EVALUATION AND ENHANCEMENT OF *BETA* COLLECTIONS FOR THE EXTENSIFICATION OF AGRICULTURAL PRODUCTION

(GENRES - CT95 - 42)

Terminal Project Report

IACR-Broom's Barn Experimental Station

Higham, Bury St Edmunds, Suffolk, IP28 6NP, UK.

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1. Project Background

Sugar beet production in Europe is significantly affected by a range of biotic and abiotic agents such as disease and drought stress. Although the current control strategies used to minimise these production constraints, based on the use of pesticides and irrigation, are effective they are relatively expensive and have potentially long-term negative effects on the environment. Ideally, control should be achieved through the use of sugar-beet cultivars that are inherently resistant to disease or tolerant of drought. However, in most cases, this option is not available to growers, as such cultivars do not exist.

The shortfall in the availability of resistant/tolerant cultivars has been recognised by the sugar-beet industry and attempts are being made to improve the situation. However, a major obstacle to improving the availability of resistance/tolerance in sugar beet is the narrow genepool from which most current cultivars are derived. This limits the potential for identifying new sources of genes that can be introgressed to develop novel cultivars. To broaden the genepool available to breeders, a consortium of European research institutes and sugar-beet breeding companies, led by Dr Lothar Frese of BAZ, Braunschweig, Germany, organised a project ('**EVALUATION AND ENHANCEMENT OF *BETA* COLLECTIONS FOR THE EXTENSIFICATION OF AGRICULTURAL PRODUCTION' - GENRES - CT95 - 42**) to identify new resistance/tolerance genes in closely-related *Beta* species. This group of plants was recognised as a potentially valuable source of genes as they are exposed to the same abiotic and biotic factors as sugar beet but have a much more variable response to them. Furthermore, many species are sexually compatible with sugar beet, making introgression of genes relatively straightforward.

2. GENRES Project– IACR Broom's Barn

2.1 Introduction

The role of IACR-Broom's Barn (participant P8) within the project was to evaluate *Beta* germplasm for resistance to Beet Mild Yellowing Virus (BMYV) and Beet Yellows Virus (BYV), seedling damping-off caused by the fungi *Aphanomyces cochlioides* and *Pythium ultimum*, and powdery mildew (*Erysiphe betae*) and for tolerance to drought stress. The project at Broom's Barn, co-funded by the European Union and the British Beet Research Organisation (BBRO), commenced in October 1996 with the appointment of two researchers specifically tasked to undertake the work under the guidance of Dr Mike Asher. The project was successfully completed in 2001.

2.2 Project tasks

Four tasks were set for IACR-Broom's Barn (P8) in the project document. Using the original task numbers, these were:

R 2.2	Screening of 600 accessions for *Aphanomyces* and *Pythium* resistance, respectively.
R 2.3	Screening of 600 accessions for BMYV and BYV resistance (rapid test procedure), respectively.
R 2.5	Screening of 600 accessions for *Erysiphe* [powdery mildew] resistance.
R 2.7	Screening of 600 accessions for drought tolerance and salinity.

The milestones of completing these tasks within the 5-year timeframe set out in the project document were achieved. The outcome of the experimental work will be described according to these task numbers.

2.3 *Beta* germplasm evaluated

Seed of 600 *Beta* accessions were provided by Dr. Frese, BAZ, for evaluation. The number of accession provided in each year of the project varied, thus: 1996 – 200 accessions; 1998 – 174 accessions; 1999 – 179 accessions; 2000 – 47 accessions.

The identity of the accessions and their country of origin are given in Figs. 1 & 2 (3 accessions were of unknown identity; 27 accessions were of unknown country of origin). Most *Beta* accessions were of cultivated origin, i.e., fodder (10.2%), garden (20.3%), leaf (20.7%) and sugar (5.2%) beets, but there was a significant number of wild species including members of the sections *Beta* (38.5%), *Corollinae* (2.5%) and *Procumbentes* (1.7%). The most common wild species tested was *B. vulgaris maritima*, or sea beet. Most accessions were collected from Southern Europe (48%), but a significant amount came from Northern Europe (24%), the Middle East (9%) and the former Soviet Union (9%).

Fig. 1. Identity of *Beta* germplasm

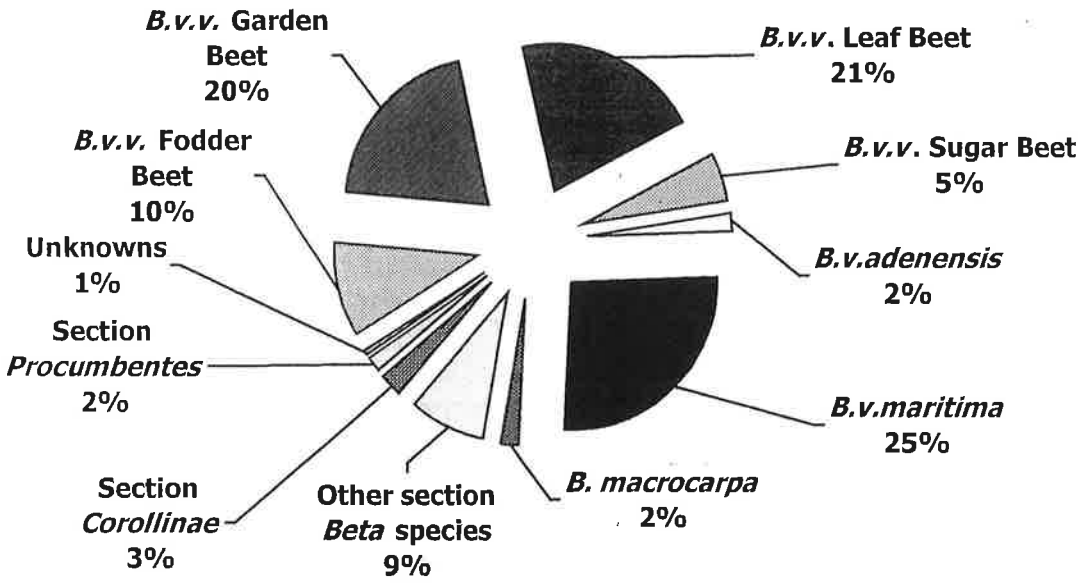
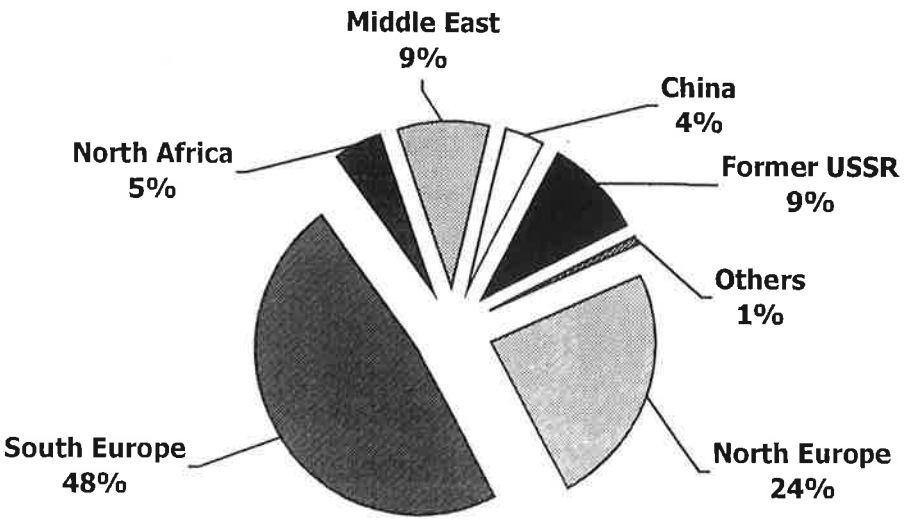


Fig. 2. Origin of *Beta* accessions



2.4 Experimental Approach

To achieve the project milestones, each task was divided into two parts. The first involved the development of suitable screening methods from existing protocols to ensure proper differentiation of resistance/tolerance responses of individual *Beta* accessions. The amount of development varied; for BMYV no changes were required to existing protocols but for drought stress tolerance a radically different approach was needed to that originally proposed.

The second part of each task involved the resistance/tolerance screening of the 600 *Beta* accessions selected for evaluation. Whilst attempts were made to screen all 600 accessions at least once it was not always possible. Some accessions, particularly those of the sections *Corollinae* and *Procumbentes*, either failed to produce sufficient seedlings despite several attempts to do so under optimal conditions or were unsuitable for screening in the field (powdery mildew screening). Where results are not available, this will be indicated at the relevant points in the report.

After initial screening, it was policy to re-test, where resources and seed quantities permitted, accessions identified as highly resistant or tolerant to ensure the original observations were correct and not due to failures in the methodology, e.g., they were not disease 'escapes'. For this reason, the final data sets for all resistance/tolerance traits included here supersede any previously reported data, as they will include information from repeated tests (approximately 800 in total) completed during the last year of the project and hitherto unpublished. Re-testing of accessions of the sections *Corollinae* and *Procumbentes* was often not possible as viable seed was in short supply.

Although the experimental protocols used in the project were reported in full in the 3rd GENRES annual report (June 1999), minor alterations in the methods of analyses of certain traits, in particular drought stress tolerance and *Pythium* resistance, have been introduced. Therefore, for the sake of clarity, the experimental protocols, with any modifications, have been included in this report; where discrepancies occur readers should consider the protocols in this report as the correct version – see Appendix 1.

2.5 Disease resistance data

The format used for reporting results from each of the disease resistance evaluation programmes is identical – appendices 2 to 6. The methodology used to evaluate each accession (columns: **'treatment', 'method'**) and the number of tests conducted (**'# tests'**) is given. The details of each experimental protocol used are described in relevant section of appendix 1.

Each accession is assigned a resistance score (**'Score'**), based on a internationally standardised scale of 1-9, where 1 = no or extremely low symptom expression and 9 = extremely high symptom expression. The score for each accession has been derived from the mean value of disease incidence (**'mean'**) of all replicates tested under controlled conditions. To minimise any potential inter-experimental variation, mean values are 'normalised' by relating levels of infection observed in each accession to those seen in a standard (sugar beet cultivar Saxon, except for powdery mildew where cv. Sandra was used) included in all tests for each trait **('% infect rel. to standard')**.

A measure of the variability of disease resistance responses within each accession is given by the range of infection observed (**'range: lower'; 'range: upper'**), the standard deviation (**'SD'**), standard errors (**'SE'**) and % coefficient of variance (**'%CV'**).

2.6 Drought tolerance data

A different approach to that used for the disease evaluation programmes was required for the interpretation of drought tolerance data. Unlike disease resistance evaluation, where the extremes in reaction (completely healthy; death) can be clearly defined and a range of responses in-between measured and quantified with relative confidence, responses to drought stress are more ambiguous and complicated by the inherent size of individual accessions and their relative water requirements. It was not possible to relate drought tolerance directly to absolute differences in growth under normal and stressed conditions as this would have resulted in inherently small accessions, which require less water, appearing more tolerant than larger types.

Therefore, a method of analysis was developed that compensated for the confounding factor of inherent plant size. In essence, the drought stress response of each accession was compared to the mean drought stress response of all accessions, the latter being described by a mathematical equation derived from the relevant data (Fig. 3). Individual accessions were assigned a drought tolerance score based on their relative performance in these analyses. The method of assessing drought stress tolerance has meant that much of the information provided in the database is different to that for the disease resistance traits - Appendix 7. The methodology used to evaluate each accession (columns: **'treatment', 'method'**) and the number of tests conducted (**'# tests'**) is given. The details of the experimental protocol are given in appendix 1F.

The mean dry weight of plants, both unstressed (= control treatment) (**'wt control: mean'**) and stressed **('wt stress: mean')**, for each test accession (**'TA'**) are given, plus the relevant measures of variation including standard deviation (**'SD'**) and the %CV (**'%CV'**).

The amount of weight loss due to drought (**'weight reduction'**) in each test accession was determined from the mean, thus:

$$\textbf{Weight reduction TA} = 1 - \frac{\textbf{mean dry weight stressed plants TA}}{\textbf{mean dry weight control plants TA}}$$

Results from tests where no significant differences (ANOVA – $p<0.05$) in the weights of the unstressed and stressed plants of the standard were discarded.

To improve the mathematical estimation of the mean drought stress response of all accessions, data were normalised relative to the standard treatment (sugar beet cv. Saxon) included in the relevant experiment to minimise inter-experimental errors. The need to normalise data was judged necessary because it was impossible to control the environment rigorously enough to ensure accessions were exposed to exactly the same conditions in each experiment (sixty experiments in total were undertaken). For example, plants grown in a glasshouse for four weeks prior to screening, were exposed to different light and heat regimes depending on the time of the year, leading to seedlings of differing size at the start of the experiment. Therefore, the inherent weight of each test accession (equivalent to the mean weight of the controls of each accession) is described as measure relative to the standard, rather than an absolute value, thus:

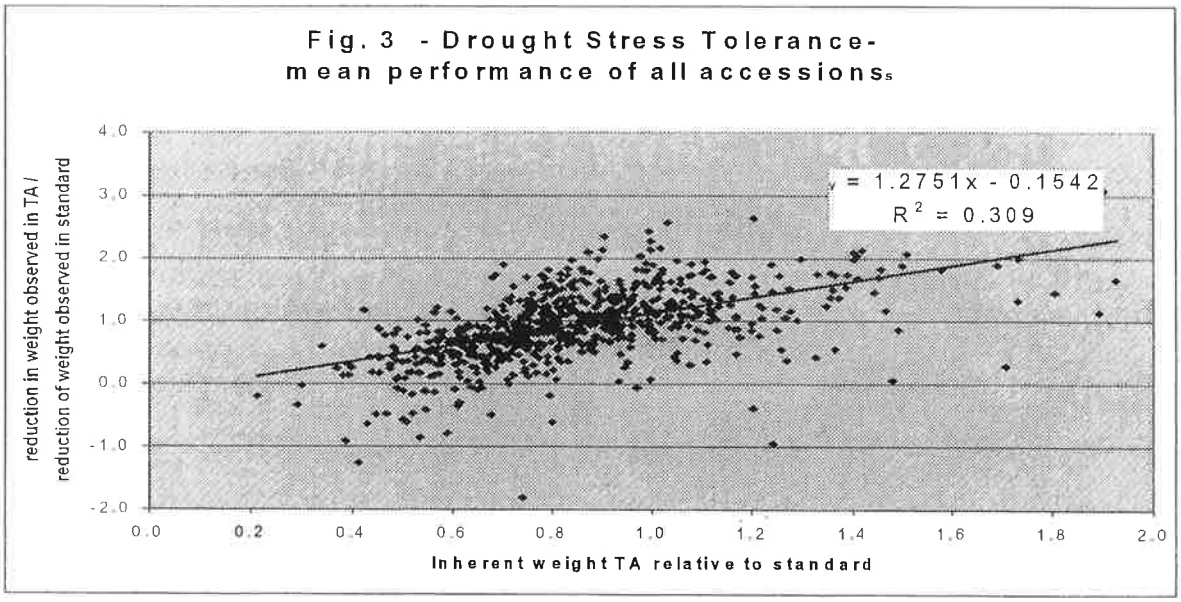
$$\text{'Inherent Weight 'TA'} = \frac{\text{mean dry weight: control}}{\text{mean dry weight: standard}}$$

The standard will always score 1; any value below 1 indicates the accessions were inherently smaller than the standard, and vice-versa. In much the same manner, the weight reduction observed in the test accession when stressed (**Weight reduction: TA**) was expressed relative to the weight reduction observed in the standard included in the same experiment (the latter value given in the column **'Weight reduction: standard'**) and calculated in the same manner described above), thus:

$$\text{'Reduction ratio 'TA' to standard (observed)} = \frac{\text{weight reduction: TA}}{\text{weight reduction: standard}}$$

The standard will always score 1; ratios below 1 indicate that the weight reduction in the test accessions was lower than the standard and vice-versa. Subsequently, the transformed data were plotted and a mathematical equation describing the relationship determined (Fig. 3 - linear regression: $y = 1.2751x - 0.1542$; $R2 = 0.31$; 730 observations). From this equation, it was possible to determine the weight reduction expected for each accession at their given inherent weight (**Reduction ratio 'TA' to standard (expected)'**). The deviation of the 'observed' value from the 'expected' was used to determine the **'Drought Stress Score'**: accessions where the observed values were greater than the expected were deemed to be less-tolerant, and hence the higher drought stress scores, and vice-versa.

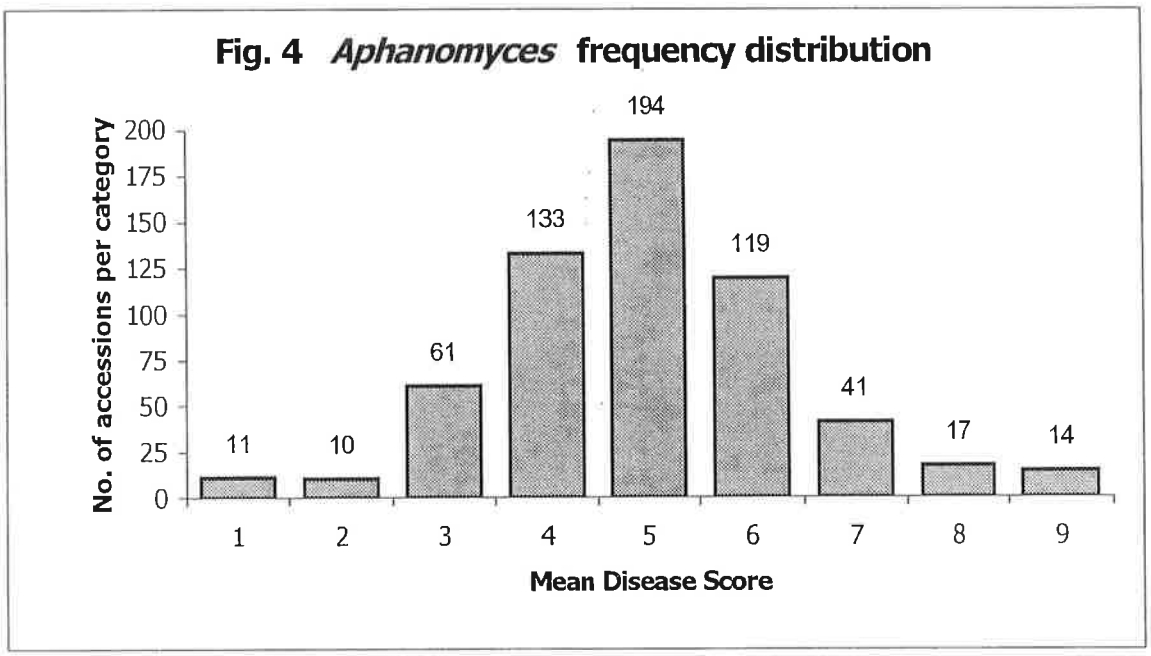
Fig. 3 - Drought Stress Tolerance-
mean performance of all accessions



3. Results from the Screening Programmes

3.1 Task R 2.2 - Screening of 600 accessions for *Aphanomyces* resistance

Screening of *Aphanomyces* resistance has been completed. All 600 accessions were screened at least once using an experimental protocol developed in the early part of the screening programme; in total, 702 tests (102 repeats) were undertaken. The evaluation method used was successful in allowing clear discrimination between different accessions, a view supported by the normally distributed frequency distribution pattern – Fig. 4. A summary of *Aphanomyces* infection per species and details of the experimental protocol are given in Table 1 and Appendix 1A. The complete *Aphanomyces* database is given in Appendix 2.



Eleven accessions (or 2% of the total) were identified with a resistance score (RS) of 1 - Table 2. Most were species of the section *Corollinae* (9 accessions). The only other accessions to have an RS of 1 were two examples of the non-cultivated species *B. vulgaris* and *B. v. maritima*, although the former example must be viewed with care due the poor germination rate observed (<25% - see comments below). A *B. macrocarpa* accession that had an RS value of 1 in early screening (and reported in the 2001 annual report) was re-graded to 2 following re-testing. By comparison the standard sugar-beet cultivar (cv. Saxon) included in all tests had an RS of 5 (as did several other commonly grown sugar-beet cultivars).

At first sight, these results may be considered disappointing as the accessions of the section *Corollinae* are not sexually compatible with sugar beet and cannot be included in conventional breeding programmes. However, they would be extremely valuable in any transgenic breeding programme for *Aphanomyces*, if the technical and socio-political challenges can be overcome.

Table 1 – Summary of *Aphanomyces* disease resistance in *Beta* species

Beta Species	No. accessions tested	Mean Score	Range of Scores	Accessions – RS1		Accessions – RS 2	
				No.	%	No.	%
Section *Beta* - (i) cultivated forms							
B.v. vulgaris – fodder beet	61	5.2	3-9	0	0	0	0
B.v. vulgaris – garden beet	122	5.4	2-9	0	0	1	1
B.v. vulgaris – leaf beet	124	5.5	3-9	0	0	0	0
B.v. vulgaris – sugar beet	31	5.0	3-7	0	0	0	0
Section *Beta* - (ii) non-cultivated forms							
B. macrocarpa	12	3.6	2-5	0	0	1	8
B. patula	1	3.0	3	0	0	0	0
B. vulgaris	51	4.9	1-9	1	2	1	2
B.v. adanensis	12	5.0	3-7	0	0	0	0
B.v. maritima	158	4.6	1-9	1	1	4	3
Section *Corollinae*							
B. corolliflora	7	1.7	1-4	4	57	6	86
B. intermedia	1	2.0	2	0	0	1	100
B. lomatogona	1	1.0	1	1	100	1	100
B. macrorhiza	5	1.4	1-2	3	60	5	100
B. trigyna	1	1.0	1	1	100	1	100
Section *Procumbentes*							
B. patellaris	6	4.0	3-5	0	0	0	0
B. procumbens	3	5.0	3-7	0	0	0	0
B. webbiana	1	3.0	3	0	0	0	0
Unknown	3	5.7	5-7	0	0	0	0
Total/Mean of All Accessions	600	5.0		11	2	21	4

If the pool of highly resistant accessions was expanded to include those with RS of 2, the number and, importantly, the diversity of accessions increased. It should be stressed that RS 2 accessions are significantly more resistant than any sugar beet cultivar currently available on the market. The position of the section *Corollinae* as the most resistant group in relation to *Aphanomyces* would be reinforced; 14 out of the 15 accessions tested fell into RS categories 1 & 2. However, the number of resistance sources in the section *Beta*, which is fully compatible with sugar beet, rose to seven (or 1.2% of the total tested). Wild species of the section *Beta*, in particular *B.v. maritima* and *B. macrocarpa*, appeared to be most promising. Only one cultivated beet, a garden beet, appeared to show any significant resistance; collectively the cultivated beets fared relatively poorly. Species of the section *Procumbentes* (*B. patellaris, B. procumbens, B. webbiana*) were no better collectively than those of the section *Beta*; all three species tested had examples with RS values of 3.

The resistance scores of 22 accessions (Table 3) must be viewed with care as poor germination rates (<25% of seeds germinated) may have distorted the results, thus giving a misleading impression of their inherent resistance. Previously, these results were discarded and new tests undertaken with fresh seed (see 2000 annual report), but unfortunately in many cases this failed to improve the validity of the results. Therefore, it has been decided to include these results in the final *Aphanomyces* data set for the sake of completion, but additional work may be necessary to verify the reported results.

Table 2 – *Aphanomyces* evaluation – accessions with RS of 1.

Type	**Accession numbers**
Corolliflora	2524, 2982, 2987, 3221
Lomatogona	2502
Macrorhiza	3199*, 3200, 8539
Maritima	5763
Trigyna	3707*
Vulgaris	5215*
* = germination rate under 25%	

Table 3 – *Aphanomyces* evaluation – accessions with low germination rates (<25%)

Type	**Accession numbers**
Garden Beet	10245
Leaf Beet	3102
Maritima	177, 182, 200, 450, 2301, 3735, 5726, 7077, 7088
Macrocarpa	3183
Macrorhiza	3199
Patellaris	1645, 3229, 6535, 6542
Procumbens	1630, 1663
Trigyna	3707
Vulgaris	2213, 5215
These results have been highlighted in the final *Aphanomyces* data set thus (*)	

3.2 Task R 2.2 - Screening of 600 accessions for *Pythium* resistance

In total, 597 accessions were screened for *Pythium* resistance; accessions 3199, 3200 (both *B. macrorhiza*) and 3707 (*B. trigyna*) failed to germinate in all tests, thus preventing an assessment of their resistance potential being made. 734 tests were undertaken, including 137 re-tests, by the end of the project.

As with the *Aphanomyces* resistance screening programme, it was necessary to develop the method originally proposed to enhance differentiation. Fixing the inoculum dose for the *Pythium* test was more complicated because of the pre-emergence infection cycle of the pathogen and the variable germination rate of individual accessions. Most *Pythium* infection occurs pre-emergence (unlike *Aphanomyces* where practically all infection occurs post-emergence) and therefore it was necessary to distinguish between non-emergence of seedlings due to seed viability and that due to *Pythium* infection. This was achieved by including an uninoculated control so seedling emergence under both normal and disease-challenged conditions could be determined, and by subtraction an estimation of seedling loss due to disease made. Although successful, this approach required more seed then originally envisaged and delays occurred to allow more to be obtained. Details of experimental protocol are given in Appendix 1B.

The data have been fully assessed and results per species are summarised in Table 4. The distribution curve (Fig. 5) indicated that the data was slightly skewed towards resistance (mode of 4). This effect was attributed to the relatively low severity of the test rather than higher levels of inherent resistance to *Pythium*. The skewness observed in the distribution curve is less pronounced than that described in previous annual reports (1999, 2000). This change reflected results from re-tests completed in 2001, where a number of apparently 'resistance' accessions identified in early screens were re-graded and given higher RS values. The complete *Pythium* database is given in Appendix 3.

Fig. 5 *Pythium* frequency distribution

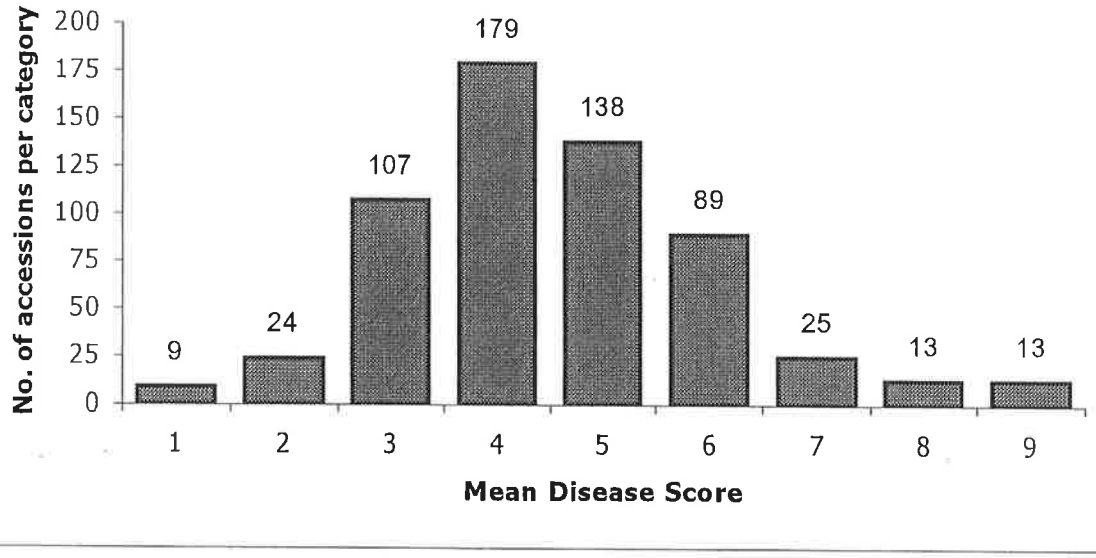


Table 4 – Summary of *Pythium* disease resistance in *Beta* species

Beta Species	No. accessions tested	Mean Score	Range of Scores	Accessions – RS1		Accessions – RS 2	
				No.	%	No.	%
Section *Beta* - (i) cultivated forms							
B.v. vulgaris – fodder beet	61	4.8	3-9	0	0	0	0
B.v. vulgaris – garden beet	122	4.6	1-9	3	2	8	7
B.v. vulgaris – leaf beet	124	4.7	2-9	0	0	3	2
B.v. vulgaris – sugar beet	31	4.7	2-7	0	0	1	3
Section *Beta* - (ii) non-cultivated forms							
B. macrocarpa	12	5.1	1-9	1	8	1	8
B. patula	1	4.0	4	0	0	0	0
B. vulgaris	51	4.9	2-9	0	0	3	6
B.v. adanensis	12	4.6	3-9	0	0	0	0
B.v. maritima	158	4.2	1-9	5	3	16	10
Section *Corollinae*							
B. corolliflora	7	5.1	4-6	0	0	0	0
B. intermedia	1	6.0	6	0	0	0	0
B. lomatogona	1	6.0	6	0	0	0	0
B. macrorhiza	3	5.0	5	0	0	0	0
B. trigyna	-	-	-	-	-	-	-
Section *Procumbentes*							
B. patellaris	6	4.2	2-6	0	0	1	17
B. procumbens	3	4.3	3-5	0	0	0	0
B. webbiana	1	3.0	3	0	0	0	0
Unknown	3	4.7	3-6	0	0	0	0
Total/Mean of All Accessions	[redacted] 597	4.5		9	2	33	6

Nine accessions (or approximately 2% of the total) were identified with an RS of 1 (Table 5), a frequency similar to that found for *Aphanomyces*. However, unlike *Aphanomyces*, all the most resistant accessions belonged to the section *Beta*. The highest resistance was observed in three accessions of garden beet, five of *B. v. maritima* and one of *B. macrocarpa*. The importance of the section *Beta* as a source of resistance was enhanced when accessions with an RS of 2 were considered. In total, there were 32 accessions, or 6%, of the section *Beta* with RS values of 1+2. Again, garden beets and *B. v. maritima* accessions look most promising. The standard sugar-beet cultivar (cv. Saxon) had an RS of 4.

Although species of the sections *Corollinae* and *Procumbentes* performed as well as those in section *Beta* collectively, there was only one example, a *B. patellaris* accession of the section *Procumbentes*, with an RS value below 3.

The potential problem of poor germination rates influencing scores was also observed in screening for *Pythium* resistance. Twenty-one accessions were affected, but unlike *Aphanomyces*, none of the accessions graded as RS 1 or 2 were affected by this problem. Those accessions where resistance scores must be viewed with caution are listed in Table 6.

Table 5 – *Pythium* evaluation – accessions with RS of 1.

Type	**Accession numbers**
Garden Beet	2584, 6801, 6850
Macrocarpa	3194
Maritima	2666, 3734, 7067, 8463, 9479

Table 6 – *Pythium* evaluation – accessions with low germination rates (<25%)

Type	**Accession numbers**
Fodder Beet	3746, 7593
Garden Beet	7345
Leaf Beet	1223, 2589
Maritima	450, 2301, 3546, 3798, 7194, 9461
Macrorhiza	3199, 3200
Patellaris	3229, 6535
Procumbens	1630, 1645, 1663
Trigyna	3707
Vulgaris	2213, 2231
These results have been highlighted in the final *Pythium* data set thus (*)	

3.3 Task R 2.3 - Screening of 600 accessions for BMYV resistance

In total, 595 accessions were screened for BMYV resistance; accessions 3199, 8539 (both *B. macrorhiza*) and 3707 (*B. trigyna*) failed to germinate in all tests, whilst insufficient seed of accessions 25 (*B. v. maritima*) and 5215 (*B. vulgaris*) was available to undertake testing. 684 tests were completed, including 84 re-tests, by the end of the project. Results from the early tests indicated that the screening methods were appropriate for differentiating between resistant and susceptible accessions, and therefore required no modification. Details of the experimental protocol are given in appendix 1C.

A summary and a histogram displaying the near-normal distribution of mean infection by BMYV across all accessions are given in Table 7 and Fig. 6. The complete BMYV data base is given in Appendix 4. In total, 30 accessions (5%) were identified as highly resistant, with RS of 1. Collectively, the most highly resistant accessions came from the sections *Corollinae* and *Procumbentes*; many individual plants within these accessions had undetectable levels of virus indicating possible immunity. However, as indicated before, the value of these accessions in conventional sugar-beet breeding programmes is limited because of compatibility problems.

By comparison, the species of the section *Beta* were, on average, more susceptible to BMYV infection. However, there were individual accessions within the section *Beta* that were highly resistant; nine accessions of this section had an RS of 1 (compared with the standard sugar beet cultivar Saxon with an RS of 6). Collectively, garden beets and the single example of *B. patula* were the best sources of potential resistance genes. Other promising candidates included a fodder- and leaf beet. Accessions with an RS of 1 are listed in Table 8.

The importance of garden beets as sources of BMYV resistance increased when RS 2 accessions were considered as well; 34 garden beets, or 28% of those tested, had very high levels of resistance. *B. vulgaris* and *B. v. maritima* were the best of the non-cultivated members of the section *Beta*, with nine accessions apiece with RS of 2.

Fig. 6 BMYV frequency distribution

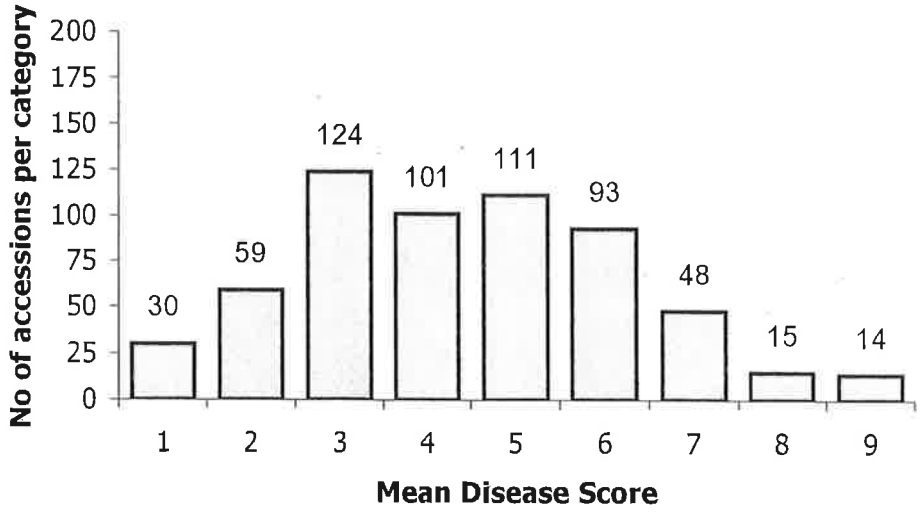


Table 7 – Summary of BMYV disease resistance in *Beta* species							
***Beta* Species**	**No. accessions tested**	**Mean Score**	**Range of Scores**	**Accessions – RS1**		**Accessions – RS 2**	
				No.	**%**	**No.**	**%**
Section *Beta* - (i) cultivated forms							
B.v. vulgaris – fodder beet	61	4.2	1-9	1	2	6	10
B.v. vulgaris – garden beet	122	3.5	1-9	6	5	34	28
B.v. vulgaris – leaf beet	124	5.4	1-9	1	1	5	4
B.v. vulgaris – sugar beet	31	4.4	2-9	0	0	2	6
Section *Beta* - (ii) non-cultivated forms							
B. macrocarpa	12	4.8	3-6	0	0	0	0
B. patula	1	1.0	1	1	100	1	100
B. vulgaris	50	4.4	2-8	0	0	9	18
B.v. adanensis	12	5.9	4-9	0	0	0	0
B.v. maritima	157	4.7	2-9	0	0	9	6
Section *Corollinae*							
B. corolliflora	7	1.0	1	7	100	7	100
B. intermedia	1	1.0	1	1	100	1	100
B. lomatogona	1	1.0	1	1	100	1	100
B. macrorhiza	3	1.0	1	3	100	3	100
B. trigyna	-	-	-	-	-	-	-
Section *Procumbentes*							
B. patellaris	6	1.0	1	6	100	6	100
B. procumbens	3	1.3	1-3	2	67	3	100
B. webbiana	1	1.0	1	1	100	1	100
Unknown	3	4.3	3-6	0	0	1	33
Total/Mean of All Accessions	595	4.4		30	5	89	15

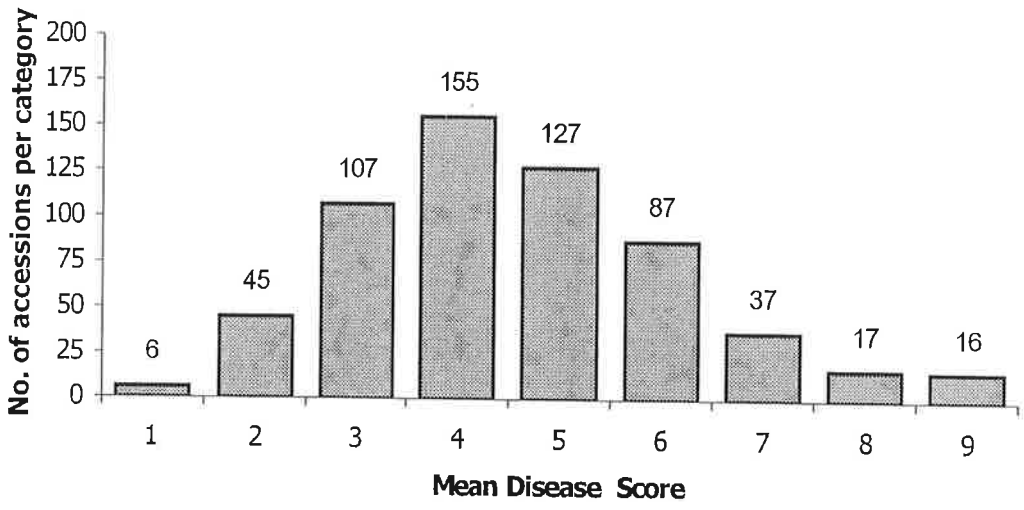
Table 8 – BMYV evaluation – accessions with RS of 1	
Type	**Accession numbers**
Corolliflora	2326, 2524, 2971, 2982, 2987, 3221, 7144
Fodder Beet	7430
Garden Beet	2676, 3965, 4874, 6223, 7479, 7493
Intermedia	3222
Leaf Beet	6353
Lomatogona	2502
Macrorhiza	3200, 8541, 8548
Patellaris	1645, 3229, 5938, 6535, 6542, 7042
Procumbens	1630, 3242
Patula	3197
Webbiana	3244

3.4 Task R 2.3 - Screening of 600 accessions for BYV resistance

Screening for BYV resistance has been completed. Of the 600 accessions tested, no results were forthcoming from three accessions, 3199, (*B. macrorhiza*), 3707 (*B. trigyna*) and 5215 (*B. vulgaris*), for the reasons alluded to in section 2.4. Early testing indicated that the original screening method was too insensitive to clearly differentiate between resistant and susceptible *Beta* accessions. The reason for the lack of sensitivity was not fully established but a change in the BYV polyclonal antibody used in the ELISA test and the shortening of interval between inoculation and testing increased the range of responses, making discrimination between accessions possible. All subsequent screening was undertaken using this modified method; the experimental protocol is given in appendix 1D. By the completion of screening, 855 tests had been undertaken including 258 re-tests.

A summary of the final results and a histogram displaying the near-normal distribution of mean infection by BYV across all accessions are given in Table 9 and Fig. 7. The complete BYV database is given in Appendix 5. Six accessions (1%) were identified as highly resistant (RS 1): half of these were in the sections *Corollinae* and the remainder in the sections *Procumbentes* (1 accession) and *Beta* (2 accessions) – see Table 10.

Fig. 7 BYV frequency distribution



If accessions with an RS of 2 were also considered, the number of potential sources rose to 51 (8.5%). Accessions with RS values of 2 are still highly resistant when compared with the standard sugar-beet cultivar (cv. Saxon), which had an RS of 6. Although species from the sections *Corollinae* and *Procumbentes* were collectively more resistant than those of section *Beta*, they exhibited more variability in response than when challenged with BMYV. Nevertheless, accessions of *B. intermedia* *B. lomatogona*, *B. macrorhiza* and *B. patellaris* were extremely resistant. When considered collectively, no section *Beta* species outperformed any other. However, there were a number of fodder-beets, garden-beets, leaf-beets, *B. vulgaris* and *B. maritima* accessions that had RS values of 1 or 2.

Table 9 – Summary of BYV disease resistance in *Beta* species							
***Beta* Species**	**No. accessions tested**	**Mean Score**	**Range of Scores**	**Accessions – RS1**		**Accessions – RS 2**	
				No.	**%**	**No.**	**%**
Section *Beta* - (i) cultivated forms							
B.v. vulgaris – fodder beet	61	4.5	2-8	0	0	6	10
B.v. vulgaris – garden beet	122	4.5	1-9	1	1	8	7
B.v. vulgaris – leaf beet	124	4.7	2-9	0	0	11	9
B.v. vulgaris – sugar beet	31	4.7	2-9	0	0	1	3
Section *Beta* - (ii) non-cultivated forms							
B. macrocarpa	12	5.3	3-8	0	0	0	0
B. patula	1	5.0	5	0	0	0	0
B. vulgaris	50	4.3	1-7	1	2	4	8
B.v. adanensis	12	5.9	4-9	0	0	0	0
B.v. maritima	158	4.6	2-9	0	0	11	7
Section *Corollinae*							
B. corolliflora	7	2.7	2-4	0	0	3	43
B. intermedia	1	1.0	1	1	100	1	100
B. lomatogona	1	1.0	1	1	100	1	100
B. macrorhiza	4	2.3	1-4	1	25	3	75
B. trigyna	-	-	-	-	-	-	-
Section *Procumbentes*							
B. patellaris	6	3.7	1-6	1	17	1	17
B. procumbens	3	4.7	2-9	0	0	1	33
B. webbiana	1	3.0	3	0	0	0	0
Unknown	3	5.7	5-6	0	0	0	0
Total/Mean of All Accessions	597	4.6		6	1	51	9

Screening for BYV resistance was conducted with an ELISA test, in which a polyclonal antibody was used. This method proved reliable in the majority of cases where accessions were tested twice, or more, to confirm resistance, but occasionally results were more variable; in each case the lowest resistance score was included in the database. A new BYV ELISA test using a monoclonal antibody was introduced in the summer of 2001 at IACR-Broom's Barn; this should prove more sensitive in detecting the virus than the old test and may improve reliability of results in future tests. However, a small comparative test, where the old and new methods were tested together, showed that most results correlated, thus indicating that the old method was adequate for discriminating between accessions.

Table 10 – BYV evaluation – accessions with RS of 1

Type	**Accession numbers**
Garden Beet	9154
Intermedia	3222
Lomatogona	2502
Macrorhiza	3200
Patellaris	3229
Vulgaris	5166

3.5 Task R 2.5 Screening of 600 accessions for *Erysiphe* [powdery mildew] resistance.

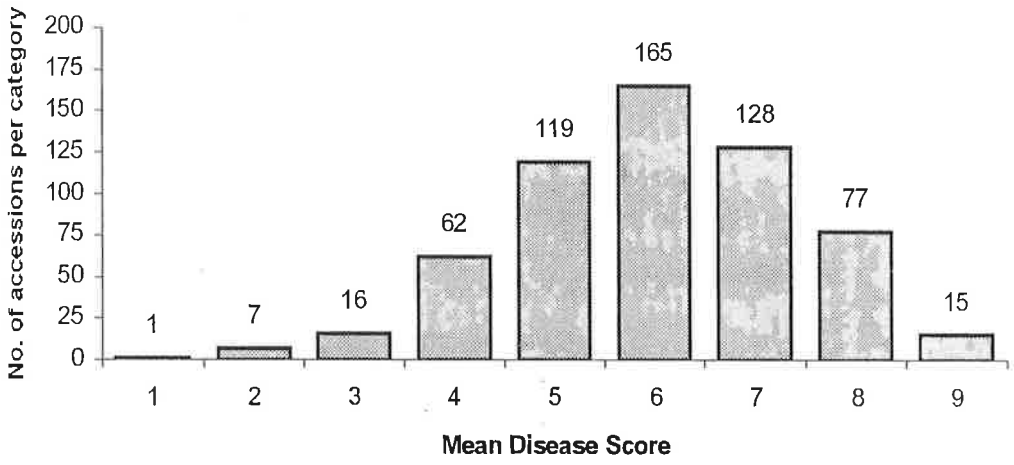
The screening programme for powdery mildew resistance has been successfully completed. Unlike the other resistance/tolerance traits, screening for mildew resistance was conducted primarily in the field. Annual epidemics of mildew are sufficiently consistent at IACR-Broom's Barn to permit such screening, a view firmly supported by the results obtained during the duration of this programme. The original screening protocol worked well throughout the tests and therefore warranted no changes. However, it was important to ensure annual types were cut back regularly up until about a month before disease assessment to prevent premature senescence of plants.

Accessions in the sections *Corollinae* and *Procumbentes* could not be field-tested, as it was anticipated they would be unable to survive in the cool temperate climate of Eastern England. Instead, a novel glasshouse screening method was developed specifically for these species. The data collected from these glasshouse tests (which included accessions that were also screened in the field so correlations could be made) indicated that the method was adequate for evaluating the resistance of these more sensitive species. However, because of problems with seed quantities and germination rates it was not possible to test all accessions of the sections *Corollinae* and *Procumbentes.* Details of all the methods used in screening for powdery mildew resistance are given in Appendix 1E.

In total, 590 accessions were tested using both methods, 575 in the field and 15 in the glasshouse; there were 80 re-tests in the field. The remaining ten accessions, mostly from the sections *Corollinae (B. corolliflora* 2524, 2971, 2982; *B. macrorhiza* 3199) and *Procumbentes* (*B. patellaris* 5938, 6535, 7042; *B. procumbens* 1630, 1663; *B. webbiana* 3244) were not tested.

The data from these tests are summarised in Table 11 and Fig. 8. The complete database is given in Appendix 6. The frequency distribution curve was slightly skewed towards susceptibility; this reflected the difficulties of exactly co-ordinating data collection with the precise time of maximum range in infection levels in the field. The results indicated that only one accession, a leaf beet (IDBB 1098), had an RS of 1, equivalent to 0.2% of all accessions tested.

Fig. 8 Powdery Mildew frequency distribution



When accessions with RS values of 2 were included, the number of resistance sources rose to eight (1.3%). More importantly, most of the highly resistant accessions were of the section *Beta* (garden- and leaf-beets) and therefore fully compatible with sugar beet, making them extremely useful in conventional breeding programmes. Collectively, species within the section *Procumbentes* showed above average resistance to powdery mildew, whereas species of the section *Corollinae* were extremely susceptible. The standard sugar beet cultivar Sandra had an RS of 6.

Table 11 – Summary of Powdery Mildew disease resistance in *Beta* species							
***Beta* Species**	**No. accessions tested**	**Mean Score**	**Range of Scores**	**Accessions – RS1**		**Accessions – RS 2**	
				No.	**%**	**No.**	**%**
Section *Beta* - (i) cultivated forms							
B.v. vulgaris – fodder beet	61	6.3	4-8	0	0	0	0
B.v. vulgaris – garden beet	122	5.7	2-8	0	0	3	2
B.v. vulgaris – leaf beet	124	6.0	1-9	1	1	3	2
B.v. vulgaris – sugar beet	31	6.1	4-9	0	0	0	0
Section *Beta* - (ii) non-cultivated forms							
B. macrocarpa	12	7.3	5-8	0	0	0	0
B. patula	1	5.0	5	0	0	0	0
B. vulgaris	51	5.8	3-9	0	0	0	0
B.v. adanensis	12	6.7	5-8	0	0	0	0
B.v. maritima	158	6.1	3-9	0	0	0	0
Section *Corollinae*							
B. corolliflora	4	7.0	7	0	0	0	0
B. intermedia	1	8.0	8	0	0	0	0
B. lomatogona	1	7.0	7	0	0	0	0
B. macrorhiza	4	7.3	7-8	0	0	0	0
B. trigyna	1	8.0	8	0	0	0	0
Section *Procumbentes*							
B. patellaris	3	2.7	2-4	0	0	2	67
B. procumbens	1	3.0	3	0	0	0	0
B. webbiana	-	-	-	-	-	-	-
Unknown	3	7.0	3-6	0	0	0	0
Total/Mean of All Accessions	590	4.5		1	0.2	8	1

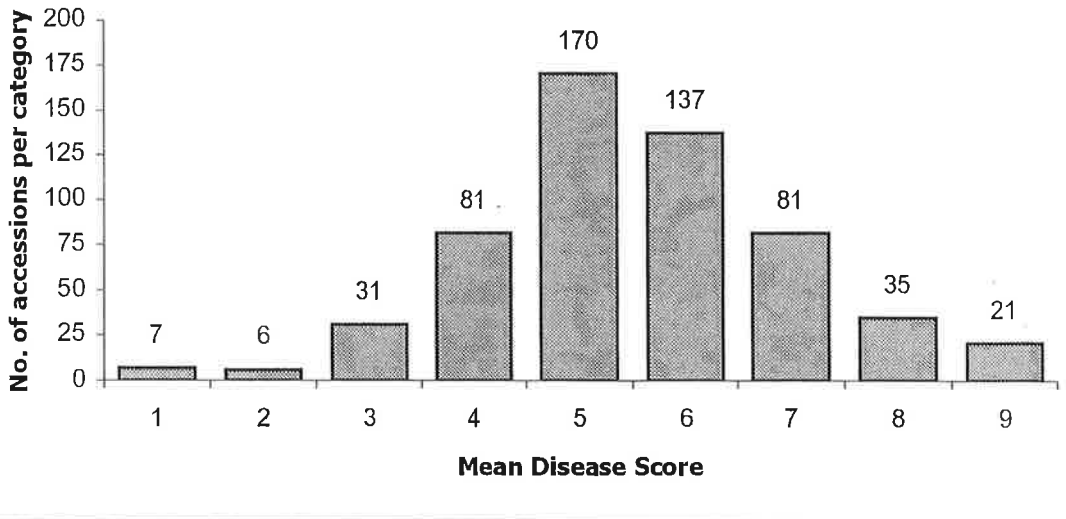
3.6 Task R 2.7 Screening of 600 accessions for drought tolerance and salinity

In total, 594 accessions were screened for drought stress tolerance in 60 experiments (equivalent to 910 individual tests) by the completion of the project. Data from twelve experiments (or 178 tests) were discarded as analysis indicated that drought stress conditions in those experiments were not severe enough to cause significant differences in growth between control and stressed plants. Six accessions were not tested because of insufficient seed; these were accessions 3296, 3626, 4129, 6336, 6829 and 8644. Of the 594 accessions where screening was attempted, 25 (Table 12), primarily of the section *Corollinae* and *Procumbentes,* failed to germinate in adequate numbers. Therefore, data are only available for 569 accessions.

The experimental approach used to screen for drought stress was significantly altered in the early part of the programme when it was discovered that the originally proposed method, based on measuring differences in photosynthetic activity in stressed and unstressed leaves, was inadequate to distinguish between accessions. The novel method (see Appendix 1F for description) subsequently developed seemed to overcome these deficiencies. The method of analysis of results obtained has been given in section 2.6.

The results obtained from screening, largely limited to species of the section *Beta* for the reasons stated above, are summarised in Table 13 and Fig 9. The graph indicated that the distribution of responses had a near-normal distribution. An examination of the results showed that, individually, there was a wide range of responses: the complete drought stress database is given in Appendix 7. Collectively, there was little difference between the cultivated members of the section *Beta*, although there were individual leaf and garden beets which had drought stress scores (DS) of 1 and 2. Early indications that the sugar-beet accessions looked promising were not fully verified after all testing was completed. The sugar beet standard cv. Saxon scored 5. *B.v. adenesis* appeared collectively to have the highest drought tolerance amongst the non-cultivated forms of the section *Beta*, but individually accessions of *B. macrocarpa* and *B.v. maritima* fared best under drought conditions. A list of the best accessions is given in Table 14.

Fig. 9 Drought Stress Tolerance frequency distribution



The frequency of *B.v. maritima* accessions (5 accessions or 3% of the total) with DS1 may indicate that this species, given its maritime habitat, is the most likely sources of tolerance genes. However, none of the remaining 150 accessions of *B.v. maritima* were better than the cultivated forms, and, in several cases, were much worse. This, perhaps surprising, observation is in line with observations made in field tests at IACR-Broom's Barn where *B.v. maritima* accessions were generally no better than sugar beet at withstanding drought stress. It should be stated that, in most cases, it was impossible to re-test these promising *B.v. maritima* accessions because of a lack of seed. Despite the inability to test many accessions of the sections *Corollinae* and *Procumbentes*, the limited results available indicated that these groups should not be ignored. A *B. webbiana* accession had an RS of 2.

Although individual accessions were assigned DS values that reflect their relative performance in these tests, care must be taken in interpreting these results. In the absence of standards, either drought tolerant or susceptible, the allocation of scores from 1 to 9 was arbitrary, covering the range of all results obtained. The method itself appears to be consistent: results indicated, that for the majority of accessions tested twice or more, results were similar. However, how these observations in a controlled environment (CE) room might translate to a field situation is unknown; it is possible that the range of growth responses observed were small in reality, leading to minimal differences in the field. The relationship between observations in CE rooms and the field is being studied by plant physiologists at IACR-Broom's Barn. Finally, of course, the test devised was unable to select for drought tolerance based on superior rooting ability. This may be an important component in the field.

There were no separate tests for salinity conducted during this programme as it was believed that the mechanisms responsible for salinity tolerance would be identical for those for drought stress tolerance.

Table 12 – Drought Stress – accessions not tested because of poor germination

Type	Accession numbers
Corolliflora	2326, 2524, 2971, 2982, 2987, 3221, 7144
Intermedia	3222
Lomatogona	2502
Macrorhiza	3199, 3200, 8539, 8541, 8548
Maritima	25, 3798
Patellaris	1645, 3229, 6535, 7042
Procumbens	1630, 1663, 3242
Trigyna	3707
Vulgaris	5215

Table 14 – Drought Stress tolerance – accessions with RS of 1

Type	Accession numbers
Leaf Beet	6168
Macrocarpa	3183
Maritima	200, 3350, 6106, 7078, 7088

Table 13 – Summary of Drought Stress Tolerance in *Beta* species

Beta Species	No. accessions tested	Mean Score	Range of Scores	Accessions – RS1		Accessions – RS 2	
				No.	%	No.	%
Section *Beta* - (i) cultivated forms							
B.v. vulgaris – fodder beet	59	5.5	3-9	0	0	0	0
B.v. vulgaris – garden beet	121	5.5	2-9	0	0	2	2
B.v. vulgaris – leaf beet	122	5.6	1-9	1	1	4	3
B.v. vulgaris – sugar beet	31	5.7	3-9	0	0	0	0
Section *Beta* - (ii) non-cultivated forms							
B. macrocarpa	12	5.3	1-9	1	8	1	8
B. patula	1	6.0	6	0	0	0	0
B. vulgaris	50	5.7	3-9	0	0	0	0
B.v. adanensis	12	4.8	3-8	0	0	0	0
B.v. maritima	155	5.7	1-9	5	3	5	3
Section *Corollinae*							
B. corolliflora	-	-	-	-	-	-	-
B. intermedia	-	-	-	-	-	-	-
B. lomatogona	-	-	-	-	-	-	-
B. macrorhiza	-	-	-	-	-	-	-
B. trigyna	-	-	-	-	-	-	-
Section *Procumbentes*							
B. patellaris	2	4.5	3-6	0	0	0	0
B. procumbens	-	-	-	-	-	-	-
B. webbiana	1	2.0	2	0	0	1	100
Unknown	3	5.0	4-6	0	0	0	0
Total/Mean of All Accessions	569	5.5		7	1	13	2

4. Potential for Exploitation and Future Work

The output from the various screening programmes conducted at IACR-Broom's Barn may be exploited in several ways. These can be summed up as follows

(a) Release of resistance/tolerance data to sugar-beet breeders
(b) Pre-breeding of disease resistant accessions
(c) Determination of genetic control of disease resistance expression.
(d) Development of molecular markers to resistance genes to promote marker-assisted selection in future breeding programmes
(e) Continued research on drought stress tolerance in the laboratory and the field

All the data generated at IACR-Broom's Barn will be place in the public domain by the project co-ordinator, Dr Frese of BAZ, Germany, so that sugar beet breeders and researchers may use them for developing future work programmes. Publication will occur in several ways, including on the Internet via the existing IDBB database and by the preparation of papers for scientific and other interested journals.

A programme of pre-breeding of disease resistant accessions has commenced at IACR-Broom's Barn so that novel resistance genes identified during the screening programmes can be introgressed into sugar beet breeding lines, which in turn, will be incorporated into commercial breeding programmes. The potential of this work has been recognised by the European Union and two years' funding to support this work has been provided as part of the EU-CRAFT programme.

The identification of disease resistance in *Beta* accessions in these screening programmes will lead to an increased understanding of the genetic control of these traits and, eventually, new molecular technologies that would radically alter the approach to selection of resistance and breeding. At present, despite having several novel sources of disease resistance, little is known about the relevant genes and how they function, e.g., whether different sources have different genes, whether genes are allelic, closely linked, or amenable to recombination to enhance resistance. Currently, the genetics of disease resistance is being investigated (e.g., whether resistance is monogenic and estimating its heritability) using molecular marker technology, and already some genes for disease resistance derived from wild *Beta* germplasm have been mapped. Also, molecular markers for a major gene conferring partial resistance to BNYVV (Rhizomania) and for quantitative trait loci (QTLs) determining resistance to powdery mildew derived from *Beta v. maritima* have been successfully located. Such investigations will enhance the introgression of disease resistance genes from wild sources into sugar-beet.

The development of molecular markers will also provide breeders with the opportunity to accelerate resistance screening in breeding programmes by replacing phenotypic testing, which is laborious and can be prone to environmental variation, with more consistent genotypic tests. DNA samples from test plants can be screened for the presence of molecular markers for a particular disease resistance gene.

The significance of drought stress on sugar beet production in Europe has been fully realised in recent years. Unfortunately, the mechanisms that govern drought stress tolerance in *Beta* species, in particular how the trait is controlled genetically, are not as fully understood as in disease resistance, making manipulation of novel sources of drought tolerance more difficult. Work has commenced at IACR-Broom's Barn to investigate these aspects of drought stress more systematically, leading ultimately to more tolerant sugar beet cultivars. As part of this programme the results collected on drought stress tolerance in CE rooms as part of the GENRES programme will be utilised so selections for field testing can be made.

5. Publications

The following publications have been prepared during the course of the programme at IACR-Broom's Barn:

1. Luterbacher, MC, Smith JM, Asher MJC (1998)
Sources of disease resistance in wild *Beta* germplasm.
Aspects of Applied Biology Vol 52, 423-430

2. Luterbacher MC, Smith JM (1998)
Improving disease resistance and drought-stress tolerance in sugar beet using wild *Beta* germplasm.
British Sugar Beet Review Vol 66 (4), 26-29

3. Luterbacher MC, Smith, JM, Asher MJC (2000)
Disease resistance sources in different Beta populations (publication of poster)
Proceedings of the 63rd Congress of the International Institute for Beet Research. IRBB, Brussels. 459 – 464.

4. Luterbacher MC, Smith JM, Asher MJC, Frese L (2000).
Disease resistance in collections of *Beta* species
Journal of Sugar Beet Research. 37 (3), 39-47

5. Luterbacher, MC (2001)
Screening *Beta* Germplasm for New Sources of Disease Resistance in Sugar Beet
F.O. Licht International Sugar Yearbook 2001 F7-F13

6. Asher MJC, Luterbacher MC and Frese L (2001)
Wild *Beta* species as a source of resistance to sugar-beet pests and diseases.
Proceedings of the 64th Congress of the International Institute for Beet Research. IRBB, Brussels. 141 - 152.

7. Asher MJC, Luterbacher MC and Frese L (2001)
Wild *Beta* species as a source of resistance to sugar-beet pests and diseases.
International Sugar Journal. 103, 447-456

8. Ober, ES and Luterbacher MC (in press)
Genotypic variation for drought tolerance in *Beta vulgaris*
Annals of Botany

APPENDIX 1:

EXPERIMENTAL PROCEDURES FOR SCREENING *BETA* GERMPLASM

(A) *APHANOMYCES* DAMPING-OFF

(1) Basic Site Data

Country of Evaluation: Great Britain

Site of Seed Evaluation:

Name:	IACR-Broom's Barn
Latitude:	52 16N
Longitude:	00 34 E
Altitude:	75m ASL

Evaluator's name and address: IACR-Broom's Barn,
Higham, Bury St. Edmunds,
Suffolk, IP28 6NP
UK.

Evaluation Environment: Controlled Environment Room

Soil Taxonomic Classification: Not Applicable.

(2) Methods:

1.1 Method applicable for following data sets*: 3-1*

Screening for *Aphanomyces* resistance was conducted in a controlled environment room. For each accession 96 seeds (4 replications x 24 seeds) were sown in partially sterilised soil inoculated with *Aphanomyces cochlioides* (0.2% w/w), previously grown on cornmeal/sand medium for three weeks. Trays containing seeds were maintained at 22°C with high soil moisture content for four weeks. Emerged seedlings were scored individually for *Aphanomyces* infection on a 0-4 scale (0 = no infection; 4 = seedling dead) and using the mean values obtained, an adjusted disease score relative to the susceptible standard cv. Saxon for each accession was calculated. These values were transformed to a standardised 1-9 scale. The standards included in each test were the sugar beet cv. Saxon & a *B. v. maritima* accession (IDBB No. 2193).

APPENDIX 1:

EXPERIMENTAL PROCEDURES FOR SCREENING *BETA* GERMPLASM

(B) *PYTHIUM* DAMPING-OFF

(1) Basic Site Data

Country of Evaluation: Great Britain

Site of Seed Evaluation:

Name:	IACR-Broom's Barn
Latitude:	52 16N
Longitude:	00 34 E
Altitude:	75m ASL

Evaluator's name and address: IACR-Broom's Barn,
Higham, Bury St. Edmunds,
Suffolk, IP28 6NP
UK.

Evaluation Environment: Temperature Controlled Glasshouse

Soil Taxonomic Classification: Not Applicable.

(2) Methods:

1.2 Method applicable for following data sets*: 4-1*

Screening for *Pythium* blackleg resistance was conducted in a temperature-controlled glasshouse. For each accession 96 seeds (4 replications x 24 seeds) were sown in partially sterilised soil inoculated with *Pythium ultimum* (0.75% w/w), previously grown on cornmeal/sand medium for three weeks. A control treatment of 48 seeds for each accession (2 replications x 24 seeds) was also sown in uninoculated partially sterilised soil. Trays containing both inoculated and uninoculated seeds were maintained at 22°C with high soil moisture content for three weeks. Seedlings were classified as either infected or not infected; pre-emergence infection was determined from the uninoculated control. The mean % seedling infection value for each accession was transformed to standardised 1-9 scale. The standards included in each test were the sugar beet cvs. Saxon & Komet.

APPENDIX 1:

EXPERIMENTAL PROCEDURES FOR SCREENING *BETA* GERMPLASM

(C) BEET MILD YELLOWING VIRUS (BMYV)

(1) Basic Site Data

Country of Evaluation:	Great Britain
Site of Seed Evaluation:	
Name:	IACR-Broom's Barn
Latitude:	52 16N
Longitude:	00 34 E
Altitude:	75m ASL
Evaluator's name and address:	IACR-Broom's Barn, Higham, Bury St. Edmunds, Suffolk, IP28 6NP, UK.
Evaluation Environment:	Temperature Controlled Glasshouse
Soil Taxonomic Classification:	Not Applicable.

(2) Methods:

1.3* Method applicable for following data sets*: 1-1

All BMYV tests were conducted in a temperature-controlled glasshouse. Twenty-four seedlings per accession and a standard sugar beet cultivar (cv. Saxon) included in each test were grown to the two true-leaf stage and inoculated with aphids (*Myzus persicae*) carrying BMYV (viruliferous aphids were raised on *Capsella bursa pastoris* plants previously inoculated with BMYV). After four days plants were fumigated to remove aphids and grown on for four weeks under high light intensity. Using leaf discs cut from the first true leaves of each accession and the standard, the virus content was quantified by ELISA. The ELISA test was conducted with a monoclonal antibody specific for BMYV strain 1. For each accession and the standard a mean % virus content was calculated relative to a standard curve of purified virus. The % virus data from each test were adjusted relative to the standard sugar beet cultivar (cv. Saxon) to reduce variation between tests, and data transformed to a 1-9 resistance scale.

APPENDIX 1:

EXPERIMENTAL PROCEDURES FOR SCREENING *BETA* GERMPLASM

(D) BEET YELLOWS VIRUS (BYV)

(1) Basic Site Data

Country of Evaluation: Great Britain

Site of Seed Evaluation:

Name:	IACR-Broom's Barn
Latitude:	52 16N
Longitude:	00 34 E
Altitude:	75m ASL

Evaluator's name and address: IACR-Broom's Barn,
Higham, Bury St. Edmunds,
Suffolk, IP28 6NP
UK.

Evaluation Environment: Temperature Controlled Glasshouse

Soil Taxonomic Classification: Not Applicable.

(2) Methods:

1.4 Method applicable for following data sets*: 2-1*

All BYV tests were conducted in a temperature-controlled glasshouse. Twenty-four seedlings per accession and a standard sugar beet cultivar (cv. Saxon) included in each test were grown to the two true-leaf stage and inoculated with aphids (*Myzus persicae*) carrying BYV (viruliferous aphids were raised on *Tetragonia expansa* plants previously inoculated with BYV). After four days plants were fumigated to remove aphids and grown on for three weeks under high light intensity. Using leaf discs cut from the first true leaves of each accession and the standard, the virus content was quantified by ELISA. The ELISA test was conducted with a polyclonal antibody specific for BYV. For each accession and the standard a mean % virus content was calculated relative to a standard curve of purified virus. The % virus data from each test were adjusted relative to the standard sugar beet cultivar (cv. Saxon) to reduce variation between tests, and data transformed to a 1-9 resistance scale.

APPENDIX 1:

EXPERIMENTAL PROCEDURES FOR SCREENING *BETA* GERMPLASM

(E) POWDERY MILDEW

(1) Basic Site Data

Country of Evaluation: Great Britain

Site of Seed Evaluation:

Name: IACR-Broom's Barn
Latitude: 52 16N
Longitude: 00 34 E
Altitude: 75m ASL

Evaluator's name and address: IACR-Broom's Barn,
Higham, Bury St. Edmunds,
Suffolk, IP28 6NP. UK.

Evaluation Environment: Field/ Temperature controlled glasshouse

Soil Taxonomic Classification: Sandy Loam.

(2) Methods:

(a) Method applicable for following data sets*: 5-1*

Test for powdery mildew resistance in the field. One plot per accession containing 10 plants was sown in three rows in the field in May. Natural field epidemics of powdery mildew were used to infect plants, and to encourage these, plots were interplanted at regular intervals with a susceptible sugar-beet cultivar (cv. Sandra). Plants bolting or senescing were cut back to lengthen the growing period. Disease assessments on 10 plants were made in August using a 0-5 infection scale, where 0 = no infection and 5 = whole plant totally infected. The mean values obtained were transformed to the standard 1-9 scale. The standard susceptible sugar beet cultivar used was cv. Sandra.

***(b) Method applicable for following data sets:* 5-2**

As method (a) except one plot contained 25-30 plants and disease assessments are made on 25 plants in September.

***(c) Method applicable for following data sets:* 5-3**

Testing for powdery mildew in the glasshouse. This method relied on naturally occurring inoculum already present in the glasshouse for infection (artificially inoculating the accessions with mildew conidia generated on a highly susceptible sugar beet cultivar made no difference to the outcome). Thirty plants per accession were raised in 2½" pots, and grown in an enclosed glasshouse maintained at 25°C with a 16-h light regime. All accessions were assessed initially when the first symptoms were observed (approximately 8-10 weeks after sowing); subsequent observations were made at two-week intervals. The result set which showed the greatest range of responses was used to evaluate resistance.

APPENDIX 1:

EXPERIMENTAL PROCEDURES FOR SCREENING *BETA* GERMPLASM

(F) DROUGHT STRESS TOLERANCE

(1) Basic Site Data

Country of Evaluation: Great Britain

Site of Seed Evaluation:

Name:	IACR-Broom's Barn
Latitude:	52 16N
Longitude:	00 34 E
Altitude:	75m ASL

Evaluator's name and address: IACR-Broom's Barn,
Higham, Bury St. Edmunds,
Suffolk, IP28 6NP. UK.

Evaluation Environment: Controlled Environment Room

Soil Taxonomic Classification: Not applicable

(2) Methods:

***(a) Method applicable for following data sets*: 6-1**

Seeds of the accessions to be tested were steeped in the fungicide Thiram for 24 h prior to sowing. 40 seedlings, previously grown in potting compost, were transplanted into 2½" pots. Four weeks after sowing, 10 seedlings of each accession were selected randomly and all plant growth above soil level was removed, dried for 24 h, and weighed: a mean dry weight value was obtained for each accession. Subsequently, 10 & 15 plants per accession were selected randomly from the remaining seedlings; these acted as the control (i.e., unstressed) and stressed test plants respectively. Plants were separated and placed in a controlled environment room set at 23°C/18°C 16h-day/8h night. Control plants were watered daily and stressed plants twice a week. In both cases, when watered the compost was saturated to capacity. Eight weeks after sowing all plants, both unstressed and stressed, were dried and mean dry weights determined. From the data collected, the reduction in growth & the growth rate of plants under the stressed and unstressed regimes could be calculated.

The success of each test was determined by the performance of the sugar beet standard (cv. Saxon) included in each test; if no significant differences ($P<0.05$) in weight of stressed and unstressed plants of Saxon were observed the data were discarded. Data from all the successful tests were normalise with reference to the standard, (sugar beet cultivar Saxon) included in each test, and subsequently used to calculate the mean drought stress response for all accessions. The deviation of the response of individual accessions from this mean relationship was used to gauge their stress tolerance; those with a better than expected response were considered more likely to tolerate physiological drought stress. The range of deviations observed were divided into nine classes of equal size; drought tolerance scores (scale 1-9) were assigned arbitrarily to each of these nine classes with a score of 1 assigned to those deemed to be most tolerant.