The Synthetic *Beta* Core Collection – State of the Art

L. Frese

Federal Centre for Breeding Research on Cultivated Plants (BAZ), Gene Bank, Bundesallee 50, D-38116 Braunschweig, Germany

ABSTRACT

More than 10,000 *Beta* accessions are maintained in genebanks worldwide. *Beta* core collection accessions were designated to facilitate access to useful genetic variation within large holdings. A set of core collection accessions can be selected by different procedures, depending on the crop and the type of available data. The branching and path indicator (PI) method has been used to establish a *Beta* core collection consisting of 805 accessions. The core collection currently is being screened for resistance to eight diseases and for drought tolerance within a collaborative project funded by the Commission of the European Countries. The central project database contains 2,013 evaluation results, expressed on a 1 to 9 scale. In 122 events, accessions were scored equal to or lower than '3' (= very low to low susceptibility). The preliminary analysis of the evaluation data detected 60 of the 122 events in PI132 (*B. vulgaris* ssp. *maritima*) followed by 21 events in PI135 (*B. vulgaris* ssp. *vulgaris* 'Garden Beet').

Additional Key Words: Branching method, evaluation, genetic diversity, path indicator, resistance.

The risk of screening genetically redundant material is significant when a genetic resources collection is large. To minimize this risk, Frankel (1984) suggested developing core collections that would represent the genetic diversity in a collection with minimum repetitiveness. Since then, core collections have been developed for many crops to improve the efficiency of germplasm screening (van Hintum, 1999). Data from DNA fingerprinting, RFLP and PCR-based marker and DNA sequencing techniques, morphological, yield and quality characters have

been used in cluster analysis. The resulting dendrograms display the genetic diversity of the genus *Beta* as an hierarchical tree. According to classical taxonomists (e.g. Buttler, 1977), the genus is divided into four sections or 'main branches' of the diversity tree. Jung et al. (1993) and Shen et al. (1998) described genetic relationships among sections by means of molecular markers. Their results generally agree with classical taxonomy. Within each section of the genus *Beta,* the genetic diversity is organized like side branches of a tree. Letschert (1993) divided *Beta* section *Beta* into wild species and wild subspecies based upon morphological characters and allozymes. Cultivated material of *B. vulgaris* forms four groups (Lange et al., 1998) within which individual hierarchical classification of accessions is also possible. This was shown by Holland (1956) and later by Michalik et al. (1998), using morphological traits, yield components, and RAPD. Further divisions into origin region and origin country within an individual side branch would produce an even more complex diversity tree such as proposed in Figure 1. It can be assumed that some branches of the diversity tree contain more useful genetic characters than others. The objective of this paper is to describe how evaluation data on genebank accessions can be allocated to branches of the diversity tree and how this procedure can reveal parts of the collection containing a high amount of useful genetic variation.

MATERIAL AND METHODS

Van Hintum (1995) distinguished two types of hierarchical methods suited to construct a core collection: the clustering method and the branching method. The clustering method can be applied if characterization data are available for cluster analysis. If such data are not available or do not qualify for statistical analysis, the branching method can be applied. The branching method is based on the analysis of passport data and knowledge of the genetic structures of a genepool, here the genus *Beta.*

The establishment of a core collection is an objective of the EU project GENRES CT95 42. The International Database for *Beta* (IDBB) currently holds passport information on 10,579 entries. If hybrid varieties, aneuploid, and other materials are excluded, 8,655 accessions remain. These are held by 28 institutions world-wide. A database descriptor 'Core_col' was added to the passport table of the IDBB to allow documentation of the selected core collection samples. When an accession became part of the core collection, it was tagged with a '+' in this database field. By setting the tag in the International Data Base for Beta (IDBB) a core collection accession was assigned to one of the 28 national holdings. This procedure yielded a 'synthetic core collection', a term defined

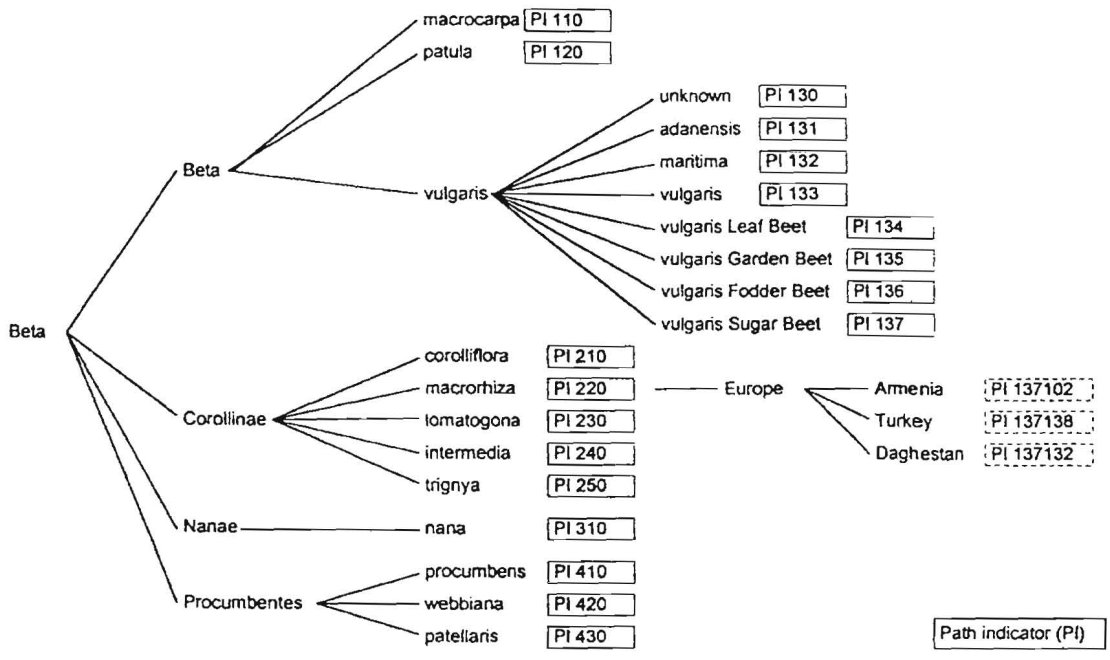

Figure 1. Construction of path indicators for *Beta*. The first digit encodes the section, the second the species, the third the subspecific level, the fourth the origin and the last two digits the origin country of the accessions.

by Knüpffer and van Hintum (1995). The set of accessions composed of samples maintained by partner genebanks of the World *Beta* Network (WBN) was called the 'Synthetic *Beta* Core Collection' (SBCC). In late 1995, a preliminary set of SBCC accessions was chosen, mainly on the basis of taxonomic and geographic criteria. If many accessions had been collected in a country, the geographic co-ordinates of the collection sites were used to choose populations in which distances between collecting sites was about 50 km.

Boukema et al. (1997) elaborated the branching method further while using path indicators to designate and describe a core collection of *Brassica oleacea*. The basic idea of path indicators is that a genepool can be divided into germplasm groups. The path indicator method has been applied in the GENRES CT95 42 project to describe the composition of the SBCC, which is being evaluated in the EU *Beta* project. To construct path indicators (PI) for *Beta*, a coding system for five categories (section name, species name, subspecific name, origin region, origin country) was established (Figure 1). A more extensive table of origin region and country codes can be requested from the author. The species name, the subspecific name, and the origin country of all accessions documented in the IDBB were used to sort the 8,655 accessions into distinct groups and to determine the total number of accessions within a group. Then the same database query was restricted to the 805 tagged core collection accessions and the database query was run again to count the number of core collection accessions represented in a group.

Five project partners of the EU project are evaluating the SBCC for resistance to eight disease agents (Rizomania, BMYV, BYV, *Aphanomyces cochlioides, Pythium ultimum, Rhizoctonia solani, Erysiphe betae, Cercospora beticola*) and to drought tolerance from 1996 to 2001. Disease resistance and drought tolerance are assessed in field and/or greenhouse experiments, either by measuring or scoring the trait. All data is then expressed on a 1 to 9 scale whereby '1' indicates very low susceptibility and '9' very high susceptibility. In total, 2,013 individual test results were sent to the BAZ Gene Bank which is coordinating the project, then recorded in a database and used for analysis by the path indicator method. Accessions with a score equal to or less than '3' (= low susceptibility) were counted per path indicator and the results have been presented in column 8 of Table 1.

RESULTS

The database query yields 353 distinct combinations of species, subspecies and origin countries when all 8,655 accession are considered

Table 1. Structure of the synthetic *Beta* Core Collection as described by the path indicator method.

Path indicator (PI)	No. of subpaths (NSP)	No. of sel. Subpaths (NSSP)	NSSP in % of NSP	No. of acc.* IDBB	No. of acc.* CC	CC %	No. of '3' scores
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
110	12	8	67	89	23	26	2
120	2	1	50	11	1	9	—
130	50	19	38	1705	93	5	5
131	3	3	100	65	17	26	—
132	31	23	74	1821	214	12	60
134	40	6	15	510	8	2	2
134	35	26	74	576	128	22	12
135	46	22	48	741	132	18	21
136	31	18	58	630	69	11	13
137	39	16	41	1478	35	2	6
210	4	2	50	110	30	27	1
220	8	2	25	67	8	12	—
230	5	2	40	236	20	8	—
240	5	1	20	267	6	2	—
250	9	2	22	102	5	5	—
310	1	0	0	44	0	0	—
410	4	1	25	59	5	8	—
420	2	1	50	30	3	10	—
430	7	3	43	114	8	7	—
Sum	**334**	**156**	—	**8655**	**805**	—	**122**

Legend: **Column 1**: PI = path indicator. **Column 2**: NSP = no. of subpaths within PI determined by a query of the International Database for Beta (IDBB). The following sample categories were excluded from the query: 'No longer in collection' - NOC and 'Not within genebank responsibility' –NOG (Frese and van Hintum, 1989). **Column 3**: NSSP = No. of selected subpaths. **Column 4**: NSSP expressed in percent of NSP. This indicates how well a taxa is geographically represented in the core collection. **Column 5**: No. of accessions documented in the IDBB and belonging to the PI. **Column 6**: No. of accessions belonging to the PI and selected for the core collection. **Column 7**: The value of column 6 expressed as percentage of column 5 indicating the weight given to a taxa during the choice of individual accession from the total holding. Low percentage = low representation of the taxa in the core. **Column 8**: Number of evaluation events resulting in a disease score equal to or lower than '3' = very low to low susceptibility.

(Table 1). If the genepool is subdivided based only on taxonomic criteria, 19 path indicators (PI) result (Figure 1). Within each PI, accessions can be grouped according to their geographic origin. In Figure 1 an example was constructed showing the subdivision of PI220 at the branching points 'origin region' and 'origin country'. If we use passport data of all 8,655 accessions selected from the International Database for Beta (IDBB), PI135 can be divided into 46 subpaths indicating that *B. vulgaris* subsp. *vulgaris* 'Garden Beet' has the widest geographic distribution of all taxonomically detailed described accessions. In the case of PI310 (*B. nana*), no subdivision occurred because this species is endemic to Greece, and the number of subpaths is only one. The number of subpaths for all PI is given in column 2 of Table 1. The number of subpaths represented in the core collection is shown in column 3 and the percentage of their representation in the core collection is given in column 4. *B. nana* is not contained in the core collection because this species cannot be multiplied easily and is very difficult to handle in evaluation projects. Therefore the value for *B. nana* in column 4 is 0% in contrast to 100% for *B. vulgaris* subsp. *adanensis*, because all possible paths and subpaths of this subspecies are represented.

Column 5 contains the total number of accessions per path indicator and column 6 the number of accessions per path indicator selected for the core collection. Column 7 shows the number of accessions in the core collection expressed as percentage of the total number of accessions per PI. For individual PIs, the CC% values deviate by a factor of 2 to 5 from the 10% of total holdings recommended by Brown (1989). In the case of *B. macrocarpa* (PI110), many countries are represented in a small total collection of this specific species, 27% of the 89 accessions have been added to the core collection. An opposite extreme is the sugarbeet (PI137). Only 2% of 1,478 sugarbeet accessions will be screened, mainly obsolete open pollinated varieties of the main breeding companies in Europe. Very low priority has been given to sugarbeet germplasm with the argument that the genetic base of sugarbeet breeding material is narrow and novel genetic variation has to be found outside this material, for instance in garden beet or the subspecies '*maritima*'. A particularly high frequency of promising evaluation data recorded for PI132 (*B. vulgaris* subsp. *maritima*) (60 counts) is encouraging and calls for further analysis.

DISCUSSION

A core collection constructed by means of the path indictor method would be perfect if all possible subpaths were represented, an allocation of accessions to each subpath would be possible and sufficient

seed of the respective accessions would be available for screening. This was not the case with the SBCC used for the EU project. As with other crops (Boukema et al., 1997), the size and composition of the SBCC was limited by seed availability. However, a refinement of the preliminary set of SBCC accessions was an important objective for the international co-operation on *Beta* genetic resources from the start.

The branching method proved to be a valuable tool for constructing the SBCC and the path indicator method was helpful for sorting the results. The path indicators imply that the germplasm group '*maritima*' contains the most genetic variation for breeding resistance to *Rhizoctonia solani, Aphanomyces cochlioides, Phoma betae, Cercospora beticola, Erysiphe betae*, beet yellowing viruses (BMYV and BYV), Rizomania and drought. After completing evaluation work within the EU *Beta* project, a more detailed analysis of the individual PIs is planned to identify branches of the diversity tree with a high frequency of accessions showing useful variation in resistance to specific diseases or drought tolerance. Such analysis also could establish whether useful traits are concentrated in specific taxonomic groups and/or geographic regions or occur at random.

Joint actions need to be undertaken by genebanks maintaining *Beta* germplasm to improve the organization and quality of the core collection. The SBCC was established in 1995 as a working collection just before the start of the EU *Beta* project and, due to time constraints, without much participation of partner genebanks in the accession selection process. A few years before the EU funded new evaluation projects, core collections for crops such as *Brassica* and barley (Boukema et al., 1997, Knüpffer and van Hintum, 1995) were established by task forces of international working groups who made decisions jointly. For the international acceptance of the SBCC as a tool for screening and research projects, it is essential that genebanks jointly agree on the composition of the SBCC, on the maintenance responsibility for core collection accessions, the degree of phenotypic variation allowed in a specific sample, and rules for distribution of seed.

The development of a core collection should be a dynamic process. Improvement of the effectiveness of the SBCC could become an objective of joint actions. The proportion of germplasm represented in an individual path ranges from 0 to 27 percent (Table 1). If less than 10% of the total holding (Brown, 1989) is required to maximize the genetic diversity in a core collection, for example 200 accessions for section *Beta* (Ford-Lloyd and Lawrence, 1993), then redundant material in a particular path needs to be identified and removed and accessions that increase genetic diversity need to be added within another path. In the case of

garden beet (PI135), Michalik et al. (1998) used horticultural characterization data and suggested selecting 15 accessions to represent the genetic diversity contained in a collection of 40 accessions (Baranski et al., in press). The large size of the world *Beta* holding may have hindered researchers from starting a detailed analysis of genetic relationships among accessions held in genebanks. An advantage of the path indicator method is that groups of accessions can be formed which are better suited to individual research projects. If molecular marker techniques are used for investigations, the data arising from individual projects could be made comparable by using a set of molecular standard accessions, as suggested by the *Beta* Coordinating Committee (BCC) of the WBN in 1998. The results of the individual projects could then be combined to develop a more detailed description of the genetic diversity within *Beta* which, in turn, would suggest changes in the SBCC and result in more efficient use of *Beta* genetic resources.

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