End of project report NAP 02-201

Summary

The goal of this project was the genetic characterization of the Swiss genetic resources of maize. The use of neutral molecular markers ensures characterization and grouping of genotypes without influences of environment and selection. A method was used that can be set up in any laboratory space requiring little financial effort. The identification of possible doubles as well as the grouping of the material on the basis of genetic distance traits was achieved. The main groups identified related to material typical for cultivation south of the Alps and north of the Alps. These groups were further divided into multiple groups of accessions. The way accessions were grouped indicated that most exchange of maize material happened within the northern parts of the country, probably because maize was more difficult to cultivate under northern climatic conditions. Based on the grouping of the accessions according to genetic distances and a software approach to maximize diversity in a subset of all accessions a core subset was defined to represent the diversity of Swiss maize in extensive trials in the future. The core subset definition was carried out in close collaboration with NAP project 02-33 (Roland Peter) so available phenotypic information could be included in the process.

Introduction

The collection and maintenance of genetic resources of crop plants has been conducted in many countries throughout the past century. Thousands of accessions are stored in gene banks world wide. To evaluate and study their use in today's agriculture by investigating every single one of them is not practicable.

Instead it has been found effective to use easily available information about accessions to classify and group them. On this basis the definition of a sample representing as much of the diversity present in all the accessions of a certain crop can be attained (Van Hintum et al., 2000). The original definition of a core set: A core set is a limited set of accessions representing, with a minimum of repetitiveness, the genetic diversity of a crop species and its wild relatives (Frankel 1984 ZITAT). This was transformed to an operational definition for a gene bank: For an individual genebank, a core collection consists of a limited number of the accessions in an existing collection, chosen to represent the genetic spectrum in the whole collection. It should include as much as possible of its genetic diversity (Brown, 1995). This kind of core set will be referred to as core subset in this study. The information used to define this can be any information that is available about the collection. The inclusion of data from neutral traits like molecular markers was found to raise the quality of a core set as well as the amount of information that is available about the accessions (Van Hintum et al., 2000).

Different screening methods for genetic polymorphism in maize existed before this study was started (Dubreuil and Charcosset, 1999; Dubreuil et al., 1999; Matsuoka et al., 2002; Pejic et al., 1998; Senior et al., 1998). The approach by Senior et al. (1998) appeared cost effective and promising because it did not require investments for new laboratory equipment.

Our goals were to group the accessions based on our genetic analysis in a way that maximizes the variation between groups and minimizes variation within groups. The clustering procedure defined by ward was designed to do exactly that (Ward, 1963). The groups resulting from the Ward approach were supposed to be compared to data on origin and history of the material. Also the possibility to define a representative core subset of all accessions under study was to be investigated.

Material

From the Swiss Gene Bank in Changins was well as private initiatives a total of 171 traditional maize landrace accessions could be obtained at the start of the project. According to their kernel phenotype, all accessions most likely belonged to the flint type of maize. Two distinct types of kernels were identified during the preparation of sowing. For one type, kernels were wider than long and rather big altogether. For the other type kernels were longer than wide and rather small. The kernel colours that could be observed were very diverse. Some accessions contained kernels of only one colour whereas others bore multiple colours.

Methods

At the starting point of the project a subset of 18 accessions was used to develop en efficient method to screen all of the material in the laboratory using Simple Sequence Repeat (SSR) markers. This subset was defined on the basis of an earlier diploma work (R. Peter, P. Piattini) and trials under controlled conditions, because extensive field trials were not feasible with 171 accessions. In addition to the 18 accessions of the subset four repeated samples of one accession (025VS) and a modern commercial hybrid (Magister) were included as controls for the analysis.

Markers were selected starting with the markers already present in the stocks of the ETH. By browsing the online genome database for maize (MGDB) the size differences of known PCR products served as a first criterion for the choice of markers. Next, one marker per chromosome was chosen by testing the markers on individuals of one accession. As an improvement, later tests were conducted with all mixtures of DNA (bulks) from the individual plants of the respective

accessions. This way the successful amplification of an SSR on the genotypes present in an accession could be tested reliably on all accessions with little effort.

SSR markers are a PCR based system. The necessary equipment is a thermal cycling system to amplify the part of the DNA that is specifically recognized by the primers of the respective SSR marker. Because of this specificity, SSR products reflect the genetic state of a certain part of a chromosome. They are sufficiently specific to detect differences in the homologous parts of chromosome pairs in diploid organisms. This is why they allow for analysis of the different alleles at a certain part of a chromosome. By amplifying DNA of one diploid individual, information on both alleles inherited from the parents can be visualized.

Genomic DNA was extracted from 10 to twelve young plants and used for PCR with the selected primers. PCR products were separated on 4 % agarose gels by means of electrophoresis (Senior et al., 1998). Gels were then stained with Ethidiumbromide solution and put on UV light where digital photographs were taken and saved to disk (Figure).

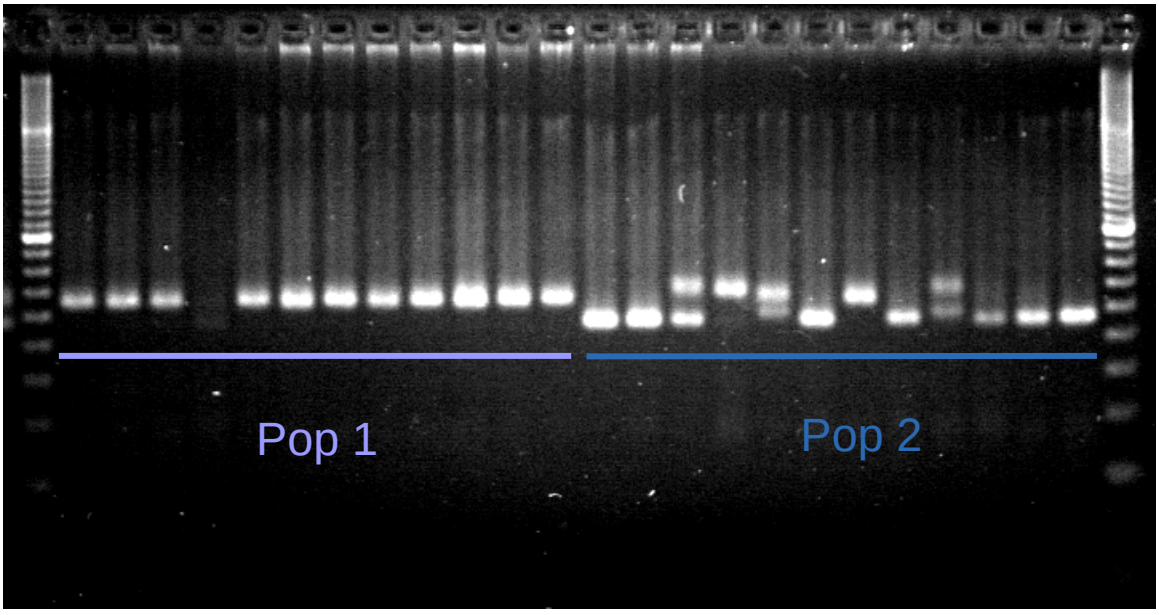

Figure x: Digital photograph showing one SSR marker applied to single plant DNA of two different accessions (Pop 1 and Pop 2). Both accessions are

represented by twelve individuals. Lanes on the left and on the right contain the 20Bp DNA ladder (Biorad) that was used as size standard.

Analysis of the digital photographs was done using Gel-Pro Analyzer 4.5. Details of the method and results on the subset will be published (Eschholz et al TAG submitted).

Because the results of the small set were encouraging the analysis was carried out on all Swiss accessions under study. This led to the conduction of more than 20'000 PCR reactions that statistical interpretations could be based on. Very few plants showed missing data for all markers indicating high quality of the DNA extracts.

Results

On the basis of the small set it could be proofed that differentiation of accessions with our approach was possible. The four repeated accessions clustered very closely together indicating that the method was capable of detecting close connection of samples. Applying the method to all of the accessions we could identify six pairs of accessions (140RV and 141RV, 147RV and 148RV, 148RV and 149RV, 038VS and 039VS, 008PR and 138AR, 034VS and 113VS) that are possibly double entries in the gene bank. In all these pairs both accessions were collected from the same geographic region. In the case of 147RV to 149RV the distance between 147RV and 149RV was small as well. Because all three of them were collected from towns close to each other, it can be assumed that they represent descendants of the same ancestral landrace.

The grouping of the accessions with the Ward method (Figure) revealed the existence of two different groups of genotypes that were further divided into subgroups. As group "A" contains the majority of the accessions from Tessin and the Posciavo valley, it can be assumed that typically southern Swiss genotypes

are represented there. It is interesting to note that also a number of accessions that were collected north of the Alps and in the Valais is part of group “A”. The number of accessions present in the two main groups indicated that the southern material contained more genetic variability than material from the north. This was confirmed also when the core subset was defined. The higher number of accessions in the cluster from the north possibly indicates more redundancy in the respective accessions. The fact that clusters exist that can be related to regions of origin (A2 to Tessin, A1 to Posciavo, B2c to Valais) likely indicated differing amounts of gene flow between regions. On this basis material from the northern regions that could not be related to a single cluster was exchanged more often between the different regions of collection than was the case for Tessin, Posciavo and Valais. This may be supported by the fact that maize cultivation north of the Alps was connected to the risk of losing the material in the field completely due to late frost events. The typical southern accessions collected north of the Alps may have been brought there as replacement for such losses. Additionally, some farmers north of the Alps might have been interested in growing maize for polenta which can be assumed as the typical use of most maize accessions from south of the Alps.

To raise the efficiency of further studies on Swiss maize genetic resources and their application regardless whether in the field or in the laboratory, a core subset of accessions was defined. Doing this on the basis of genetic data by use of a simulated annealing approach with Powermarker led to an overrepresentation of material from south of the Alps. This was the second hint at the existence of more variability in the material from the south relative to the northern Swiss material. The final core subset (Table) was constructed by including a second strategy the D allocation method (Franco et al., 2005; Franco et al., 2006) as well as phenotypic data from NAP02-33.

The inclusion of the different information for the formation of the core subset in a stepwise manner led to an optimization of the core subset. It comprised 34

accessions representing the regions of origin roughly proportional to the number of accessions that were collected from that region. The final core subset represented 95.5 % of the alleles present in all accessions and it represents a large amount of the higher pair wise genetic distances (Figure). Detailed results of these analyses will be published.

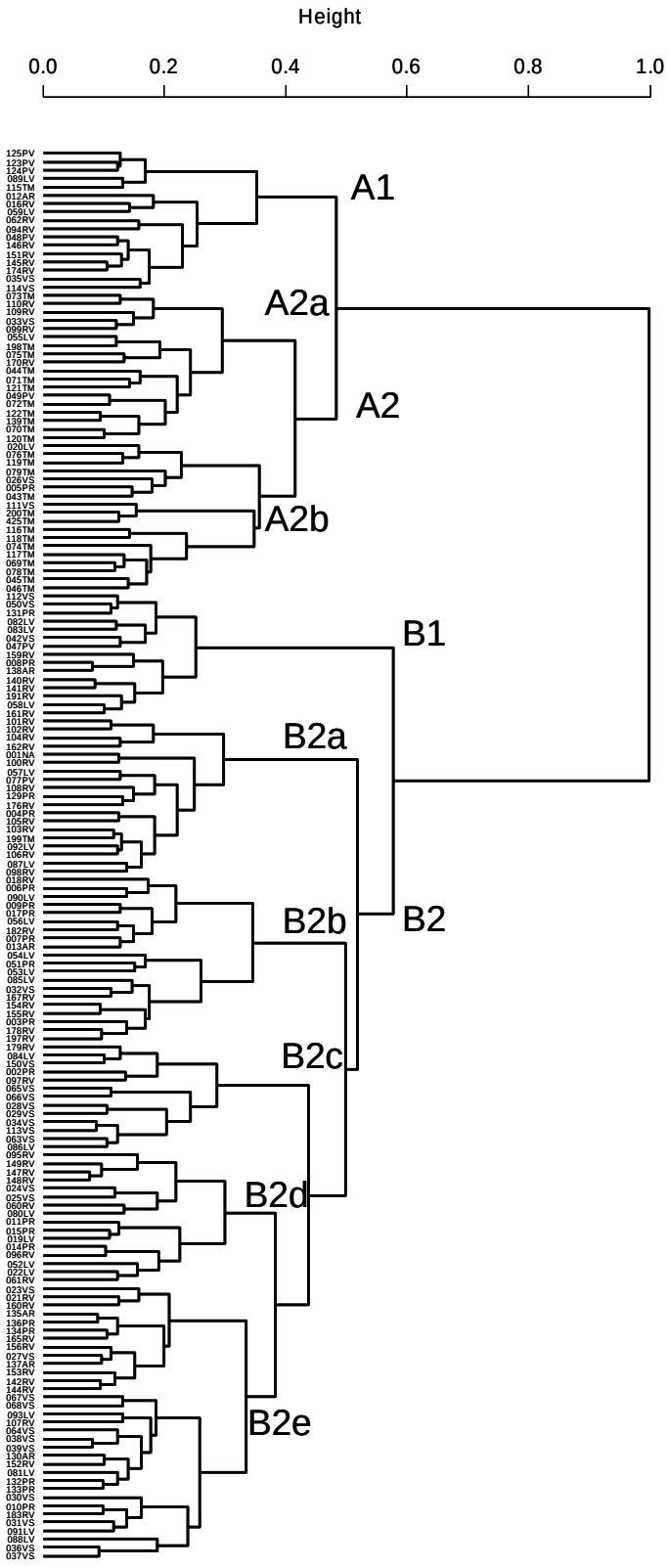

Figure x: Grouping of the whole set of Swiss accessions (169) used for the definition of the core subset.

Accessions from the gene bank were excluded if their passport data indicated foreign origins.

Table x: The Summary of the accessions included in the core subset to represent the diversity of Swiss maize material.

Final code	VARNUMBER (www.bdn.ch)	former ETH code	Gene bank No.	locality of origin	Canton	Historic code	Collector	Date of collection
002PR	13001002	HR001	ZM002	Sils im Domleschg	Graubünden	Gr.1-2	P. Christoffel	4/29/1942
003PR	13001003	HR002	ZM003	Rodels	Graubünden	Gr.2-1	F. Jecklin	4/15/1942
007PR	13001007	HR006	ZM007	Tartar	Graubünden	Gr.4-2	Joh. D. Holzner	4/12/1942
014PR	13001014	HR011	ZM014	Realta	Graubünden	Gr.8-a		
015PR	13001015	HR012	ZM015	Scharans	Graubünden	Gr.9-a		
018RV	13001018	RT001	ZM018	Pfaeffers	St. Gallen	L.1-1-a		
025VS	13001025	VS003	ZM025	Drone (Saviese)	Wallis		Louis Roten	
028VS	13001028	VS006	ZM028	Eyholz	Wallis		Ernest Heldner	
030VS	13001030	VS028	ZM030	Niedergesteln	Wallis		Leo Kalbermatten	
054LV	13001054	LT006	ZM054	Schaenis	St. Gallen	L.10-d	Alb Jud	11/30/1942
059LV	13001059	LT007	ZM059	Eschenbach	St. Gallen	L.180		
073TM	13001073	TM011	ZM073	Gerra (Verzasca)	Tessin	Sdt.13-c-2		
074TM	13001074	TM012	ZM074	Preonzo	Tessin	Sdt.15-b	Pietro Bionda	10/19/1943
079TM	13001079	TM015	ZM079	Hasenboehler	Tessin	Sdt.177		
082LV	13001082	LT010	ZM082	Mels	St. Gallen	L.8-d	Aristo Bernold	11/23/1942
094RV	13001094	RT023	ZM094	Buchs	St. Gallen	Rh.1-4-a	M. Schwendener-Hess	4/10/1942

Final code	VARNUMBER (www.bdn.ch)	former ETH code	Gene bank No.	locality of origin	Canton	Historic code	Collector	Date of collection
098RV	13001098	RT027	ZM098	Eichberg	St. Gallen	Rh.2-3	S. Fenk	4/22/1942
103RV	13001103	RT032	ZM103	Au	St. Gallen	Rh.7	Oskar Messmer	4/28/1944
104RV	13001104	RT033	ZM104	Au	St. Gallen	Rh.7-a	Oskar Messmer	4/28/1944
113VS	13001113	VS026	ZM113	Sion	Wallis	W.3-1		
114VS	13001114	VS027	ZM114	Sion	Wallis	W.3-2		
116TM	13001116	TM003	ZM116	Mesocco	Graubünden	Sdt.3-c		
119TM	13001119	TM018	ZM119	Camorino	Tessin	Sdt.11-c	Pietro Gianocca	10/16/1943
121TM	13001121	TM020	ZM121	Bodio	Tessin	Sdt.16-a	Gino Scolari	10/19/1943
125PV	13001125	PB007	ZM125	Brusio	Graubünden	Sdt.173		
132PR	13001132	VR004	ZM132	Scharans	Graubünden			
139TM	13001139	TM022	ZM139	Aquila (TI)	Tessin			
141RV	13001141	RT013	ZM141	Tamins	Graubünden			
142RV	13001142	RT014	ZM142	Trimmis	Graubünden			
149RV	13001149	RT020	ZM149	Igis	Graubünden			
160RV	13001160	RT041		Thal	St. Gallen			
176RV	13001176	RT048		Steiger, Oberriet				
179RV	13001179	RT039		Buchs	St. Gallen			
198TM	13001198	TM023		Schaub Gabrielle, Ludiano	Tessin		Schaub Gabrielle, Ludiano	1990

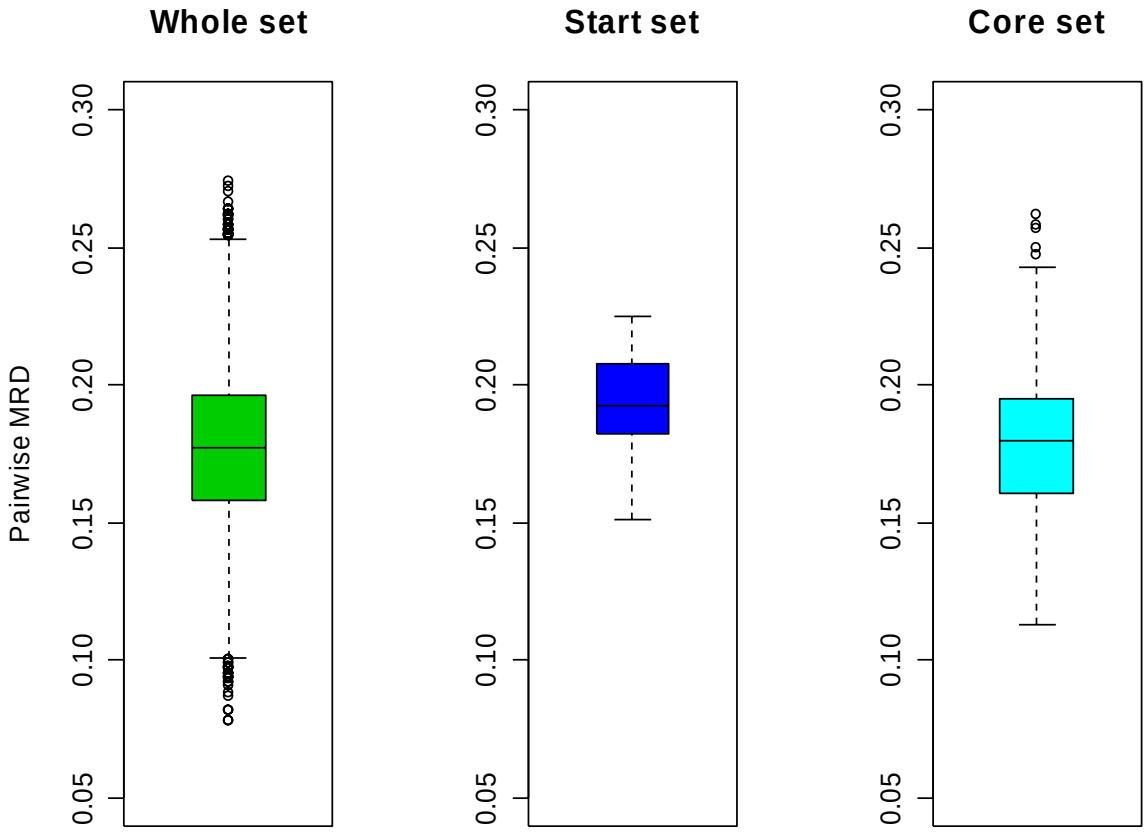

Figure x Comparison of the pair wise modified Rogers distances (MRD) calculated on the basis of the accessions part of the different sets. Whole set describes all the Swiss accessions, that were used for the definition of a core set. Start set describes a set, defined on the basis of a simulated annealing approach and core set refers to the final core subset defined by combining data from the simulated annealing approach and phenotypic characters.

Conclusions

This project delivered proof for the feasibility of genetic profiling of maize accessions with a low number of SSR markers. The identification of possible doubles in the Swiss Gene Bank may serve to reduce cost of maintenance in the future. But prior to mixing seed of these accessions their phenotypic differences should be considered as well. The core subset shall enable future projects in science or application oriented fields to screen the diversity present in the Swiss Gene Bank by using a reasonable number of accessions. Of course special characters of certain accessions that may be caused by one or only a few genes could not be taken into account in this project. If the screening of the core set should lead to the identification of traits of interest in a certain accession of the core subset, this should be followed by an investigation of accessions situated close to the accession of interest in the grouping shown here. The consideration of material from the same region of collection may also be useful.

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