.55	29.40

Table 6. Clustering of 18 sugar beet genotypes on the basis of genetic divergence

Cluster	No.	Variety
I	5	LKC-2, OPH, RK(RH), RK(GH), Ramonskaya-06
II	3	LS-7, AJ-3, Stupinizm
III	5	LS-6, AJ-4, SK-E, V-25, US-75
IV	1	IISR-2
V	1	Brasov
VI	1	2FXX77
VII	1	Dobrovicka-C
VIII	1	IISR Comp-1

Table 7. Cluster means for reduction percent (from May to June) for different parameters measured for genetic divergence in sugar beet

Cluster	Variety [†]	Root yield	Sucrose (%)	Recovery (%)	Recoverable sugar	Root survival
I	2, 12, 16, 17, 18	36.06	3.07	6.18	38.89	23.43
II	5, 6, 7	54.06	10.79	14.43	61.34	31.45
III	4, 7, 11, 14, 15	57.97	3.10	2.61	59.63	36.65
IV	3	23.67	3.42	1.58	26.92	27.14
V	8	5.96	6.59	8.59	13.95	35.78
VI	10	60.37	3.49	6.70	62.48	33.08
VII	13	34.90	2.66	2.41	44.64	25.25
VIII	1	44.00	3.41	5.76	48.62	24.90

[†] See Table 1 for variety names.

Table 8. Average intra- and intercluster D² values

Cluster	I	II	III	IV	V	VI	VII	VIII
I	3.24	10.72	7.84	3.39	6.21	4.86	18.50	6.39
II		7.18	16.71	21.52	18.26	7.92	6.43	14.18
III			6.06	8.20	21.59	2.49	35.51	25.44
IV				0.00	3.68	19.26	31.79	8.46
V					0.00	17.64	16.93	10.00
VI						0.00	22.46	9.66
VII							0.00	18.10
VIII								0.00

reduction in cluster V (Brasov), followed by clusters IV (IISR-2) and I (LKC-2, OPH, Ramonskaya, RK(RH) and RK(GH)). Thus, if we take recoverable sugar per hectare as a criterion for high-temperature tolerance, we may infer that the varieties of clusters I, IV and V are relatively tolerant to high temperatures compared with other varieties because they show less reduction in recoverable sugar (Srivastava *et al.* 1989).

Intra- and intercluster D² values are given in Table 8. Intracluster values ranged from 0 to 7.180. The value was highest in cluster II. Maximum genetic diversity (35.51) was present between clusters III and VII. This is very interesting because varieties represented in these two clusters have their geographic origin in India, Poland, UK, Russia, USA and Czechoslovakia. They are followed by clusters IV and VII (31.79). The minimum genetic diversity was observed between clusters I and IV followed by clusters IV and V. This clearly indicates that genotypes of these clusters (I, IV and V) are related to each other. The fact that

IISR-2, RK(RH), RK(GH) and LKC-2, which had genetic material from Ramonskaya-06, have a small genetic distance among them clearly suggests that these groupings are quite authentic. Similar results were reported by Kapur *et al.* (1987) in a previous study.

The mutual relationship of different clusters was determined from data obtained from intercluster distances between different clusters. These results also indicate that selecting one genotype from each cluster and crossing them in a diallel model may lead to more variability and a higher chance for selecting appropriate genotypes for high temperature tolerance.

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Pre-breeding for major disease resistances in sugar beet in Iran

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Abstract

The leaf spot disease (*Cercospora beticola*), the curly top virus disease and powdery mildew (*Erysiphe betae*) are causing some problems in Iranian sugar beet growing areas. The paper describes the evaluation and resistance breeding programme of the SBSI. Germplasms with tolerance to *C. beticola* and curly top have been developed recently and are being improved.

Introduction

The sugar beet has its origin as a domesticated, sugar-producing crop in Germany. From this region, it has spread around the world, mainly in the temperate zones. In some areas of introduction it has become a crop of only local importance, while in others, it has been grown on a large scale. In several areas into which sugar beet has been introduced, its value as an agricultural crop plant depends, to a large extent, on successful disease control.

Beet curly top virus virtually destroyed the sugar beet industry in the western USA in the 1920s, and continued to be the principal factor limiting production until 1940. In the absence of control measures (including resistant varieties and cultural methods) sugar beet could still be grown only in limited areas of the western USA. Yellow wilt, first observed in Argentina in the 1920s, caused the complete collapse of the sugar industry in that country and, furthermore, limited the distribution of sugar beet growing in Chile. Attempts to extend the cane sugar factory operations in the southern USA by using sugar beet roots as an additional raw material failed completely because of the damage caused by two rots, *Rhizoctonia* crown rot and *Sclerotium* root rot.

In Iran, sugar production from sugar beet goes back exactly 100 years. From the beginning, sugar beet growers have had to deal with different, numerous insect pests and diseases, which still remain a problem today. Incidence of severe curly top in the southern and central provinces causes great losses, as in any sugar beet growing region. Powdery mildew is observed in all sugar beet fields, and would cause considerable damage if it were not controlled. Additionally, *Cercospora* leaf spot is one of the most important diseases in our territory, particularly in the Khuzestan province, which has a large sugar beet cultivation in autumn and consequently suffers from reduced sugar yield.

A new disease, named beet wart, has appeared in recent decades in Khuzestan. Unfortunately, the existence of rhizomania has been confirmed in Fars province, and is assumed to exist in other parts of Iran. Disease management requires preventive measures. Application of chemical compounds, apart from inducing the selection of pathogen strains resistant to the active substances, can pollute the environment and threaten human health. The safest management of plant diseases is through resistant varieties. For this reason, the Sugar Beet Seed Institute (SBSI) has given resistance breeding in sugar beet a high priority since the breeding programme started in 1985. Through the evaluation of the sugar beet

germplasm, SBSI *Beta* Gene Bank has succeeded in obtaining resistant sources against beet curly top, *Cercospora* leaf spot, and powdery mildew.

Cercospora leaf spot

Cercospora leaf spot is one of the most important and widespread fungal diseases of sugar beet around the world, and particularly in the humid and warm climates. It causes extreme damage to quality and yield of sugar beet. This disease is found in central regions of the USA from Ohio and Michigan to Colorado, and, in Europe, it has spread in the regions that have humid and warm weather such as Spain, Italy, Greece, Austria and southern parts of Germany. The disease has been found in Japan, China, Turkey, Pakistan, Egypt, India and Azerbaijan (former Soviet Union), where it also can cause significant sugar yield losses.

In Iran, *Cercospora* leaf spot has been reported in Khouzestan (Ahwaz and Dezful), Bandar abbas, Ardabil and the Caspian Sea bank. In fields of sugar beet crops in other provinces, the aforementioned disease occurs intermittently and infections are limited. The direct effect of *Cercospora* infection is a disordering of the physiology, which consequently decreases the root yield and sugar content. The indirect effect is the stimulation of leaf growth. When new leaves are formed, sugar reserves are mobilized and consumed and the undesired α -amino nitrogen increases. These biochemical effects and further disease-dependent physiological disorders result in the well-known damage. The roots of diseased plants contain a considerable amount of nitrogen, which reduces the amount of recoverable sugar and increases the undesired molasses.

In 1925, the USDA produced varieties resistant to *Cercospora* leaf spot and introduced variety US 212 to Iran as the first *Cercospora*-resistant germplasm. Other resistant varieties have been produced in collaboration with US companies and experts in Europe. The Italian varieties released as Cesena, Mezzano 71 and Rovigo 581 are valuable genetic resources for *Cercospora* resistance breeding. These varieties probably were derived from the material developed by Munerati, who worked at the research institute of Rovigo in Italy around 1910. He crossed sugar beet with *B. vulgaris* subsp. *maritima* which had been collected in the Po estuary.

The mechanism of resistance has been investigated. Harrison and his colleagues have clarified that a phenolic compound named Tri-hydroxytryamino is involved in the resistance expression. The relevant oxide compound acts as a toxin against *Cercospora beticola*, the causal agent. The resistant varieties contain much more of the phenolic compound. Hecker and his coworkers determined that the amount of Tri-hydroxytryamino in sugar beet leaves is controlled by four or more genes.

Although this disease cannot be regarded as a factor restricting the sugar beet production in Iran, according to the policy of SBSI, screening and research to find sources of resistance are considered necessary. Consequently, a search for sources of resistance among breeding material was started in 1987 and is being continued. In 1987, a framework planning was put forward and investigations duly executed. In this study, 263 lines of sugar beet, including diploid and tetraploid lines together with 13 foreign germplasm sources, were evaluated in Ghaem shahr, which has favourable natural epidemic conditions for *Cercospora* leaf spot (25-30°C and 90-96% humidity). This evaluation was done according to the KWS disease scoring table (0= resistant to 9= highly susceptible). The evaluated lines with scores of 2 or less were selected, and, in 1989, tested more precisely in 5 rows of 10-m length. All plants were evaluated and the plants rated higher than 3 were discarded. Thirteen diploid and tetraploid lines showed tolerance. Among them were two lines, one diploid and one tetraploid, that were better than the

others, and their roots were sent to Karaj. The improvement of the breeding material has continued, i.e. seeds harvested from single tolerant plants were evaluated and selected again in Ghaem shahr, according to the scheme described above.

Determination of the O-type trait of selected, single plants by crossing them with CMS plants under isolation in cages also was accomplished. Some plants showed an O-type character and, at present, are being used in our breeding programme. Thus far, four pairs of CMS and their equivalent O-type, six diploid open-pollinated lines, and seven tetraploid lines are being further improved and utilized in our breeding programme. Since 1987, 695 sugar beet breeding lines of different ploidy levels, along with collected landraces from different parts of our country, have been evaluated in Ghaem shahr.

Beet Curly Top Virus (BCTV)

The early history of curly top is somewhat obscure because it was confused at first with other diseases and injuries. It was apparently observed on garden beets in Nebraska as early as 1888. Extensive losses were reported in California in 1989, and the following year it was reported from all the western states where sugar beet was grown, indicating that it had a wide distribution before it was recognized as a distinct disease. Within a few years, it was discovered that curly top is caused by a virus transmitted by a leafhopper, *Circulifer tenellus* (Baker). This vector is widely distributed over the western USA, as well as parts of Canada and Mexico. For many years, curly top was known only in North America, and it was generally assumed that the disease was native to the western USA. However, it was determined in 1946 that the beet leafhopper is an introduced species that probably came from the Mediterranean area where several other species of *Circulifer* occur. In 1958, it was reported that curly top was widely distributed in Turkey, and later reports indicated that it was present in other semi-arid areas of Europe, Africa and Asia.

BCTV is one of the most important and destructive diseases known to sugar beet in the southern provinces of Fars and Kerman, as well as in Esfahan province in central Iran. It is most prevalent in the Fasa region in Fars. The reports show that 90% of beet plants are infected by BCTV in this region. Pre-breeding for resistance to BCTV included screening and repeated mass selection in breeding material of sugar beet. Evaluation of sugar beet germplasm to gain sources of resistance to BCTV is one of the most important objectives of the sugar beet breeding programme of SBSI. Screening of beet germplasm was conducted in Darab and Fasa in 1989 and 1990. Four tolerant lines selected from a screening programme during 1985 to 1988 have been compared in the field with a sensitive variety named IC1. To protect the vectors, no insecticides were used during the growing season.

The first evaluations were conducted 45 days after sowing. It was noticed that the vector was abundant in late June. The percentage of infested plants was calculated in different treatments. Evaluations were made again in mid-August. Some plants had already died and some did not show any symptoms. They were well developed and seemed to be healthy. To avoid any experimental error in data-processing, all data from the first disease scoring were used for statistical analysis. To confirm the field results, a study was conducted in the greenhouse at the Fars Agricultural Research Center (ARC) between 1989 and 1990. To collect inoculum, four clay pots were filled with sterile soil and sown with the IC-

sensitive variety. In early June the infected vectors were captured from a sugar beet farm in Fasa and transferred to ARC. The vector (*Neolitrus haematocephus*) was separated from other leafhoppers by the Plant Pest and Disease Research Laboratory and put on the beet plants in four of the above-mentioned pots, and each pot was covered by a small cage. The leafhoppers bred and their population increased. The distinct symptoms of BCTV appeared on the beet plants. This phenomenon indicated that the vector carried the virus.

The seed of four selected lines and the IC variety were sown in clay pots. A randomized block design was used for comparison (5 treatments, 2 pots per treatment, 4 replications). Surplus seedlings were discarded, and one seedling in 4-leaf stage kept per pot. Each pot was covered with a gauze cloth 15 days after infection. Evaluation of infested plants was made on the basis of percentage of infested plants as follows: 1 = plants without symptoms, 2 = plants with slight symptoms, 3 = plants with relatively high symptom expression, 4 = plants with high symptom expression and clear infection, i.e. sensitive reaction.

Evaluations of the tested lines were used for statistical analysis and final conclusions. After performing the workplan in the first year and recording the data during the growing season, the data were analyzed by analysis of variance and the treatments were compared by the LSD method. A significant difference between treatments and check at $P=1\%$ level could be detected. The 16396 line with 4.94% infection and 16402 line with 7.56% infection were more resistant than the other cultivars. Under greenhouse conditions, although the differences proved not to be significant between treatments, lines 16402 and 16396 were again superior in comparison with others.

Based on the complex statistical analysis of data collected in the field and the greenhouse in two successive years, it can be stated that there is a significant difference among treatments at $P=1\%$ and that the 16402 line is superior. The above-mentioned lines can be recommended as curly top tolerant germplasm.

Screening and utilizing *Beta* genetic resources with resistance to Rhizoctonia root rot and Cercospora leaf spot in a sugar beet breeding programme

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Abstract

The United States Department of Agriculture - Agricultural Research Service (USDA-ARS) has had a sugar beet (*Beta vulgaris* L.) breeding programme at Fort Collins, CO, for about 70 years. During that time, this programme has provided the sugar beet seed industry with germplasm having excellent resistance to Rhizoctonia root rot (caused by *Rhizoctonia solani*) and Cercospora leaf spot (caused by *Cercospora beticola*). This germplasm has been developed by mass selection and recurrent mass selection techniques in nurseries in which artificial epiphytotics of these diseases have been created. Because of the higher heritability of genetic resistance to Rhizoctonia root rot, a higher level of resistance to this disease than to Cercospora leaf spot is seen in sugar beet germplasm. Much diverse germplasm has been screened to find new and varied sources of resistance to these diseases. Because of the difficulties in evaluating the disease resistance of annual wild beet germplasm (*B. vulgaris* subsp. *maritima* and other wild relatives), this germplasm has not been utilized as often as germplasm with a biennial reproductive system. To find additional sources of host plant resistance and create a more diverse genetic base in our resistant germplasm, especially in resistance to Cercospora leaf spot, we need to find better ways to screen and incorporate annual wild beet germplasm into the commercial genepool.

Introduction

Fort Collins, Colorado, is located at the foothills of the Rocky Mountains, just east of the Continental Divide, about 60 km south of the Wyoming border. This elevation is 1500 m, and the rain shadow of the Rocky Mountains causes an arid environment, characterized by a low relative humidity and average precipitation of about 400 mm. Approximately 1.3 of the 4.4 million hectares of crop land in Colorado is irrigated, as is almost all of the 17 334 ha of sugar beet (*Beta vulgaris* L.) grown in Colorado (Anonymous 1996). The warm summer days with cool nights have made this area an excellent climate in which to produce sugar beet. Sugar beet was first grown in Colorado in the last quarter of the 19th century and has been processed in Colorado since the processing plant in Greeley was established in 1902.

There has been a USDA-ARS sugar beet research effort in Colorado since the mid-1920s (then called the Bureau of Plant Industry). In 1925, ARS began a resistance breeding programme to combat the devastation caused by the curly top virus and Cercospora leaf spot. Initiated in Rocky Ford, Colorado, after a few years the leaf spot resistance breeding project moved to Fort Collins. The original team was a collaborative effort of USDA scientists with Colorado, Michigan, Minnesota and New Mexico Agricultural Experiment Stations along with the sugar beet companies that operated east of the Rocky Mountains. It consisted of G.H. Coons, D. Stewart, J.O. Gaskill and F.G. Larmer. Agronomic testing at the various locations was done over the years by J.G. Lill, G.W. Deming, S.B. Nuckols,

J.O. Culbertson, R.W. Henderson, O.E. Reece and G.J. Hogaboam (Coons *et al.* 1955). The original effort concentrated on developing open-pollinated varieties with resistance to *Cercospora* leaf spot. As more scientists were added (L. Powers, R. Hecker, G. Maag, G. Smith, E. Ruppel) to the programme over time, it expanded to include research and development of germplasms with resistance to *Rhizoctonia* root rot, *Aphanomyces* black rot, curly top and storage rots. Breeding efforts at Fort Collins since have been focused on the development and release of germplasm with enhanced resistance to *Rhizoctonia* root rot and *Cercospora* leaf spot. The Fort Collins environment has proven extremely valuable in these efforts. The arid climate, low organic matter content of the soils, and hot, dry wind are not conducive to the development of soilborne or foliar diseases. Therefore, when artificial epiphytotics are created to test sugar beet for resistance to *Rhizoctonia* root rot or *Cercospora* leaf spot, there is little confounding of the results by the presence of other diseases.

Development of a screening nursery for *Cercospora* leaf spot

Cercospora leaf spot is a foliar disease caused by the fungus, *Cercospora beticola* Sacc. It has been an intermittent problem in sugar beet growing areas where the summer can be hot and humid (Red River Valley, Michigan, Ohio and, less often, Great Plains growing areas). A severe epidemic can cause up to a 42% loss of gross sugar (Smith and Ruppel 1973; Smith and Martin 1978), which can mean up to a 43% relative dollar loss (Shane and Teng 1992). Along with the resistance to curly top virus, resistance to *Cercospora* leaf spot was one of the first goals of the USDA-ARS sugar beet research programme at Fort Collins.

Early researchers depended on natural epiphytotics to provide the disease pressure necessary to make selections for resistant germplasm, and it was quickly realized that it was necessary to manage the screening nurseries in such way as to promote the development of the disease (Stewart 1948). However, evaluations are most effective when there is a uniform, moderately severe epiphytotic. At Fort Collins, since 1956, artificial inoculation and management of the nursery micro-environment have been employed successfully to create the development of such epiphytotics (Ruppel and Gaskill 1971). The arid climate and low relative humidity, combined with a careful crop rotation (sugar beet/barley/barley/barley/sugar beet), have allowed this to be done in such a manner that there is rarely any other disease present in the leaf spot nursery to confound the results.

The buffer rows of the screening nursery always are planted with a leaf spot susceptible synthetic variety (SP351069-0), and at 49-46 days after inoculation (3rd or 4th cycle of infection), infected leaves are harvested into burlap bags and dried on a tarp in the open air (under a roofed shed). The leaves are rebagged and hung in an unheated, well-ventilated building where they are stored over the winter. Because of the low relative humidity, there generally is no mould on their leaves. Inoculum for the screening nursery comes from these infected leaves saved from the previous year.

To prepare the inoculum, burlap bags of dried leaves are wetted and hand-rubbed in galvanized tubs containing about 50 L of water. As the leaf material is broken up and the spores released, water is squeezed from the leaves, rubbed leaves are discarded and more leaves are added until the full amount has been processed – about 30 bags in four tubs. A sieve is used to remove the remaining debris and this stock spore suspension is diluted to 94.5 L in each tub. The spore

suspension is well mixed in (and between) two 189-L drums to assure uniformity and then used immediately.

A metal drum with about 210-L capacity is mounted on the back of a Farmall-A^{®1} tractor and used to feed the spore suspension into a spray boom mounted on the back of the tractor. Three nozzles are used to cover each row (one 5X nozzle on the centre and one 10X nozzle to cover each side) spraying at 0.69 MPa while the tractor travels at approximately 5.6 km/hr. We spray spore suspension at about 468 L/ha. The field is inoculated in the late afternoon (15:00 - 17:00), which allows *Cercospora* to infect the plants without drying in the hot sun or being washed off by continual sprinkling immediately after inoculation.

The field is lightly fertilized, about two-thirds of the recommended rate (~132-165 kg/ha available nitrogen, Draycott 1993), to prevent excessive foliage and planted in mid-April to early May (two-row plots 3.65 m long, 56 cm apart, planted with three replicates). Beets are thinned (about one plant every 20 cm within row) 30 days after emergence. In early June, 8-16 rows of maize are planted around the nursery to provide a windbreak and help keep the humidity high within the nursery. Sugar beets are inoculated twice – once in late June or early July and then again 7-10 days later. At this time of year, we normally have high daytime temperatures (30-37°C) in Fort Collins and relatively high night-time temperatures (above 15°C), conditions which promote disease development.

To provide high relative humidity in the plant canopy, the field is lightly sprinkled with overhead sprinklers the day before inoculation and then briefly, to wet the leaves, immediately preceding inoculation. After inoculation, the nursery is intermittently sprinkled for 4-5 days from early morning until sundown. This keeps the sugar beet leaves wet and the relative humidity high, which promotes disease development. Once the first mature leaf spores are observed, 14-21 days after inoculation, the plants are intermittently sprinkled (½ hour on, ½ hour off) from morning until sundown on every other day (3 days each week).

Varieties are evaluated with a 0 to 10 scale, with 0= no leaf spot and 10 = complete defoliation; 0 or 10 are never observed (Dr E.G. Ruppel, pers. comm.). Evaluations are made every 7 days and begin once differences among lines can be detected and continue until the peak of the epiphytotic (generally 49-56 days after inoculation). Two checks, one susceptible and one resistant, are included in each experiment. The resistant check generally ranges from 3 to 4 and the susceptible from 6 to 8 during the peak of the epiphytotic. An analysis of variance (ANOVA) test is used on each date of evaluation for each experiment in the nursery and, if significant, an LSD means separation is used. Means across all dates cannot be analyzed and are not useful, because they can lead to erroneous conclusions about the level of resistance in any variety tested. (For example, one entry receives four ratings of 7, 3, 3, 3 and another of 3, 3, 3, 7. Both would have a mean of 4. However, the first may have been 'super susceptible', losing most of its blighted leaves before the second date of evaluation and, therefore, the second would be preferable, because it would have already produced an adequate yield before succumbing; E. Ruppel, pers. comm.). These techniques has been reviewed in the literature (Gaskill *et al.* 1967; Smith and Gaskill 1970; Ruppel and Gaskill 1971).

Development of a screening nursery for *Rhizoctonia* root rot

¹ Mention of a trademark or manufacturer by the USDA does not imply its approval to the exclusion of other products or manufacturers.

Rhizoctonia root or crown rot, caused by *Rhizoctonia solani* Kühn anastomosis Group 2-2 (AG-2-2), is endemic in sugar beet growing areas. J. Gaskill began a breeding programme for *Rhizoctonia* resistance at Fort Collins in the early 1960s. The first two resistant cultivars from this programme, FC 701 and FC 702, were released in 1966 (Gaskill 1968). Early breeding efforts relied on mass selection or recurrent field selection to improve population for resistance to root rot (Hecker and Ruppel 1977b). J. Gaskill realized early that a natural epiphytotic did not produce a consistent, uniform disease pressure necessary for successful recurrent mass selection for *Rhizoctonia* resistance (Pierson and Gaskill 1961). It was necessary to define the parameters for producing a successful, artificially induced epiphytotic.

The isolate of *R. solani* used to create the artificial epiphytotic today (R-9) is the same “B-6” that was used by Pierson and Gaskill (1961). It has been stored in the refrigerator at 4°C (for the short term) or in a freezer at –3 to –4°C (for longer periods) with no apparent loss of viability. R-9 is in AG-2-2 IIIB and is as virulent on sugar beet as any isolate of *R. solani* that we have tested (unpublished data). There has been no report of cultivar × isolate interaction (Ruppel 1972), nor have any of our tests shown any sign of cultivar × isolate interaction (unpublished data); therefore, we continue to use a single isolate inoculum instead of multiple isolate mixtures. Inoculum of the isolate is tested in the greenhouse each year to assure its virulence before being used to create the artificial epiphytotic in the field.

Currently, we mass-produce our *R. solani* inoculum on moist, autoclaved barley grain. We use 14.2-L stainless steel pans with lids (~ 50 × 29 × 10 cm) that fit into our steam sterilizer. The pan is filled with barley and distilled water (in a ratio of approximately 1 L barley to 600 ml water) and soaked overnight. It then is sterilized at 250°C (0.145 MPa) for 2 hours, removed, and cooled. The barley is colonized with *R. solani* that has been grown on PDA for 7 days and aseptically transferred to the pans of sterile, steamed barley. The fungus is allowed to grow under aseptic conditions for 21 days at 28°C, when the colonized barley is air-dried in large paper trays. The dried *Rhizoctonia*-colonized barley is ground in a Wiley® mill No.1 (Arthur H. Thomas Co., Philadelphia, PA) until it passes through a No. 3 sieve. It is stored refrigerated (~4°C) in paper bags until use. Each year, we normally produce about 24 pans of inoculum (~100 L of barley), which yields between 40 and 45 kg of ground barley inoculum.

Field plots are 3.7-m single rows 56 cm apart, planted in five replicates. Beets are thinned to 20 cm between plants. The beets are planted late (mid-May) to reduce the risk of hail damage and assure that the beets are not too large when inoculated in early July. Shortly before inoculation, a plant count is made of the number of plants in each plot. About 63 days after planting the beets are inoculated with a Gandy Ezee Flow® granulate applicator (Gandy Manufacturing Co., Owatona, MN 55060). Inoculum is placed in a 10-cm band over the centre of each row and into the beet crowns. Two passes are made (one in each direction), delivering a total of 7.2 g of inoculum for each 3.7-m row. Immediately after inoculation, the field is cultivated at a fast rate of speed to throw soil into the plant crowns, which accelerates disease development (Schneider *et al.* 1982). Irrigation is used to promote the epidemic:

- overhead sprinklers for 4 hours immediately after inoculation to wet the inoculum, carry it into beet crowns, and help it stick to the plant
- 2 hours of overhead sprinkling each day (at mid-day) for 5 to 6 days

- irrigation thereafter as needed via sprinklers or furrows.

Plants are harvested in late September, and each plant is visually evaluated on a scale of 0 to 7 (Table 1).

Table 1. Disease class description for visually evaluating *Rhizoctonia*-infected sugar beet roots

Score	Symptom description
0	No visible lesions
1	Superficial, arrested, dry lesions at point of inoculation; or small, delineated, scattered, scurfy, non-active lesions on taproot. No canker, rifts or rotting
2	Shallow, dry-rot canker in centre of crown; or active lateral lesion(s) affecting no more than 5% of root tissue
3	Deep, dry-rot canker at point of inoculation (crown); or extensive lateral lesion(s) affecting no more than 25% of tissue; usually cracks or cankers are in lesion areas
4	Extensive rot of upper half of taproot, with cankers, rifts or lesions up to 5 mm deep
5	From more than 50 to 75% of taproot blackened, with rot extended well into the interior; roots usually misshapen, with cracks and rifts
6	Entire root blackened, except for extreme tip; most foliage dead, but some small, green leaves in centre of crown
7	Plant dead; 100% rotted

A disease index (DI), the percentage healthy plants, and the percent plants with DI of 0 through 3 are calculated from these evaluations (see below).

DI = $\Sigma(\text{Disease class} \times \text{number of roots within that class}) / \text{Total initial number of plants within plot}$

Percent healthy roots = $[\text{Roots within classes } \Sigma(0 + 1) / \text{Initial roots within plot}] \times 100$

Percent plants with DI of 0 through 3 = $[\text{Roots within classes } \Sigma(0 + 1 + 2 + 3) / \text{Initial roots within plot}] \times 100$

An ANOVA test can be used to test for significant differences among lines for each of these parameters. Percentages need an angular or arcsin transformation for ANOVAs. This technique has been described in the literature a number of times over the past 30 years (Pierson and Gaskill 1961; Gaskill 1968; Hecker and Ruppel 1977b; Ruppel *et al.* 1979; Schneider *et al.* 1982; Ruppel and Hecker 1988).

Germplasm sources

Cercospora leaf spot resistance

Progress in increasing leaf spot resistance was made early through mass selection of open-pollinated sugar beet varieties (Skuderna 1925; Peterson and Cormany 1952; Oldemeyer and Zielke 1967). However, it quickly was realized that mass selection within open-pollinated varieties was not going to bring the quick results that had been seen in the development of resistance to curly top virus and, therefore, a programme of inbreeding was developed. This programme was started with germplasm developed by W.W. Tracy at Fort Collins (Coons *et al.* 1955), which he had received from F.J. Pritchard, who had been inbreeding sugar beet for genetic studies (Pritchard 1916). Mass selection was continued within

these inbred populations and lines. This resulted in 1937 in the release of US 217, which was a synthetic variety of five inbreds (Coons 1936). A series of varieties was developed from this germplasm – US 200 X 215, US 200 X 216, US 216 X 226, etc. – and widely used in areas prone to *Cercospora* leaf spot epiphytotics (Coons *et al.* 1954, 1955). Another source of leaf spot resistance that came into this country was germplasm originally developed in Italy from crosses with wild sea beet (*B. vulgaris* subsp. *maritima* (L.) Arcang.) by Munerati and improved by Italian commercial breeders (Coons *et al.* 1955). This material found its way into Great Western varieties GW 304 and GW 359 (source = Cesena), and ARS scientists used Mezzano 71 as the source to develop US 201 (Lewellen 1992), which became the new standard for resistance to leaf spot.

The sugar beet breeding programme at Fort Collins, which has continued to develop *Cercospora* leaf spot resistant germplasm, has relied heavily on these early releases. Three groups of germplasm releases have come out of the programme. Those designated as FC 900s are multigerm pollinator populations, mostly self-fertile, segregating for genetic male sterility (*aa*), with combined leaf spot and curly top resistance. The FC 500 germplasms are leaf spot resistant O-types with CMS equivalents, and FC 600 germplasms are combined leaf spot and curly top resistant O-types with CMS equivalents. In an attempt to increase the level of *Cercospora* leaf spot resistance, these populations have been inbred continually.

FC 901 (Gaskill *et al.* 1967) (SL 932 × SP581813-00) was an attempt to combine two curly top resistant inbreds (SL 932 = F_1 [CT5 and CT9A]) with a US 201 derivative to produce a multigerm pollinator with combined disease resistance. FC 902 (Smith and Gaskill 1979a), FC 903 and FC 904 represent intercrossed progenies selected within this original germplasm pool of FC 901. US 201 also was important in the original germplasm that went into the FC 500 series of germplasm released to the industry from 1963 through 1969. Three O-types/CMS pairs were officially released and registered in Crop Science (Smith and Gaskill 1979b) (FC 502/2, FC 504, and FC 506). FC 502 is derived from Savitsky's V.F.S. no.715 (SP561012-0) × US 201. FC 502/2 (Smith and Gaskill 1979b) is an S_2 subline of FC 502, FC 504 (Smith and Gaskill 1979b) is derived by selfing from US 216 × SLC 101, and FC 506 (Smith and Gaskill 1979b) was derived from FC 504 × FC 502/2.

The FC 600 series, which has been released beginning in 1965, built on this germplasm and combined it with sources of curly top resistance. FC 601 comes from a population built on SL 202, SLC 122-0, US 201 and US 22/3; FC 602 is an S_2 inbred derived from this population; FC 603 is from a population comprised of SL 202, SWLC 122-0, US 201, US 22/3 and US 22/4. FC 604 (Smith and Ruppel 1979) originated from 632028s1, which is an early generation parent of FC 601. FC 605 (Smith and Ruppel 1979) is from 632028s1 × FC 601, and FC 606 (Smith and Ruppel 1980a) is from FC 605 × (FC 601 × 662119s1). The breeding line 662119s1 is the F_2 of (C2563 × 611227-001); 611227-001 was one-half SLC 122-0 and a mixture of US 216, SLC 101, and US 226. FC 607 (Smith and Ruppel 1980b) is from the three-way cross (FC 504 X FC 502/2) × FC 605 and FC 609 (Smith and Ruppel 1988) from (FC 504 X FC 502/2) × 662119s1.

A major problem in the development of sugar beet with resistance to *Cercospora* leaf spot has been the loss of vigour due to the continual inbreeding, which was noted by Coons *et al.* (1955) early on and has been a concern ever since (McFarlane 1971). The development of hybrid varieties, with the resultant

heterosis, has ameliorated this problem to some extent, but seed production on O-type male and CMS females still is a problem. This is seen in germplasm from both the FC 500 and FC 600 series.

***Rhizoctonia* root rot resistance**

Lewellen (1992) provided an excellent review of the development and origins of the Great Western Sugar Co. (GWS) sugar beet germplasm (as well as the rest of the US germplasm), which formed the basis of the *Rhizoctonia* root rot resistance breeding programme by the USDA-ARS scientists at Fort Collins, CO. The first two resistant varieties, FC 701 and FC 702 (Hecker and Gaskill 1972), were released in 1966 (Gaskill 1968). FC 701 was mass-selected from the open-pollinated variety GW 674-56C, and FC 702 from C817, which was a synthetic derived from 359-52R, the GWS open-pollinated variety from which GW 674-56C was derived. FC 703 (Hecker and Ruppel 1977a) resulted from FC 702 × FC 701, FC 705 (Hecker and Ruppel 1979) from a synthetic of FC 701 origin, FC 707 (Hecker and Ruppel 1979) was derived from a pool of C817 and GW 674-56C germplasm, FC 709 (Hecker and Ruppel 1988) from FC 702-5 × FC 701-5, which are sib-lines of FC 702 and FC 701. FC 712 (Hecker and Ruppel 1986) is a composite cross of the most resistant Fort Collins germplasm (almost entirely of GWS origin). All of these germplasms – FC 701, 702, 703, 705, 707, 709 and 712 (and derived sib-lines) – trace 100% of their origin back to the open-pollinated GWS line GW 359-52R. This series of germplasm has become relatively more inbred and more resistant to *Rhizoctonia* root rot over time.

When the *Rhizoctonia* breeding project at Fort Collins was taken over by R.J. Hecker, in addition to continuing to develop the sources used by J.O. Gaskill, he sought other, more diverse, sources of *Rhizoctonia* root rot resistance. FC 704 (Hecker and Smith 1979) is a root rot resistant red sugar beet developed from a GWS heterogenous population known as 'German red beet'. FC 706 (Hecker and Smith 1979) shares FC 703 parentage and a mixture of SP5831-0 (partially derived from *B. vulgaris* subsp. *maritima*), US 226, and US 400 parentage. FC 708 (Hecker and Ruppel 1981) is derived from FC 701 and a pool of curly top leaf spot resistant germplasm (US 22/3, US 22/4, US 35/4, US 201, SLC 122 and SLC 202). It was the first O-type/CMS pair with *Rhizoctonia* root rot resistance that was released. FC 710 (Hecker and Ruppel 1991) arises from the sources crossed with FC 703 to create FC 706. FC 711 (Hecker and Ruppel 1983) was mass-selected from two heterogeneous Japanese breeding lines. FC 718 is composed of selections within four Russian, open-pollinated populations, and FC 719 is a cross between a Polish variety and FC 702-5 and FC 701-5 (Panella *et al.* 1995). FC 726 (Panella and Ruppel 1996b) is from a cross of FC 703/3 and a fodder beet (Peramono), which showed some resistance to *Rhizoctonia*.

Some of the most recent releases from Fort Collins – FC 716, FC 717 and FC 728 – have incorporated germplasm from commercial varieties to improve the agronomic desirability of those germplasms (Panella *et al.* 1995; Panella and Ruppel 1996b). Although there is some curly top resistance in the FC 600 series of leaf spot resistant germplasm, a greater focus now is being placed on germplasm with multiple disease resistance – FC 708, FC 715, FC 715CMS, FC 721, FC 721CMS, FC725 and FC728 (Hecker and Ruppel 1981; Ruppel *et al.* 1995; Panella and Ruppel 1996b). This emphasis on multiple disease resistance is continuing with the major effort placed on germplasm resistant to *Rhizoctonia* and *Cercospora* and some attention to the single gene source of rhizomania resistance.

Breeding progress

Progress in breeding for resistance to *Rhizoctonia* has been quicker than for *Cercospora* and the level of resistance in *Rhizoctonia*-resistant germplasm is greater.

The DI for our highly *Rhizoctonia*-resistant check averages 1.7 (0 to 7 scale) over the last 10 years, and that for our *Cercospora*-resistant check averages 3.8 (0 to 10 scale). Initial rates of *Rhizoctonia* inoculum (4.8 g) have been increased to 7.2 g for each 3.7 m of row because of an overall higher degree of resistance in commercial and experimental materials being evaluated.

Greater breeding progress has been made in *Rhizoctonia*, primarily because of the higher heritability of resistance to *Rhizoctonia* found in sugar beet. Resistance to *R. solani* in sugar beet is polygenic, involving at least two loci, two or three alleles, and modifying genes in some populations (Hecker and Ruppel 1975). Broad-sense heritability is about 0.65 (Hecker and Ruppel 1975). The relatively high heritability has allowed for quick and successful development of increased host plant resistance through mass selection, and incorporation of this resistance into commercial varieties (reviewed by Panella and Ruppel 1996a). Current germplasm has a level of resistance to root and crown rot in which there was no yield loss under heavy disease pressure (Ruppel and Hecker 1994).

Estimates of broad-sense heritability of resistance to *Cercospora* have ranged from 12 to 71%, with an estimated 4 or 5 genes responsible for the resistance (Bilgen *et al.* 1969; Smith and Gaskill 1970). Narrow-sense heritability estimates of about 24% compared well with measured (realized) heritability values, and environment was responsible for 44 to 62% of the variation in the same test (Smith and Ruppel 1974). It is this large environmental variation that has made it difficult to make quick progress in developing *Cercospora* resistance through mass selection. This also has made incorporation of high levels of leaf spot resistance into varieties with superior agronomic performance difficult (Smith and Campbell 1996). In many areas, resistant varieties require some fungicide application to provide adequate levels of protection against *Cercospora* (Miller *et al.* 1994).

Use of Plant Introduction (PI) germplasm in the Fort Collins programme

The National Plant Germplasm System Beta collection has over 2000 PI accessions. The material that has been used most often in breeding comes from the taxa *B. vulgaris* subsp. *vulgaris*, which includes all of the biennial sugar beet types, and *B. vulgaris* subsp. *maritima*, which contains the closely related wild sea beet and has both annual and biennial types. Germplasm with a biennial flowering habit is not only easier to introgress but also much easier to screen. Annual wild beets often have a fibrous root system, which may test resistant to *Rhizoctonia* through simple, mechanical blocking of penetration by the fungus. This type of resistance is lost once the wild beet germplasm has been introgressed into a genetic background of a commercial sugar beet with tuberous root.

At the latitude of Fort Collins (40°35'N), annual beets flower early and the senescence and small size of their leaves makes it very difficult to get a good estimation of the level of *Cercospora* resistance. Nonetheless there have been attempts to use *B. vulgaris* subsp. *maritima* as a source of resistant germplasm. Most of the *Cercospora*-resistant germplasm owes its origin to the material that came out of Munerati's programme in Italy, in which *B. vulgaris* subsp. *maritima* was the donor of resistance genes (Lewellen 1992). However, since that time, there have been very few efforts to locate and incorporate new sources of resistance to *Cercospora* into this narrow germplasm base. Much of the success in the Fort Collins breeding effort is due to the diversity present in the GWS open-pollinated varieties that formed the base genepool from which the mass selection programmes began. This is especially true of the *Rhizoctonia* resistance breeding

programme, which drew so heavily on these populations for its early releases. Four germplasms released from the programme (FC 706, FC 708, FC 710 and FC 712) have *B. vulgaris* subsp. *maritima* in their pedigree from sources other than GWS open-pollinated varieties.

I believe that there is a need to continue to infuse our disease-resistant germplasm with a broader genetic base than we have today. As commercial hybrid parents become more inbred, it is important that the germplasm base from which these parents are developed has the diversity necessary to provide for maximum gain through heterosis. It is time to revisit the use of wild beet germplasm in developing new pools of disease-resistant germplasm, perhaps with techniques similar to the model presented by Bosemark (1989). Munerati's success, and the research of others, has shown that it can be done if we have the persistence to do it (Bilgen *et al.* 1969; Doney 1993).

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Beta evaluation and sugar beet enhancement from wild sources

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Abstract

The systematic evaluation of the US National Plant Germplasm System (NPGS) *Beta* Collection was initiated in 1987. Emphasis was on a set of priority descriptors identified by the Sugar Beet Crop Advisory Committee (CAC), now the Crop Germplasm Committee (CGC). Nine scientists with expertise in the priority descriptors agreed to perform this evaluation on a limited but continuous basis. Between 30 and 60 accessions have been evaluated each year. As of 1995, about one-fourth of the nearly 2000 accessions in the *Beta* collection have been evaluated. Evaluation data are recorded in the Germplasm Resources Information Network (GRIN) and can be accessed via Internet, phone modem or pcGRIN software. Valuable characters such as male sterility, monogerm seed and resistance to Beet Yellow Virus, powdery mildew, Erwinia root rot, Cercospora leaf spot, *Rhizoctonia*, curly top and rhizomania have been identified. Concurrent with evaluation, a sugar beet enhancement programme utilizing wild germplasm was commenced. Wild accessions (mostly *B. vulgaris* subsp. *maritima*) of different types and geographic background were crossed to sugar beet. Following two recombination generations to allow for the mixing of wild and domestic genes, plants were selected for root shape. After four cycles, roots began to approach sugar beet in appearance. Several fifth-cycle selections crossed to a common sugar beet inbred gave root yields equal to commercial sugar beet hybrids. Four of the better lines were released to industry in 1995. New releases are expected every 2-4 years.

Introduction

The major activities of germplasm resources groups can be grouped into a series of steps, one building upon another. These steps can be likened to a pyramid where each succeeding tier depends on the strength and quality of the preceding tier. If all tiers are not in place the capstone cannot be secured. With *Beta* germplasm resources activities (Fig. 1), the "Total *Beta* Genetic Variation" serves as the foundation. Building upon this foundation to secure all available genetic variation requires careful and systematic collecting efforts. These collections are of little value if they are not maintained. The next tier is, therefore, the proper maintenance of this valuable material. Collecting and maintenance, although important in preserving the genetic material, do little toward guaranteeing its use. The collection will not be used unless it has some value. The evaluation of the genetic material, the next tier, is essential to the user community. Even after the evaluation process, many of the beneficial genes in the wild material are in genetic backgrounds that are undesirable for commercial use and need to be enhanced or transferred into more desirable backgrounds before the final incorporation into commercial cultivars. The final step or capstone is the development of commercial cultivars or hybrids. In the United States, this step is almost totally in the realm of private breeding companies. All the tiers up to the final step usually are turned over to curators and public geneticists. Unfortunately, only a few dedicated researchers and scientists are involved in the bulk of the development of genetic

resources. This paper focuses on the *Beta* evaluation and enhancement efforts in the United States.

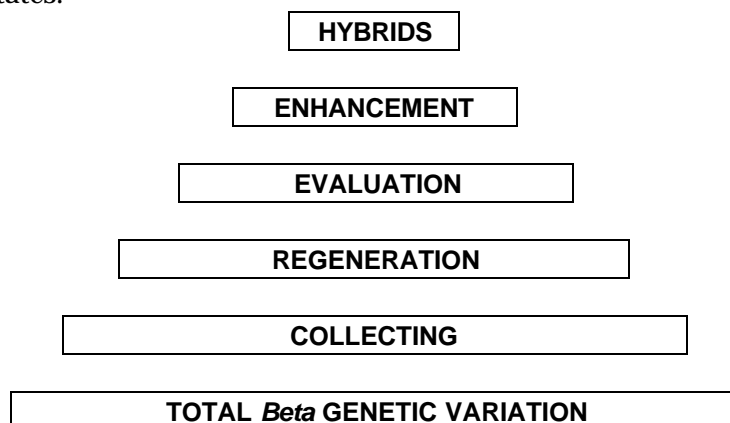


Fig. 1. The major activities of germplasm resource researchers can be grouped into a series of steps, one building upon another.

***Beta* evaluation**

Early evaluation of the US National Plant Germplasm System (NPGS) *Beta* collection was conducted by J.O. Gaskill and J.A. Elder in 1960. They evaluated the collection for leaf spot (*Cercospora beticola*) and Rhizoctonia (*Rhizoctonia solani*) resistance, bolting and pigmentation of stem and leaf. The same year C.L. Schneider evaluated the collection for Black Rot (*Aphanomyces cochlioides*) resistance. Since 1960 (owing to collecting expeditions, germplasm exchanges and germplasm releases) the collection has expanded from about 300 to near 2000 today.

In 1987, the Sugar Beet Crop Advisory Committee (CAC), now the Crop Germplasm Committee (CGC), initiated a systematic evaluation of the collection (Doney 1987). Emphasis was placed on a set of descriptors identified as high priority by the CAC. Nine scientists, with expertise in the priority descriptors, agreed to perform this evaluation on a limited but continuous basis (Table 1). The priority descriptors have changed little even though several of the original evaluators have retired. Between 30 and 60 accessions have been evaluated each year. As of 1995, about one-fourth of the nearly 2000 accessions in the *Beta* collection have been evaluated. Evaluation data are stored in the Germplasm Resources Information Network (GRIN) and can be accessed via Internet (<http://www.ars-grin.gov>), phone modem (+1 (301) 504-6227) or pcGRIN software. Valuable characters such as male sterility, monogerm, frost tolerance and resistance to Beet Yellow Virus, powdery mildew, Erwinia root rot, Cercospora leaf spot, *Rhizoctonia*, curly top and rhizomania has been identified (GRIN 1996).

Table 1. Original *Beta* germplasm evaluators, location and the descriptors they evaluate

Evaluator	Institution	Location	Descriptor
Doney, D.L./Kern, J.	USDA/ARS, Amer.Crystal	Moorhead, MN	Agronomic/Hort.
Lewellen, R.	USDA/ARS	Salinas, CA	Virus Yellow, RZ
Whitney, E.	USDA/ARS	Salinas, CA	Erwinia root rot

Yu, R.	USDA/ARS	Salinas, CA	Cyst nematode
Brown, T.	BSDF	Kimberly, ID	Curly top
Smith, G.	USDA/ARS	Ft. Collins, CO	Cercospora leaf spot
Hecker, R.	USDA/ARS	Ft. Collins, CO	<i>Rhizoctonia</i>
Anderson, A.	NDSU	St. Thomas, ND	Root maggot

Enhancement

Pest-resistant germplasm identified in the evaluation programme is being incorporated into useful sugar beet germplasm. These efforts are located at Salinas, CA for Beet Yellow Virus, rhizomania and Erwinia root rot; at Ft. Collins, CO for *Rhizoctonia* and leaf spot; and at Fargo, ND for root maggot resistances. Since pest resistance is often found in wild materials that carry many undesirable characters, the incorporation is long term and requires many selection cycles. Some of this material is close to release or in the process of being released to the user community.

Concurrent with this, a sugar beet enhancement programme utilizing wild germplasm to broaden the genetic base of sugar beet breeding pools was commenced at Fargo, ND (Doney 1993). This project evolved into a multistage development programme with crosses between sugar beet and wild germplasms initiated in 1986, 1990 and 1994 (Table 2). The sugar beet or female side of the cross was different for each set of crosses, i.e. a cms sugar beet inbred was used for the female in the 1986 crosses, a sugar beet inbred segregating for genetic male sterility was used as the female in the 1990 crosses, and a self-incompatible sugar beet inbred was utilized to obtain crossing in the 1994 scheme (Table 2). In all cases, two cycles of random mating were generated prior to selection. This was done to obtain recombination between the wild and domesticated germplasm. Harvesting seed from only male-sterile plants in each cycle of the 1986 and 1990 crosses maintained male sterility in the succeeding population and prevented selfing. The following scheme was used in the 1994 crosses: (1) 10 plants from each accession were crossed separately to 10 plants of the self-incompatible sugar beet inbred, (2) for each accession, 10 plants from each of the 10 F_1 plants (total of 100 plants) were intercrossed to generate the F_2 seed, and (3) equal numbers of seed from each of the F_2 plants were bulked and grown to produce the F_3 seed. This method was used to ensure that the genetic variation existing within each accession was represented and recombined with sugar beet germplasm prior to the first selection generation.

Following the recombination cycles, each new population was planted in field selection trials. Spacing within the selection trials was 55 cm between rows and 30 cm between beets within the rows. About 3 weeks prior to the regular sugar beet harvest, the selection trials were dug and plants were selected for sugar beet shape (smooth and free from 'sprangling'). Selected roots were intercrossed in isolated random mating crossing blocks and seed was harvested only from male-sterile plants to generate the next selection cycle.

Table 2. Crosses for enhancement from wild sources

1986 crosses	1990 crosses	1994 crosses
L53cms X WBs (Mixture)	<u>Regional population</u>	R376-43 (Self-incompatible)
L53cms X WB 252 (Greece)	• 3747(aa) X <i>B. maritima</i>	X
L53cms X WB 172 (Italy)	(Denmark)	50 individual accessions from:
	• 3747(aa) X <i>B. maritima</i>	• UK
	(Belgium)	• France
	• 3747(aa) X <i>B. maritima</i>	• Ireland
	(Ireland)	• Denmark
	• 3747(aa) X <i>B. maritima</i>	• Belgium
	(Middle East)	• Channel Islands
	• 3747(aa) X <i>B. macrocarpa</i>	

- 3747(aa) X *B. patula*
 - 3747(aa) X *B. atriplicifolia*
-

Results

After five cycles of mass selection for root shape, roots in several populations were beginning to resemble sugar beet roots. Individual family lines from one of the most promising populations (L53cms X WB 252) were crossed with a sugar beet inbred and the resulting hybrids tested in a replicated field trial for root and sugar yield. On the basis of these results, four of these lines were released to the user community in 1995 (Doney 1995). The results of 2 years of replicated field trials for these four lines are given in Table 3. Data were consistent over years, and only the combined data are given in Table 3. Two lines (y322 and y387) gave root yields equal to or greater than the commonly used commercial hybrid check included in the tests. All lines were lower in sugar percentage and total sugar yield than the commercial sugar beet hybrid. However, line y322 was not significantly different from the commercial hybrid in sugar yield.

Table 3. Sugar yield, root yield and sucrose percentage for released germplasm (lines from the L53cms X WB252 cross, followed by five cycles of selection for root shape) and a commercial hybrid; data are the summary of 2 years of field tests

Entry	Sugar yield (kg/ha)	Root yield (t/ha)	Sucrose (%)
v317	5960	46.6	13.0
y318	5042	40.3	12.2
y322	6647	55.1	12.3
y387	6093	51.3	12.3
Commercial hybrid	6995	53.0	13.3
LSD 0.05	537	2.1	0.4

It should be noted that selection was for root shape only and that all lines are carrying significant amounts of wild germplasm. This suggests that growth genes from wild sources can be readily transferred into elite sugar beet breeding pools.

Populations from crosses made in 1990 have advanced through two cycles of random mating for recombination with domestic sugar beet germplasm and are in the second cycle of selection for root shape. Some lines are beginning to show a positive response; producing sugar beet-like roots. One population (from the 3747ms X *B. atriplicifolia* cross) exhibited enlarged semi-sprangled roots in the first cycle of selection. This population appears to be approaching near-sugar beet type roots faster than the other crosses. Crosses made in 1994 have advanced through two cycles of random mating and will be ready for root-shape selection in 1997.

These efforts, although long term, are beginning to bear fruit. In some crosses, progress is slow and discouraging. Patience and perseverance are required. Utilizing the wild subspecies *B. vulgaris* subsp. *maritima* as the source of wild germplasm, progress is possible. Because of the broad geographic distribution of *B. vulgaris* subsp. *maritima*, many diversified genes are found within subpopulations of this taxon. It is, therefore, a very desirable source of non-sugar beet growth genes for broadening the genetic base of sugar beet. This is one of the first demonstrations of transferring genetic variation for growth from wild relatives into domestic germplasm.

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Posters

Evaluation of *Beta* germplasm for disease resistance and stress tolerance

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Screening *Beta* germplasm for resistance to several diseases and for tolerance of drought stress will be undertaken with funds provided jointly by the EU and the UK Sugar Beet Research and Education Fund. Approximately 500 accessions of *Beta* section *Beta* and hard-seeded *Corollinae* and *Procumbentes* species will be evaluated over the period 1996-99. Resistance to the soil-borne seed diseases caused by *Pythium ultimum* and *Aphanomyces cochlioides* will be separately evaluated in controlled environment tests using partially sterilized soil mixed with artificial inoculum of each pathogen. Resistance to Beet Yellow's closterovirus (BYV) and beet mild yellowing luteovirus (BMV) will be separately evaluated in glasshouse tests by inoculating plants with aphids carrying known virus strains, and testing by ELISA. Powdery mildew (*Erysiphe betae*) resistance will be assessed in specially designed 'disease nurseries', using rows of a susceptible sugar beet variety to spread the disease. Drought and salinity stress tolerance will be evaluated by determining the ability of leaves to continue physiological functions when subjected to osmotic stress. Leaf discs from glasshouse-grown plants will be exposed to mannitol solutions and their photosynthetic efficiency (Fv/Fp) measured using a Plant Efficiency Analyser.

Influence of sugar beet breeding on a wild beet population in Italy

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There is a potential risk of genetically modified (transgenic) plants transferring genes via pollen to wild relatives of the commercial varieties. This possibility is the basis of many of the scenarios concerning the potential risk of increased invasiveness of inadvertently formed transgenic wild relatives. This study is prompted by the pending commercialization of a transgenic sugar beet developed to provide resistance to the soil-borne virus disease, rhizomania. There is a high probability that transgenic varieties may influence wild beets in the seed-production area of northern Italy. For this reason a survey of the local wild beet population was conducted in 1994/95, before transgenic beets and their offspring could become established.

Wild beets (*Beta vulgaris* subsp. *maritima* (L.) Arcang.) were found at locations as a part of natural vegetation (*Cakiletea maritimae* association *Atriplicetum tatarici*) between Trieste and Cesenatico. In root probes of 60 representative plants no virus infestation was found, indicating that the introgression of transgenic virus resistance would not increase the fitness of the wild beets in this area.

Greenhouse studies on morphology and life-cycle attributes demonstrated actual gene flow between conventional seed beet and the examined wild beet population.

Resistance to blackleg of beet: adaptive potential of *Beta* L. genetic resources

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More than 80 fungi cause blackleg of beet, and they differ in their degree and rate of activity. In the course of this research, seven major species of pathogens have been identified and found to have differing frequencies of occurrence under different conditions (Table 1). For example, *Pythium debaryanum* has been found to possess high virulence.

Resistance to blackleg of beet is controlled by several genes. No beet varieties have been found to be immune to the disease. The ability to resist the disease largely depends on the plants' vitality and is characterized by differing degrees of tolerance. The percentage of beet accessions differing in their response to the causative pathogen is comparatively low.

Testing for resistance in 263 beet accessions has been carried out in two stages: (1) field evaluation, and (2) artificial infection (involving both individual species of the pathogen and complex inoculum). The percentages of relatively resistant accessions were 5 forage beet, 7 table beet and 9 sugar beet accessions (Table 2).

The percentage of resistance to blackleg of beet in accessions belonging to 8 variety types of garden beets, 8 of fodder beets and 2 of sugar beets was different. After artificial inoculation, relative resistance was shown by garden beet accessions of the Zelonolistnaya type by the following indices. Average point of affection (APA) was 2.13 and extent of disease spreading (EDS) was 70.8%. Resistant accessions of fodder beets belonged to the Red Otofte variety type (APA 2.1, EDS 79%). Inoculation of relatively resistant sugar beet accessions yielded 2.14 APA and an EDS of 74.6%.

Reaction of beet accessions to individual pathogen populations did not vary distinctly. Accessions that showed relative resistance after artificial inoculation maintained their resistance under exposure to a different population of the pathogen. Hybrid plants were less susceptible to the disease. Blackleg of beet development after artificial inoculation was 64 and 67% for initial diploid and tetraploid forms and 60% for triploid hybrids, while the APA was 1.8, 2.0 and 1.67, respectively. Hybrid beets are characterized by a rapid growth rate at the initial stages: 100-seed weight was 25.5 g for triploids, 21.6 g and 20.1 g for diploids and tetraploids, respectively.

Resistant accessions of beet differed from the susceptible ones by the number of root hairs. Seedlings of disease-resistant accessions had, as a rule, a larger number of root hairs, which accelerated plant growth and development. Also, the formation of adventitious roots has been noted in the seedlings as a response to the infection by the beet blackleg pathogen.

The 11S-globulin sugar beet seed storage protein has shown the highest percentage of polymorphic electrophoretic patterns in early ripening accessions (19.2 spectra types) and a lesser degree of polymorphism in late-ripening ones (14.5 spectra types). The percentage of major spectra types occurring in early ripening accessions was 45.8 versus 9.5 in late-ripening ones. Leaves of the disease-resistant accessions transpired considerably less moisture during the day than susceptible ones. This fact underlines the impression that resistant types are generally more vigorous.

Table 1. Pathogens causing blackleg of beet that have been identified in the microflora at different research sites

Pushkin Laboratories of VIR (Leningrad Region) 1992-94	Maikop Experiment Station of VIR (Maikop town, Krasnodar Territory) 1994	Samarkand Region (Ishitikhan town, Uzbekistan), 1993
1. <i>Pythium debaryanum</i>	1. <i>Fusarium</i>	1. <i>Fusarium</i>
2. <i>Phoma betae</i>	a) <i>F. oxysporum</i>	a) <i>F. oxysporum</i>
3. <i>Fusarium</i>	b) <i>F. solani</i>	b) <i>F. solani</i>
a) <i>F. oxysporum</i>	c) <i>F. culmorum</i>	c) <i>F. culmorum</i>
b) <i>F. culmorum</i>	2. <i>Phoma betae</i>	
c) <i>F. solani</i>		
4. <i>Aphanomyces cochliodes</i>		
5. <i>Rhizoctonia solani</i>		

Table 2. Beet accessions divided by end use and grouped by degree of resistance to blackleg of beet (Pushkin Laboratories of VIR, 1992-94)

Degree of resistance	No. of accessions	Naturally infested fields			No. of accessions	Artificial inoculation		
		APA [†]	DDD [‡]	EDS [§]		APA	DDD	EDS
Garden Beet	106							
I	18	0.70	33.0	17.3	—	—	—	—
II	48	1.34	59.2	34.6	4	1.66	63.2	41.3
III	34	2.01	77.6	49.0	32	2.26	76.7	53.9
IV	6	2.66	82.9	66.5	31	2.76	85.3	68.3
V	—	—	—	—	4	3.27	92.8	81.7
Fodder Beet	124							
I	24	0.73	40.9	18.2	—	—	—	—
II	51	1.24	58.3	31.4	2	2.68	74.3	42.5
III	35	2.00	80.0	50.0	32	2.06	77.9	51.7
IV	13	2.86	89.4	71.3	34	2.79	90.3	70.0
V	1	3.29	90.1	82.4	6	3.50	95.9	87.3
Sugar Beet	142							
I	28	0.51	26.2	12.9	—	—	—	—
II	59	1.54	58.6	32.1	9	1.57	67.1	41.5
III	39	2.12	83.1	57.2	37	2.07	77.1	51.7
IV	16	2.69	85.5	67.1	40	2.82	86.6	70.5
V	—	—	—	—	3	3.39	94.9	84.7

[†] APA - Average Point of Affection.[‡] DDD- Degree of Disease Development.[§] EDS - Extent of Disease Spreading.**Table 3.** Rate of occurrence of major spectra types in sugar beet accessions differing by time of ripening

Accessions	No. of major	No. of common	Percentage of occurrence
Early ripening	16.3	19.2	45.8
Medium ripening	10.0	19.5	42.0
Late ripening	3.5	14.5	9.5

In summary, plant genetic resources of the genus *Beta* L. contain certain resistances, within the range of 5-9%, to blackleg of beet, the disease affecting beet seedlings. F₁ hybrids characterized by early ripening and rapid growth rate at the initial stages manifested a lesser degree of susceptibility to the disease. As a rule, early ripening beet accessions possessed a higher degree of polymorphism than the late-ripening ones. The former developed more root hairs than the late-ripening accessions, and their leaves had a better water-retaining capacity, thus enhancing their resistance to the disease.

Genetic diversity of fodder beets

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Fodder beet (*Beta vulgaris* subsp. *vulgaris* Fodder Beet) presents the greatest genetic diversity among cultivated forms of beets. The diversity mainly concerns the morphological traits of roots and agricultural values (root shape and position in soil, yield and dry matter content). Fodder beet varieties are divided into four basic groups by shape: (1) taproot (Mammoth type), (2) cylindrical (Eckendorfer type), (3) oval (Barres type), and (4) conical (Fig. 1). Additionally, intermediate forms can be found as a result of crossings among these groups. In these groups, roots with white, yellow, orange and red skin are present. Variation in root yield, leaf yield and dry matter content was found in fodder beet varieties and populations evaluated in our collection (Fig. 2). There are great differences in root yield and dry matter content, and the results confirm the well-known negative correlation between these two features. As with sugar beets, the main breeding aim in fodder beets is the development of highly productive monogerm hybrid varieties adapted to the new cultivation technologies. Only the Owen's CMS from sugar beet is used in hybrid production. Utilization of this source has led to the narrowing in diversity of root size and dry matter content. The roots of hybrids are mainly conical, especially the lower part. The dominance of this shape type above all others is clear, and it is in agreement with the previously obtained results describing inheritance patterns. The dry matter content ranges from medium to high. Hybrids with low dry matter content and high root yield have not yet been obtained.

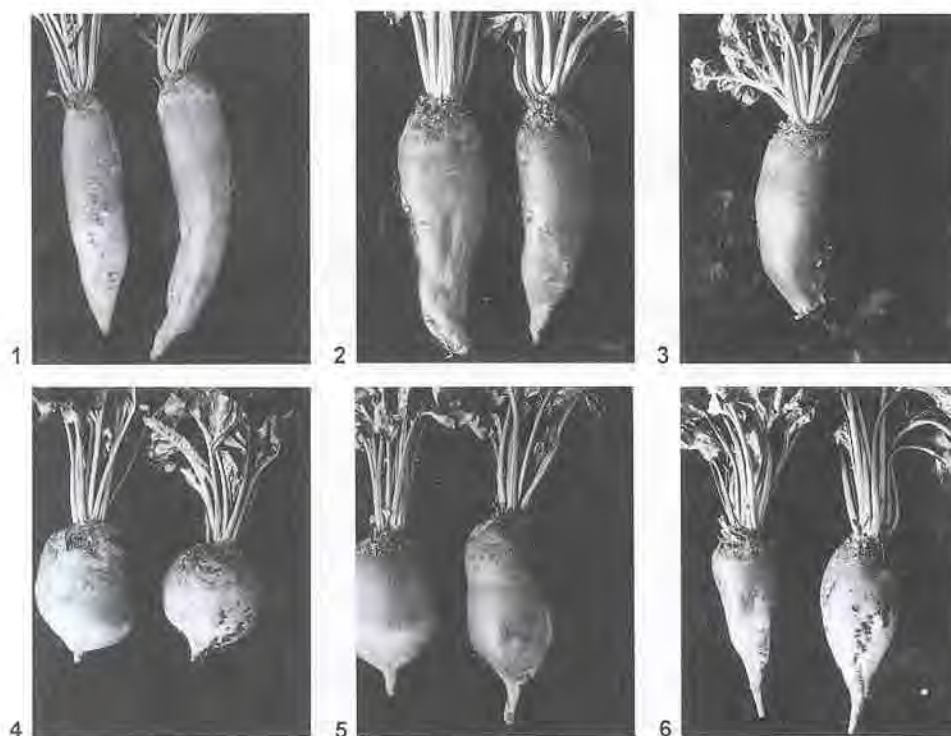
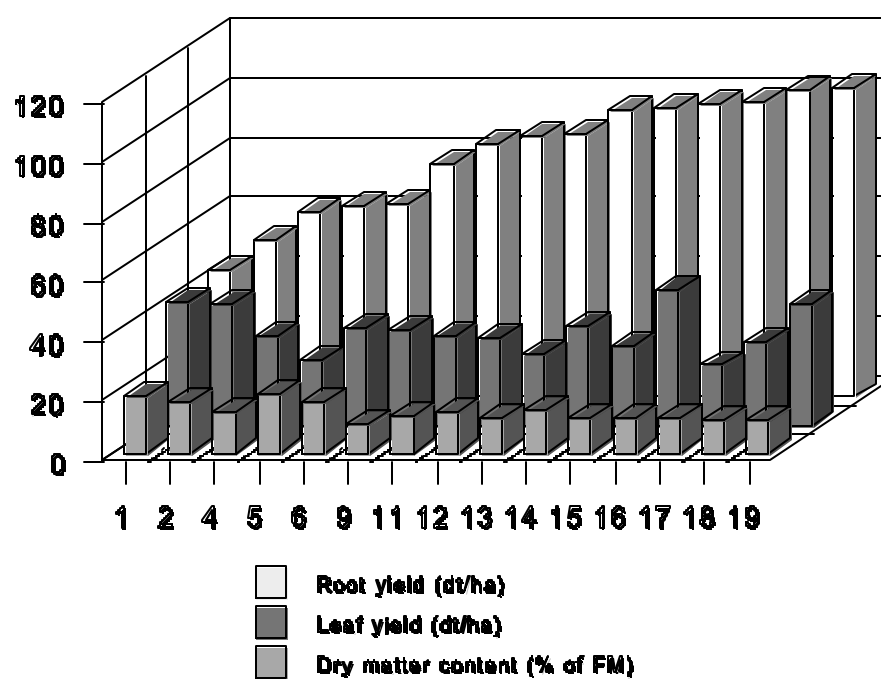


Fig. 1. Beet shape: 1, 2 = taproot; 3 = oval; 4 = circular; 5 = cylindrical; 6 = conical.



No.	Accession no.	No.	Accession no.
1	88217	13	88225
2	88250	14	88239
4	88201	15	88242
5	88204	16	88246
6	88202	17	88232
9	88269	18	88248
11	88205	19	88244
12	88205		

Fig. 2. Yield characteristics of some Polish Fodder Beet accessions.

Towards a French network for beet genetic resources

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In France, the management of beet genetic resources is undertaken by a cooperative network of different partners from state organizations (Ministry of Agriculture, BRG, CTPS, GEVES, INRA, University of Lille), from professional organizations (GNIS, ITB, FNAMS), and from private breeding companies. The network members agree on a common pool, on maintaining, evaluating and distributing the genetic resources. The different partners, together in a Piloting Board, are managing the network (giving the main directions), assisted by a Coordination Section (in charge of activities and technical organization).

The French Beet Network is defined in relation to (as a part of) the existing World *Beta* Network. The material, which is introduced into the collection (National Collection) as a priority, is that for which France will assume responsibility. The following material will be considered as part of the National Collection:

- the French cultivars arising from the French catalogue (A and B lists)
- the populations and the old cultivars with a French origin (e.g. diploid populations stored in INRA)
- the wild material arising from explorations on French territory
- the material well known for the presence of identified genes
- the material arising from the dynamic management programme, associating wild and cultivated beets (programme initiated in IIRB).

By the use of efficient criteria to evaluate the variability, we will try to limit redundancy and material losses, taking into account the limited size of the national collection.

The Piloting Board will decide on the new entries and exchange of the national collection every year. The management of the National Collection is governed by maintenance and preservation rules. The cultivated material is stored with specific and defined conditions for storage and renewal. The wild material, as a principle, is preserved *in situ*. For evaluation and distribution, the Piloting Board will decide on supplying seed from the areas of origin. Every year the evaluation programme will be decided by the Piloting Board in conjunction with the WBN. The Coordination Section will be in charge of database enrichment (with IDDB as the model).

For most of the material in the collection, seed distribution is totally free for the network partners. This also applies to non-members as long as the reciprocity principle of exchanges is respected (an invoice could, however, be issued, even for reciprocal requests – the Piloting Board will decide). Access to the material arising from the dynamic management programme is restricted to its working members. By decision of the working members, new members also could have access to this material.

Variation patterns in Swiss chards

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Swiss chards probably were the first cultivated beets selected from *Beta vulgaris* subsp. *maritima* in the Near East. This germplasm was later introduced to Europe, North Africa, China, India and other countries. Continued selection by farmers gave rise to a great diversity of leaf and petiole shapes, colour types, daylength reactions and vernalization requirements. Swiss chards are held in many collections today. However, an internationally accepted classification system capable of reliably transmitting descriptive information on Swiss chards does not exist. Classical diagnostic keys for Swiss chards were published by Krasochkin (1959) and Helm (1957). The latter titled his paper 'Attempt of a morphological-systematic grouping of the species *Beta vulgaris*' which already indicates some classification difficulties.

Krasochkin (1959) and later Burenin (1986) developed a classification system partly based on the geographic origin of the material and defined taxa like *B. cicla* subsp. *cretica*. Helm (1957) divided *B. vulgaris* subsp. *vulgaris* into *convar. vulgaris* (Swiss chards) and further into *provar. vulgaris* (leaf use) and *provar. flavescens* (petiole use). In addition, within each provariety a number of formae were distinguished. Owing to a continuous variation, it proved to be difficult to clearly delineate *provar. vulgaris* from *provar. flavescens* (Frese 1991).

In a field experiment, 74 accessions from various parts of Europe were grown and described by 17 morphological and agronomic characters. The data set was subjected to single linkage cluster and principal component analysis. The first two principal components absorbed only 62.4% of the total variation, indicating a moderate separation of the accessions. Where clear separation of taxa is difficult, classical determination keys will remain of limited use. Therefore, it is suggested to develop a descriptive database that can store, retrieve and disseminate information on germplasm in a more flexible and efficient way than a classification system based on botanical names.

Screening of leaf beet germplasm has yielded some interesting results. Accessions held at Nyon (Switzerland) seem to contain BNYVV resistance (field test done by Dr E. Biancardi, Italy); Chinese Swiss chards (testing done by Dr E. De Ambrogio, Italy) showed excellent tolerance to *Cercospora beticola*; male sterility occurs in Turkish accessions of Swiss chard; Krasochkin (1959) reported tolerance to *Peronospora* in Swiss chards. These four examples show that Swiss chards are an interesting source of inherited traits useful for sugar beet enhancement programmes, and deserve more attention not only as a neglected vegetable crop but also as an underutilized source of genetic variation. A better understanding of the structure of diversity of this crop is a first step towards its purposeful conservation, evaluation and use.

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Evaluation of some Egyptian wild types of beet (*Beta vulgaris* subsp. *maritima*)

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Abstract

Twenty-six wild types of beet (*Beta vulgaris* subsp. *maritima*) were collected in May/June 1992 from different districts representing the northwestern coast, the delta of Egypt and Upper Egypt. Agronomic observations were taken in 1992/93, 1993/94 and 1994/95 in the Western Nubaria area and in Alexandria, Egypt, and in 1995 in Fargo, North Dakota, USA. The goal was to evaluate the 26 accessions for agronomic traits: germination, leaf colour, growth habit, leaves, roots, bolting, seed-setting and seed-ripening. Differences in these characteristics were found among these accessions in both Egypt and the USA. The 26 accessions of wild beet also were evaluated at six locations in the USA by different evaluators for resistance to curly top, *Rhizoctonia*, leaf spot, cyst nematode, root aphid, rhizomania, virus yellows, root maggot and *Aphanomyces*. Considerable differences were recorded among the 26 accessions in their resistance to the different diseases. The objective of this study was to evaluate the usefulness of these wild types of beet (*B. vulgaris* subsp. *maritima*) in sugar beet (*B. vulgaris* subsp. *vulgaris* L.) breeding programmes in Egypt, the USA and worldwide.

Introduction

Although *B. vulgaris* subsp. *maritima* is considered the most important wild species for the improvement of sugar beet, other wild relatives should be collected, evaluated and utilized. Hijner (1952), Winslow (1954) and Golden (1959) found that three *Procumbentes* species, i.e. *Beta patellaris* Mog., *Beta procumbenes* Chr.Sm. and *Beta webbiana* Mog., were resistant to the cyst nematode, *Heterodera schachtii* Schm., which is the most important nematode pest of sugar beet (Steele 1984). Some success in developing resistance to *H. schachtii* has been achieved through the introgression of *B. patellaris* genes into the sugar beet genome through interspecific hybridization and repeated backcrosses (Savitzky 1975; Speckmann and De Bock 1982; Heibroek *et al.* 1983; Löptien 1984).

Collecting of the wild relatives of beet began many years back. The first US efforts were by George H. Coons of the USDA-ARS in 1925 (Coons 1975). He collected seed of *B. vulgaris* subsp. *maritima* along the coasts of southwestern France, the southeastern coast of England, and the coast of Italy near the mouth of the Po River. Another collecting expedition of his in 1935 went to England, France, Spain, Portugal, the Madeira Islands, the Canary Islands, Italy, Greece and Turkey.

Wild relatives and landraces of *Beta* are found in China, India, Central and European Asia; along the Mediterranean Coasts of Europe and Africa, and the European shores of the Atlantic Ocean. The first historical documentation of *Beta* germplasm collecting was by Munerati (Biancardi and Biaggi 1979). In Egypt, sugar beet production and breeding research began less than 15 years ago. Collecting of wild beet (*B. vulgaris* subsp. *maritima*) from Egypt was jointly initiated by M.A. El Manhaly (Egypt) and D.L. Doney (USA) in 1990 and the first

collecting was done by El Manhaly, Badawy and Doney in 1992. Twenty-six accessions were collected at that time.

The objective of this study was to evaluate the 26 accessions for utilization in sugar beet breeding programmes in Egypt, the USA and worldwide.

Materials and methods

Twenty-six accessions of wild beet (*B. vulgaris* subsp. *maritima*) were collected in May/June 1992 from three different districts in Egypt: (1) the northwestern coast, (2) the delta of the Nile, and (3) Upper Egypt. The seeds were divided between Sugar Crops Research Institute, Giza, Egypt and USDA Agricultural Research Service as cooperative research partners. Passport data for the 26 accessions are given in Table 1.

Evaluation of agronomic traits

Egypt

In November 1992, each of the 26 accessions was planted in one 10.5-m² plot at the Nubaria Agricultural Research Station (NARS). Seed was sown on 14 November 1992. Counts were taken 3 weeks later for percentage germination. Observations on the other traits, such as growth habit, leaf erectness, leaf blade pigmentation, hypocotyl colour, bolting percentage and dates, seed-setting date, male sterility and germs/ball were made in January, February and March 1993. In November 1993, 26 pots of 50-cm diameter were used to study the flowering at Alexandria. Observations were made on the same date as for the 1992 study. At the end of December 1994, the previous experiment was repeated to study the effect of planting date on the flowering of wild types of beet.

USA

The 26 accessions were planted in Fargo, North Dakota on 18 May 1995. The following were recorded: growth habit, leaf erectness, leaf blade pigmentation, leaf hairiness, leaf thickness, petiole length (mm), petiole width (mm), leaf blade length (mm), leaf blade width (mm), petiole colour, hypocotyl pigmentation, external root colour, main colour flesh, root shape, flower stem pigmentation, male sterility, multigerm, and bolting tendency (% on given dates). Most observations were made in late July. The percent of plants bolting was measured on 22 June and 20 July.

Evaluation for diseases and insect resistance in the USA

With the exception of WB 1001, 1002, 1006, 1007 and 1015, the accessions were evaluated for disease resistance. Table 2 lists the evaluators, locations and diseases for which they were evaluated.

Results and discussion

Evaluation for agronomic traits

Egypt

Table 3 list the values for the agronomic traits of the 26 accessions evaluated in Egypt. Percentage germination differed among the accessions. The highest

germination rates were for the Upper Egypt *maritima*, which ranged between 88 and 94%. Four accessions from the Delta (WB 1008, 1010, 1011 and 1012) ranged

Table 1. Egyptian collection of *Beta vulgaris* subsp. *maritima*, 1992. See key below for column definitions

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
WB1001	05/15	Matruh	Matruh	Village 15	0.6W	31.1N	27.0E	20	BK	200	2	2	6	Collected by Manhaly
WB1002	04/20	Behira	Damanhur	Village 15	1.0E	30.8N	29.4E	30	BK	9000	2	2	2	Collected by Manhaly
WB1003	05/28	Noubaria			0.50NW	30.7N	29.3E	25	BK	5000	2	2	2	Collected by Manhaly, believed to have come in water from Delta
WB1001	06/22	Noubaria			0.6W	30.7N	29.4E	25	SP	6000	2	2	2	On ditch bank, believe came in water from Deha
WB1005	06/23	Matruh	Al Metane		4.ON	31.5N	26.5E	20	SP	500	2	6	6	Around 5-yr-old greenhouse
WB1006	06/24	Dabah	Dabah		1.OE	31.1N	27.7E	25	SP, BK	25	1	2	2	95 km east of Matruh, around greenhouse
WB1007	06/24	El Hamem	El Omiad		1.OS	30.8N	29.0E	30	SP	10	2	3	3	3 km south of Hotel Adia, between greenhouses
WB1008	06/25	Alexandria	Village 2		area	31.0N	30.2E	25	SP, BK	50	1	12	12	Along ditch by garbage dump
WB1009	06/25	Alexandria	Village 1		area	31.0N	30.2E	25	SP, BK	200	2	12	12	Along ditch bank, many plants, dry
WB1010	06/25	Alexandria	Village 3		area	31.0N	30.2E	25	SP, BK	500	2	12	12	Along canal bank
WB1011	06/25	Alexandria	Village 4		main	31.0N	30.2E	25	SP, BK	5000	2	12	12	A few groups along canal
WB1012	06/25	Alexandria	Village 7		one	31.0N	30.2E	25	SP, BK	5000	2	12	12	Along canal, 1 green with red seed, & prostrate
WB1013	06/26	Alexandria	Village 10		area	31.0N	30.2E	15	SP, BK	5000	2	1	1	Very large field, seg stem, seed & growth habit
WB1014	06/26	Alexandria	Village 7		area	31.0N	30.2E	15	BK, SP	5000	2	1	1	Scattered along bank of ditch
WB1015	06/27	KafrAsh	Shaykh		3KW	31.1N	30.9E	15	BK	5000	2	12	12	Large group planted for comparison with SB
WB1016	06/27	Kafr Ash	Shaykh		1KW	31.1N	30.9E	15	BK	5000	2	12	12	Many small dry plants along ditch
WB1017	06/7	Bela	El Owywa		1KNW	31.1N	31.2E	15	BK, SP	5000	2	12	12	Scattered along bank of ditch
WB1018	06/7	Dumyat	Kafr Saad			31.3N	31.5E	15	BK, SP	500	2	12	12	Around greenhouses, very dry
WB1019	06/28	Port Said	Ext. Farm		area	31.1N	32.2E	15	BR	500	2	3	3	Plants scattered around greenhouses
WB1020	06/28	Port Said			31KS	30.8N	32.2E	15	BK	5000	2	3	3	Port Said Agric. Project, around greenhouses
WB1021	06/29	Fayum	Abo Khalaf		Farm	29.2N	30.9E	25	SP	500	2	2	2	Along canal bank
WB1022	06/29	Fayum	Harfosh		Farm	29.2N	30.9E	25	BK	5000	2	2	2	Small field planted by farmer for green veg.
WB1023	06/29	Baru Suwaf	El Azhary			29.0N	31.0E	25	SP	25	1	2	2	Plants by bridge, some coll. by family nearby
WB1024	07/01	Luxor	El Awania		Nile	25.3N	32.7E	82	BK	1000	12	12	12	Col. by farmer in May to plant for green veg.
WB1025	07/01	Luxor	El gamal			25.1N	32.6E	82	SP, BK	5000	12	12	12	Along Nile, neighbour children brought some plants
WB1026	07/01	Luxor	El Odysat			25.0N	32.5E	82	BK		12	12	12	Annant, coll. by farmer to sell at market
1	text 6	Col. Type	Name/description	6	text 5	Distance in km from the nearest city or town	11	numeric	Sampling area in square meters	12	numeric	Plants sampled - number	13	numeric
2	text 5	Wb number	Date of collecting	7	text 6	Latitude (N or S)	12	numeric	Uniformity (1=uniform, 2=segregating, 3=mixed)	13	numeric	Soil texture (1=clay, 2=silt, 4=fine sand, 6=coarse sand, 7=fine sand)	14	text 48
3	text 3	Subspecies name	Government (country)	8	text 6	Longitude (E or W)	15	text	Notes					
4	text 10	Nearest town or city name		9	numeric	Altitude in meters								
5	text 10			10	text 6	Sampling method (SP=single plant or BK=bulk)								

Table 2. List of evaluators, locations and descriptors evaluated

Evaluator	Location	Descriptor
A. Anderson	Fargo, ND	Root maggot
BSDF (T. Brown)	Twin Falls, ID	Curly top virus
C. Rush	Bushland, TX	<i>Aphanomyces</i>
D. Doney	Fargo, ND	Agronomic
E. Ruppel	Fort Collins, CO	<i>Rhizoctonia</i>
E. Ruppel	Fort Collins, CO	<i>Cercospora</i>
J. Michels	Bushland, TX	Root aphids
R. Lewellen	Salinas, CA	BWYV, rhizomania
S. Hafez	Parma, ID	Nematode

between 80 and 90% in germination. The germination of the remaining accessions was less than 80%. No observations were taken in 1995 owing to poor germination in the field.

Bolting: The Delta '*maritima*' flowered during the last 10 days of January, while the Fayum, Bani suwaf and Luxor types flowered 1 month later. Seed-setting occurred in February and March, respectively, for the groups.

Growth habit: With the exceptions of WB 1004 and 1013, the Delta '*maritima*' were all prostrate. WB 1004 was erect and prostrate and WB 1013 was procumbent and erect. The Fayum, Bani suwaf and Luxor types were erect to procumbent.

Hypocotyl pigmentation: Pink to red in all accessions.

Male sterility: None of the accessions was male sterile.

Germ: All accessions were multigerm and number of germs/ball ranged between two and four.

Leaf blade pigmentation: Light green to green in all types.

The observations of these traits in the second season (1994) did not differ from these.

USA

Table 4 list the values for the agronomic traits of the 26 accessions evaluated in the USA.

Bolting: Delta '*maritima*' types began bolting very early, some as early as 14 June and by 19 July most had set seed and discontinued pollinating. The Fayum and Luxor '*maritima*' began bolting later and by 20 July were just beginning to flower and pollinate.

Leaf colour: The Fayum and Luxor '*maritima*' were light green to green with no vein pigmentation. The Delta '*maritima*' were dark green. Most had a red central

vein on each leaf. The accession from Matruh (WB 1005) appeared to have darker red and more red pigmentation (not segregating for green petiole).

Growth habit: The Delta '*maritima*' were all prostrate except WB 1013, which came from a large field of wild beet near Alexandria, and was like the Fayum and Luxor accessions in all characteristics. The Fayum and Luxor '*maritima*' were erect to procumbent.

Leaves: The Delta '*maritima*' had very small leaves except as mentioned in 'Growth habit' above but the Matruh accession (WB 1005) had rounder leaves than the rest.

Roots: The Delta '*maritima*' were small in diameter and very 'sprangled'. The Fayum and Luxor '*maritima*' were sprangled but slightly swollen and larger in diameter than the Delta accessions. One accession from Luxor and one from Fayum contained some red beets that appeared to have been physically mixed.

Summary

There are basically two groups: Delta (North Atlantic type prostrate, small thick leaves, no leaf hairs) and the Luxor/Fayum (segregating for leaf size and shape, growth habit, root swelling, bolting and red types). The Luxor and Fayum accessions may have been leaf type crosses or mixed with Delta types in earlier generations (especially the two accessions with red (garden) types). The Luxor accessions have a higher frequency of procumbent-smaller leaf types than the Fayum accessions. An unusual mutant was noted in one plant of accession WB 1008. Part of the plant was normal and part had very deformed flowers and lighter foliage. The deformed flowers appeared to have reduced or extremely small aborted anthers and the ovaries were closed into a tube.

Evaluation for disease and pest resistance

Table 5 presents the evaluation data for resistance to curly top, *Rhizoctonia*, *Cercospora* leaf spot, sugar beet cyst nematode, root aphid, rhizomania, virus yellows, root maggot and *Aphanomyces*. The following reports of the evaluators summarize the behaviour of the Egyptian *B. vulgaris* subsp. *maritima* concerning disease resistance.

Key for Table 3:

- 1 Num = number of plants/plot
- 2 Grow H = growth habit: 1 = erect; 2 = erect and procumbent; 3 = procumbent; 4 = erect and prostrate; 5 = prostrate.
- 3 Leaf E = leaf erectness: 1 = prostrate; 2 = procumbent; 3 = erect.
- 4 LPig = Leaf blade pigmentation: 1 = light green; 2 = green; 3 = green red mixture; 4 = red.
- 5 Hyc = hypocotyl pigmentation: 1 = green; 2 = pink; 3 = red; 4 = mixed.
- 6 Bolt% = bolting percent and date.
- 7 Seed S% = seed-setting date.
- 8 Seed R Date = seed-ripening date.
- 9 MS = male sterility: 1 = fertile; 2 = semisterile; 3 = sterile.
- 10 Germ no/ball = number of germs/ball.

Table 3. Evaluation of agronomic traits in Egypt, 1993

WB number	Code 1 Num	2 Grow H	3 Leaf E	4 LPig	5 Hyc				
WB 1001	57	5	6	2	2				
WB 1002	48	5	8	2	3				
WB 1003	62	5	2	2	2				
WB 1004	49	4	3	2	2				
WB 1005	52	5	8	2	2				
WB 1006	37	5	2	2	3				
WB 1007	75	5	7	2	3				
WB 1008	80	5	9	2	3				
WB 1009	78	5	2	2	2				
WB 1010	88	5	2	2	3				
WB 1011	90	5	1	2	2				
WB 1012	82	5	1	2	3				
WB 1013	67	3 & 4	8	2	2				
WB 1014	32	5	6	2	2				
WB 1015	27	5	7	2	2				
WB 1016	35	5	9	2	3				
WB 1017	61	5	2	2	3				
WB 1018	75	5	8	2	3				
WB 1019	78	5	3	2	3				
WB 1020	75	5	2	2	3				
WB 1021	68	1 & 2	3 & 5	2	3				
WB 1022	72	1 & 2	2	1 & 2	3				
WB 1023	90	2	8	2	3				
WB 1024	92	2	7	2	3				
WB 1025	88	1 & 2	2	1 & 2	3				
WB 1026	94	1 & 2	9	1 & 2	3				
	Code 6 Bolt%	7 Date	8 Seed S%	9 Seed R Date	10 MS	11 Germ no/ball			
WB 1001	100	22.1	20.2	15.4	1	3 & 4			
WB 1002	100	22.1	20.2	15.4	1	3			
WB 1003	100	22.1	20.2	15.4	1	3			
WB 1004	100	18.1	20.2	15.4	1	4			
WB 1005	100	28.1	20.2	15.4	1	2 & 3			
WB 1006	100	30.1	25.2	15.4	1	2 & 3			
WB 1007	100	30.1	25.2	15.4	1	3			
WB 1008	100	31.1	30.2	25.4	1	4			
WB 1009	100	28.1	30.2	25.4	1	2 & 3			
WB 1010	100	27.1	20.2	20.4	1	3 & 4			
WB 1011	90	26.2	25.2	25.4	1	2 & 3			
WB 1012	100	28.1	20.2	20.4	1	3			
WB 1013	100	30.1	20.2	20.4	1	4			
WB 1014	70	30.1	20.2	20.3	1	3 & 4			
WB 1015	100	30.1	25.2	20.3	1	3			
WB 1016	100	30.1	25.2	20.3	1	2 & 3			
WB 1017	100	30.1	25.2	20.3	1	3			
WB 1018	100	20.1	28.2	25.3	1	3			
WB 1019	100	20.1	20.2	25.3	1	4			
WB 1020	100	20.1	20.2	25.3	1	3 & 4			
WB 1021	100	25.1	20.3	10.4	1	3 & 4			
WB 1022	100	26.2	20.3	10.4	1	2			
WB 1023	100	24.2	25.3	25.4	1	3			
WB 1024	100	28.2	20.3	20.4	1	3			
WB 1025	100	1.3	25.3	20.4	1	2 & 3			
WB 1026	100	28.2	20.3	20.4	1	2 & 3			

Table 4. Agronomic data for the Egyptian collection of *Beta vulgaris* subsp. *maritima* taken at Fargo, North Dakota USA in 1995

			Leaf E Leaf			Lf	Pet L	Pet W	Blade L	Blade W
WB 1001	562579	5	1	2	0	6	40	5-7	85-100	50-60
WB 1002	562580	5	1	2	0	6	40-70	5-8	80-95	50-70
WB 1003	562581	5	1	2	0	6	35-65	5-7	75-100	50-65
WB 1004	562583	5	1	2	0	6	30-75	5-7	80-110	55-75
WB 1005	562583	5	1	2	0	6	35-50	5-7	65-110	55-75
WB 1006	562583	5	1	2	0	6	30-50	5-7	55-90	35-60
WB 1007	562585	5	1	2	0					
W8 1008	562586	5	1	2	0	6	35-60	5-8	65-105	40-80
WB 1009	562587	5, few 1	1, few	2	0	6	35-60	6-7	75-105	60-75
WB 1010	562588	5	1	2	0	6	35-90	6-7	75-125	55-80
WB 1011	562589	5	1	2	0	6	35-50	5-7	80-90	50-70
WB 1012	562590	5	1	2	0	6	35-50	5-8	75-95	50-80
WB 1013	562591	1, few 3	8	1,	0	4	90-260	7-14	140-270	120-180
WB 1014	562592	5	1	2	0	6	35-60	6-7	85-105	60-75
WB 1015	562593	5	1	2	0	6	35-50	5-6	65-100	45-60
WB 1016	562594	5	1	2	0	6	30-60	5-8	85-100	60-65
WB 1017	562595	5	1	2	0	6	40-70	5-7	80-95	60-70
WB 1018	562596	5	1	2	0	6	35-60	5-8	85-95	60-75
WB 1019	562597	5	1	2	0	5 & 6	45-80	5-7	85-120	65-80
WB 1020	562598	5	1	2	0	6	40-70	5-7	85-95	55-70
WB 1021	562599	1, few 3	8 & 6	1 & 2	0	4	65-250	5-14	100-290	50-80
WB 1022	562600	1, few 3	8 & 6	1 & 2	0	4	70-310	7-15	120-260	70-160
WB 1023	562601	2	8 & 6	1 & 2	0	4	60-290	7-15	110-290	80-160
WB 1024	562602	2	8 & 6	1,	0	4	75-160	5-9	75-190	35-115
WB 1025	562603	1 & 2	6 & 8	1,	0	4	45-215	5-13	85-220	55-120
WB 1026	562604	1 & 2	6 & 8	1 & 2	0	4	30-150	4-13	65-275	45-150

Rating descriptors and scales

Growth habit (Grow H): 1 = erect; 2 = erect and procumbent; 3 = procumbent; 4 = erect and prostrate; 5 = prostrate.

Leaf erectness (Leaf E): 1 = prostrate; 5 = procumbent; 9 = erect.

Leaf blade pigmentation (Leaf Pig): 1 = light green; 2 = green; 3 = green red mix; 4 = red.

Leaf hairiness (Hair): 0 = hairs absent; 3 = hairs scarce; 5 = hairy; 7 = very hairy.

Leaf thickness (Lf Thick): 3 = thin; 5 = medium; 7 = thick.

Petiole length (Pet L): (minimum - maximum in mm).

Petiole width (Pet W): (minimum - maximum in mm).

Leaf blade length (Blade L): (minimum - maximum in mm).

Leaf blade width (Blade W): (minimum - maximum in mm).

Petiole colour (Pet Col): 1 = green; 2 = pink; 3 = red; .4 = mixed; 5 = yellow.

Hypocotyl pigmentation (Hy Col): 1 = green; 2 = pink; 3 = red; .4 = mixed.

External root colour (Rt Col): 1 = white; 2 = yellow; 3 = orange; 4 = red; 5 = dark red.

Main colour of flesh (Flesh Col): 1 = white; 2 = yellow; 3 = orange; 4 = red; 5 = purple.

Root shape (Rt Shape): 1 = narrow elliptic; 2 = elliptic; 3 = circular; 4 = broad elliptic; 5 = narrow oblong; 6 = narrow triangular; 7 = non-swollen; 8 = fibrous.

Flower stem pigmentation (Stem Pig): 1 = light green; 2 = green; 3 = green red mix; 4 = red.

Male sterility (MS): 1 = fertile; 2 = semi-sterile; 3 = sterile.

Multigerm (Germ no/ball): number of germs/seedball.

Bolting tendency (% on given date) (Bolt%-Date): first date = 22 June 1995; second date = 20 July 1995.

Table 4. Continued

WB No.	PI No.	Pet Col	Hy Col	Rt Col	Flesh Col	Rt Shape	Stem Pig	MS	Germ(no/ ball)	Leaf vein	Bolt%- Date
WB 1001	562579	2	3	1	1	7	2	1	3 & 4	Rd, few Gr	100-22/6
WB 1002	562580	2	3	1	1	7	2	1	3 & 4	Gr, few Rd	100 22/6
WB 1003	562581	2	3	1	1	7	2	1	3 & 4	Green	100-22/6
WB 1004	562583	2	2 & 3	1	1	7	2	1	2 & 3	Gr, few Rd	100-22/6
WB 1005	562583	2	3	1	1	7	2	1	2 & 3	Red	100-22/6
WB 1006	562583	2	3	1	1	7	2	1	3 & 2	Gr, few Rd	1100-22/6
WB 1007	562585	2		1	1	7	2	1	3 & 4		100-22/6
WB 1008	562586	2	3	1		7	2	1	2 & 3	Gr, few Rd	100-22/6
WB 1009	562587	2	3	1	1	7	2, few	1	2 & 3	Gr, few Rd	100-22/6
WB 1010	562588	2	3	1	1	7	2	1	3	Gr, few Rd	100-22/6
WB 1011	562589	2	3	1	1	7	2	1	2 & 3	Gr, few Rd	100-22/6
WB 1012	562590	2	3	1	1	7	2	1	3 & 4	Gr, few Rd	100-22/6
WB 1013	562591	1, few 2	2	1	1	7	1, few 2	1	3, 4 & 5	Green	6-2/6 100-20/7
WB 1014	562592	2	3	1	1	7	2, few 3	1	4 & 3	Gr, few Rd	100-22/6
WB 1015	562593	2	3	1	1	7	2	1	3	Green	100-22/6
WB 1016	562594	2	3	1	1	7	2, few 3	1	3 & 2	Gr, few Rd	100-22/6
WB 1017	562595	2	3	1	1	7	2	1	3 & 2	Gr, few Rd	100-22/6
WB 1018	562596	2	3	1	1	7	2	1	3	Gr, few Rd	100 22/6
WB 1019	562597	2 & 1	3	1	1	7	2	1	3 & 4	Green	100-22/6
WB 1020	562598	2	3	1	1	7	2	1	3 & 4	Gr, few Rd	100-22/6
WB 1021	562599	1, few 2	2 & 3	1	1	7	1, few 2	1	2 - 5	Green	25-22/6 100-20/7
WB 1022	562600	1, few 2	2 & 3	1	1	7	1	1	3	Green	40-22/6 100-20/7
WB 1023	562601	1, few 2	2 & 3	1, few 4	1, few 4	7	1, few 3	1	3	Green	50-22/6 100-20/7
WB 1024	562602	1, few 3	3	1, few 4	1, few 4	7	1, few 4	1	2 & 3	Green	50-22/6 100-20/7
WB 1025	562603	1, few 2	3	1	1	7	1, few 2, 4	1	2 & 3	Green	50-22/6 100-20/7
WB 1026	562604	1, few 2	2 & 3	1	1	7	1, few 2	1	2 & 3	Green	50-22/6 100-20/7

Sugar beet root aphid – J. Michels, Texas A&M Bushland Exp. Station

“Three entries, PI 562582 (WB 1004), PI 562681 (WB 1003), and PI 562587 (WB 1009) showed no signs of aphid colonization and scored lower than the resistant check, Seedex ‘Ranger’. The presence of level 3 infestation indicates that sugar beet root aphid can colonize the roots, but the absence of any level 4 infestations indicates a possibility of some resistance. All entries in the first run except ACH05 (the susceptible check), PI 562597, and PI 562591 were free of level 4 infestations and therefore may show some signs of resistance. Any entries with level 4 infestations, even in one plant, indicates that the roots are prone to high levels of infestation and would likely be susceptible in the field. In the second run, all entries except PI 562604 and PI 562599 may exhibit some resistance to sugar beet root aphid colonization.” (J. Michels, pers. comm.).

Leaf spot - E. Ruppel, USDA-ARS, Fort Collins, CO

“Inoculations were performed on July 7 and 13, and evaluations were made on August 31, September 7, and 14; the peak of the epidemic occurred around September 14. On September 14, means of the resistant and susceptible infernal controls were 3.9 and 5.9 (scale of 0 - 9), respectively, across the nursery. In 1994 (September 2), these means were 3.3 and 4.8, respectively. Means of contributor lines on September 14 ranged from 3.8 to 7.2, compared with 3.0-5.8 in 1994. Coefficients of variation across contributor tests ranged from 5.6-12.3 on September 14, indicating the uniformity of our nursery and the evaluations. PI entries ranged from 4.75 to 9.50 with a mean of 7.29 indicating nothing significantly more than the susceptible check.” (E. G. Ruppel, pers. comm.).

***Rhizoctonia* - E. Ruppel, USDA-ARS, Fort Collins, CO**

“The hot, dry weather of late July and August provided ideal conditions for the development of an excellent disease nursery. Differences among entries in all tests were highly significant ($P = 0.0001$). Mean DIs across all tests for highly resistant FC705-1, resistant FC703, and highly susceptible FC901 controls were 1.7, 2.1 and 4.3, respectively. Percentages of healthy roots were 51.2, 36.9 and 8.0 for these controls. Percentages of roots in disease classes 0 through 3 were 96.5, 94.9 and 58.2, respectively. The highest and lowest DIs for contributor lines were 6.6 and 0.9, respectively. The DIs of PI entries ranged from 4.75 to 7.00 indicating nothing significantly more resistant than the susceptible check.” (E.G. Ruppel, pers. comm.).

Curly top - T. Brown, BSDF, Kimberly, ID

“The 1995 Curly Top Nursery was again conducted in Kimberly, ID. T. Brown continues to serve a Manager and reports directly to T. Schwartz. The official tests in the nursery this year were rated by T. Brown, Dr L. Panella and D. Traveller. It was the general consensus that the 1995 Curly Top nursery was one of the best in many years. The field was treated with good agronomic practices, i.e. fertilizing, irrigation and weed control. The differentiation among lines was excellent, ratings ranging from 2 to 9. This is the first year we have identified any accession exhibiting curly top resistance. Those showing moderate resistance are from the Fayum and Luxor regions and accession WB 1013. These are all more leaf-type accessions.” (D. Doney, pers. comm.).

Table 5. Evaluation data for collecting conducted in 1995. Curly top by T. Brown (BSDF), *Rhizoctonia* and Leaf spot by E. Ruppel (USDA), Nematode by S. Hafez (U. of Idaho), Root aphid by G. Michels (Texas A & M), Rhizomania and Virus Yellows by R. Lewellen (USDA), Root maggot by A. Anderson (North Dakota State U.) and *Aphanomyces* by C. Rush (Texas A & M)

WB code	PI No.	Curly top	<i>Rhizoctonia</i>	Leaf spot	Nematode [†]	Root aphid [†]	Root maggot	<i>Aphanomyces</i>
WB 1003	562581	9.0	6.78	9.50	869	1.00	7.0	4.8
WB 1004	562582	9.0	6.37	9.50	683	1.00	7.0	4.6
WB 1005	562583	9.0	7.00	9.50	861	1.67	7.0	4.6
WB 1008	562586	9.0	6.87	9.00	750	2.67	7.0	3.4
WB 1009	562587	9.0	6.23	9.00	799	1.00	7.0	3.6
WB 1010	562588	9.0	6.81	9.00	782	1.92	7.0	5.6
WB 1011	562589	9.0	6.91	9.00	650	2.07	7.0	5.0
WB 1012	562590	9.0	6.78	9.00	921	1.50	7.0	3.6
WB 1013	562591	5.5	5.09	5.50	713	2.73	7.0	6.6
WB 1014	562592	9.0	6.79	9.00	762	2.13	7.0	4.0
WB 1016	562594	9.0	6.91	8.00	789	2.17	7.0	4.6
WB 1017	562595	9.0	7.00	9.00	839	2.33	7.0	3.6
WB 1018	562596	9.0	6.89	9.50	798	2.33	7.0	4.2
WB 1019	562597	7.5	5.03	5.50	759	2.67	7.0	3.4
WB 1020	562598	9.0	6.78	9.00	576	2.33	7.0	4.0
WB 1021	562599	6.5	5.06	5.00	846	2.67	7.0	4.4
WB 1022	562600	6.0	5.39	5.00	784	1.27	7.0	7.6
WB 1023	562601	6.5	5.72	5.00	729	1.54	7.0	6.6
WB 1024	562602	7.0	5.82	4.75	679	2.20	7.0	4.4
WB 1025	562603	6.5	4.75	5.50	433	2.00	7.0	4.6
WB 1026	562604	6.0	6.15	5.00	740	2.47	7.0	3.8
Susc. Check		6.0	4.70	5.25	594	2.47	7.0	1.5
Res. Check		4.5	1.30	3.25	NS	1.27	3.0	
LSD 0.05		0.7	0.93	0.85		0.60		

[†] Average number of cysts in the root-soil system at harvest

* Rating based on: 1= no nymphs or adults present; 2= nymphs present, no adults present; 3= nymphs present, few adults present; 4= nymphs present, many adults present.

Note: Rhizomania and virus yellows evaluation: All lines were very easy bolting and had to be removed in mid-July before hard seed-set. They bolted so rapidly that scoring them in the field was difficult. No information on virus yellows reaction was obtained. When removed from the field, the root system was examined and all appeared to be highly susceptible to rhizomania (BNYVV).

Sugar beet Cyst Nematode - S. Hafez, Univ. of ID, Parma Exp. Station

"Twenty-one sugar beet plant introduction (PI) accessions and the susceptible genotype WS-PM-9 were grown in a greenhouse in the presence of sugar beet cyst nematode. No significant differences between the susceptible check and any of the PIs were found for the number of nematode cysts, eggs or larvae in the soil-root system at harvest." (S. Hafez, pers. comm.).

Conclusions

It could be concluded that some accessions (e.g. WB 1008, 1004 and 1009) could be used in breeding programmes for root aphid resistance. Many authors have come to the conclusion that the *B. vulgaris* subsp. *maritima* types could be use as genetic resources for some disease resistance (Doney and Whitney 1990). Further study of these 26 accessions is planned. New collections of Egyptian *B. vulgaris* subsp. *maritima* will be made this spring from unexplored areas such as: areas surrounding the Red Sea, Sinai, some oases and other locations in Upper Egypt, etc. It is possible that better sources of disease resistance might be found among them.

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Cytomorphology of diploid and tetraploid beets

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Introduction

The yield potential of polyploidy in sugar beet, especially in triploids with reference to their root yield and sucrose percentage is well known. However, the polyploid varieties of sugar beet in India are generally anisoploid mixtures having diploid, triploid and tetraploid components, and therefore the true benefits of polyploidy are not derived to their desired extent. Furthermore, tetraploids are developed in countries with different specific objectives. Thus, it becomes imperative to develop and assemble our own tetraploid populations for further use in our breeding programmes (Srivastava *et al.* 1985). To breed and establish anisoploid and triploid hybrids for Indian agroclimatic conditions, development of indigenous diploid and tetraploid lines is of utmost importance. In this direction, preliminary work was initiated with three main approaches: (1) to induce and build up new populations of tetraploids from highly adapted diploid genotypes, (2) to isolate tetraploids from well-adapted anisoploid populations, and (3) to test the efficacy of stomatal parameters for screening of ploidy status of anisoploid populations, and then correlate it with cytology. Details of the findings on these aspects are discussed in this paper.

Material and methods

In the first experiment, seeds of an elite indigenous variety LS-6 ($2n=18$) which shows good yield and quality characters along with tolerance to high temperature were germinated in pot cultures. Shoot apices at 3-4 leaf stage were treated with different concentrations of aqueous colchicine solution through cotton swab method for 18 hours. The plants were thoroughly washed after removal of the cotton. The colchicine-affected plants were screened on the basis of their thick green leaves and stunted growth and were further confirmed cytologically from leaf tip squashes (Srivastava and Ethirajan 1971). Twenty-four such autotetraploids were transplanted in the hills at Mukteshwar for flowering, seed multiplication and further study. At the time of flowering young flower buds were fixed in Carnoy's solution for 24 hours and stored in 70% alcohol. Anthers were squashed in 2% acetocarmine. Pollen fertility was determined based on the percent stainability of pollen grains. In the second experiment, 208 plants from two anisoploid populations of sugar beet, designated as 'A' and 'B', were screened for various stomatal parameters from epidermal peelings of leaves stained in potassium iodide solution, and also by the chromosome numbers of individual plants through leaf-tip squashes.

Results and discussion

In the first experiment, seedling treatment with 0.2% aq. colchicine was found to be the best for induction of tetraploidy followed by 0.3%. No autotetraploids could be induced through 0.1 and 0.5% colchicine treatment. This is in agreement with the earlier studies in different plant species (Sen and Ghosh 1965; Shambulingappa *et al.* 1987; Singh *et al.* 1978; Gupta and Sinha 1978). In general, the induced autotetraploids were vigorous and had thicker stems and leaves as

well as delayed flowering and slower initial growth when compared with the diploid controls. A detailed comparative morphological study of induced autotetraploids with diploids revealed improvement of some of the characters such as plant height, leaf size and pollen diameter (Table 1). There was an increase in size of stomata but the frequency of stomata per unit area was reduced (Table 2). There also was slight reduction in pollen and seed fertility (Tables 1 and 2). Reproductive behaviour of these tetraploids and diploids was characterized through meiotic chromosomes pairing in pollen mother cells. In the diploids, regular meiosis was observed with an occasional occurrence of univalents. However, in the tetraploids, varying number of quadrivalents (1-4/cell), trivalents (0-1/cell) and univalents (0-2/cell) were present; nonetheless, the bivalent formation was most common. Mean meiotic configurations of these tetraploids at metaphase-I/diakinesis are listed in Table 3. At anaphase I and II, several chromosome disjunctional anomalies were recorded, e.g. lagging chromosomes, multipolar segregation, micronuclei formation, chromosome extension, etc. (Table 4). Such meiotic abnormalities have been reported previously in induced autotetraploids of various plant species including, *Crotolaria* (Gupta and Sinha 1978), *Vigna* (Sen and Ghosh 1965), *Cicer* (Sharma and Gupta 1987) and *Sorghum* (Magoon and Tayyab 1968). These induced tetraploids are being multiplied at Mukteshwar (in the hills) to find out the possibility of their commercial utilization.

The second approach involved screening of anisoploid populations. Two anisoploid populations, 'A' and 'B', which showed good adaptability under Indian conditions, were analyzed for their stomatal characters vis-à-vis chromosome numbers. The usefulness of stomatal characters for determining ploidy in sugar beet has already been emphasized by Srivastava *et al.* (1988). A total of 208 plants from both the populations was screened to ascertain the efficacy of this procedure (Table 5), which proved quite efficient for screening of diploids (71.74%) and tetraploids (68.63%). This suggested a strong dependence of stomatal characters (i.e. size and frequency of stomata and number of chloroplasts in guard cells) as with other morphological attributes on the ploidy level ($2n$ and $4n$) of sugar beet plants.

Table 1. Morphological characters of diploid and tetraploid sugar beet

Character	Diploid	Tetraploid	% difference
Plant growth (visual)	Normal	Better	—
Plant height (m)	0.15	1.25	+ 8.69
Leaf size (cm ²)	97.50	189.00	+ 93.85
Leaf morphology (visual)	Normal light green	Thick dark green	—
Pollen size (mm ²)	20.60	25.80	+ 25.24
Pollen fertility (%)	87.23	69.03	— 20.90
1000-seed weight (g)	27.23	14.41	— 47.08
Seed germination (%)	90.00	30.00	— 66.66

Table 2. Stomatal characteristics of diploid and tetraploid sugar beet

Stomatal characters	Diploid		Tetraploid	
	Mean	Range	Mean	Range
Stomata length (m)	29.30	18.32-33.20	46.30	40.00-57.50
Stomata width (m)	24.19	17.50-29.00	31.10	28.13-37.50
Size of stomata (m ²)	729.97	396.88-962.80	1462.75	1160.0-2018.25
Frequency of stomata/mm ²	147.11	106.92-245.28	53.86	37.74-69.18

No. of chloroplasts/guardcells	16.18	13.00-22.80	23.74	20.28-28.00
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Table 3. Mean meiotic configurations of *B. vulgaris* L. diploids and their induced autotetraploids

Ploidy	Univalents	Chromosome configurations							
		Bivalents		Trivalents		Quadrivalents		Ring	Total
		Ring	Open	Ring	Open	Ring	Open		
Diploid	0.57	1.63	7.08	8.71	—	—	—	—	—
Tetraploid	1.46	1.55	11.31	12.86	—	0.65	0.65	0.72	1.72

Table 4. Anaphase disjunctional anomalies in induced autotetraploids

Percentage of cells having:						
Stage	Normal segregation	Anomalous segregation				
		Laggards	Multipolar	Micronuclei	Chromosome extrusion	Cytomixis
A I	66.67	10.44	3.70	12.46	2.02	4.71
A II	61.89	10.02	12.16	1.27	8.22	6.44

Table 5. Ploidy level screening of anisoploid sugar beet populations on the basis of stomatal parameters and chromosome counts

Population	No. screened	Plants confirmed on				% Efficiency of stomatal parameter	
		Stomatal basis		Chromosome counts		2n	4n
		2n	4n	2n	4n		
Anisoploid A	120	29	24	21	17	72.41	70.83
Anisoploid B	88	17	27	12	18	70.59	66.67
Total	208	46	51	33	35	71.74	68.63

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Beta genetic resources in Romania

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Beta corolliflora Zoss. (with $2n=36$ chromosomes) normally has a 2-year life cycle under Romanian environmental conditions. The native material corresponds to that described in the literature with respect to flower and fruit morphology, as well as growth habit. Native *B. corolliflora* populations are tolerant to attack by *Erysiphe betae* and *Cercospora beticola* Sacc. *Beta trigyna* L. populations (with $2n=54$ chromosomes) are perennials with seeds that shatter easily. Local populations of *B. vulgaris* L. subsp. *maritima* are annual. The hypocotyl, the petiole and the seed stalks are green, red or pink. They have a good tolerance to *Erysiphe betae*, *Mamestra brassicae* L. and *Loxostege sticticalis* L. The fruits are monogerm (2%) and bigerm (30%) and the germination is 98-100%. As with all *B. vulgaris* subsp. *maritima* distributed in the Mediterranean area, local Romanian populations are outcrossing. The progeny segregate in a panmictic way, indicated by the frequency of the hypocotyl genotype in the local material. The investigated progeny was composed of 41-48% of red hypocotyls. Pink hypocotyl colour did not show up in the first panmictic generation. The Suceava Gene Bank has 24 accessions of indigenous beet cultivars, of which 18 are landraces and 6 are breeding families.

Breeding of sugar beets in China

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Breeding for disease resistance is our major objective in sugar beet research. Resistance to *Cercospora beticola* has been an important character in every new variety since the 1960s because this disease has spread widely through the sugar beet production regions of China. Through strict selection over a long term, we have developed a number of sugar beet varieties resistant to *Cercospora beticola*, for example, Tian 303, Tian 304, Tian 307 and Tian 7.

Recently, root diseases have become a serious problem. Root rots (*Fusarium* and *Rhizoctonia*) and rhizomania are spreading from the northwest to the northeast of China.

Root diseases may reduce root yield by 20-70% (Table 1) and the sugar content by 3-6%. By selection in heavily diseased fields and with ELISA techniques, some breeding lines or hybrid combinations with tolerance to root rots have been found.

Introduction of commercial seed and breeding lines from abroad and screening of valuable material is an important part of the breeding programme. Foreign varieties have a higher root yield, shorter crown and more desirable root shape than Chinese sugar beet varieties. Their superiority will be more evident in a dry year or irrigated region than in a wet year or rainy region. The disadvantages to some foreign varieties are that they are not resistant or tolerant to *C. beticola* or root rot and that the sugar content is lower than in Chinese varieties.

The other problem of national varieties is the root yield. Generally speaking, Chinese varieties have a lower root yield than some foreign varieties (about 10-20% less). We are trying to improve yield ability through pre-breeding and selecting for combination ability.

Table 1. Yields of sugar beet varieties in a heavily rhizomania-infested field
(Inner Mongolia, 1994; ck=check variety Xizuo 2)

Variety	Root yield		Sugar content		Sugar yield	
	t/ha	ck%	%	D to ck	t/ha	ck%
Tian 303	21.63	123.9	10.70	4.83	2.42	210.14
Tian 304	22.26	127.5	10.98	5.11	2.49	216.07
88302	20.33	116.4	11.02	5.15	2.32	201.91
88304	29.61	169.6	12.27	6.40	3.69	319.48
T--202	21.11	120.9	11.42	5.55	2.43	210.83
Xiezuo 2	17.46	100	05.87	0	1.15	100

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Appendix II. Acronyms and abbreviations

AARI	Aegean Agricultural Research Institute, Turkey
ANOVA	Analysis of Variance
BAZ	Federal Centre for Breeding Research on Cultivated Plants, Germany
BCC	Beta Coordinating Committee
BCTV	Beet Curly Top Virus
BGRC	Braunschweig Genetic Resources Collection, Germany
BMVY	Beet Mild Yellowing Luteovirus
BNYVV	Beet Necrotic Yellow Vein Virus
BRG	Bureau des ressources génétiques, France
BSDF	Beet Sugar Development Foundation, USA
BYV	Beet Yellowing Closterovirus
CAAS	Chinese Academy of Agricultural Sciences, China
CBD	Convention on Biological Diversity
CGN	Center for Genetic Resources, the Netherlands
CMS	Cytoplasmic Male Sterility
CPRO-DLO	Center for Plant Breeding and Reproduction Research, the Netherlands
CTPS	Comité technique permanent de la sélection des plantes cultivées, Ministère de l'Agriculture, France
ECP/GR	European Cooperative Programme for Crop Genetic Resources Networks
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization of the United Nations
FDDR	Former Deutsche Demokratische Republik
FNAMS	Fédération Nationale des Agriculteurs Multiplicateurs de Semences, France
FSV	Former Soviet Union
GEVES	Groupe d'Etude et de Contrôle des Variétés et des Semences, France
GNIS	Groupement National Interprofessionnel des Semences, Graines et Plants, France
GRIN	Genetic Resources Information Network, USA
HBC	Sugar Beet Enterprise, Poland
IC&G	Institute of Cytology and Genetics, Russia
ICAR	Indian Institute of Sugarcane Research, India
ICBN	International Code of Botanical Nomenclature
ICNCP	International Code of Nomenclature for Cultivated Plants
IDBB	International Database for Beta
IfZ	Institute of Sugar Beet Research, Germany
IHAR	Plant Breeding and Acclimatization Institute, Poland
IIRB	Institut International de Recherches Betteravières, Belgium
IISR	Indian Institute of Sugarcane Research, India
INRA	Institut National de la Recherche Agronomique, France
IPK	Institut für Pflanzengenetik und Kulturpflanzenforschung, Germany
ISCI	Istituto Sperimentale per le Colture Industriali, Italy
ISHS	International Society for Horticultural Science
ITB	Institut Technique Français de la Betterave Industrielle, France
KWS	Kleinwanzlebener Saatsucht AG, Germany

LSD	Least Significant Difference
NMS	Nuclear Male Sterility
NPGS	National Plant Germplasm System, USA
NSSL	National Seed Storage Laboratory, USA
PGRRI	Plant Genetic Resources Research Institute, Turkey
PI	Plant Introduction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restricted Fragment Length Polymorphism
SBSI	Sugar Beet Seed Institute, Iran I.R.
SES	Société Européenne de Semences
SPSB	Società Produttori Sementi Bologna, Italy
UPOV	Union pour la Protection des Obtentions Végétales, Switzerland
USDA- ARS	United States Department of Agriculture-Agricultural Research Service, USA
VIR	N.I. Vavilov Research Institute of Plant Industry, Russia
VNIIS	Mazlumov All-Russian Research Institute of Sugar Beet and Sugar, Ramon, Voronezh Region, Russia
WANA	West Asia North Africa (IPGRI Regional Office)
WBN	World Beta Network
WRPIS	Western Regional Plant Introduction Station, USA
WWW	World Wide Web