REPORT

Analysis of Rhubarb (*Rheum* x *hybridum*) collection at Julita Sweden's Agricultural Museum using molecular markers

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Materials and Methods

Material

The material represented the collection of rhubarb clones at the Julita Sweden's Agricultural Museum. The set of 19 clones were included in the analysis. Each clone is represented by two or more plants (except 'Hallonrabarber' which only has one plant). In general, one sample was taken from each plant, resulting in two samples per clone. In some cases more samples were taken from each plant, in order to investigate the purity of the clone. In total were 59 rhubarb samples analysed (Table 1).

Table 1 Rhubarb clones included in analysis

Emk Nr	Liberts Nr	Clone variety	Origin	Number of samples
R1	202	'Elmblitz'	Germany	2
R3	218	'Purpurat'	Germany	6
R4	219	'Vierländer Blut'	Germany	5
R5	215	'Karpow Liepshiego'	Poland	2
R6	216	'Wszesny Horsera'	Poland	2
R7	203	'Prince Albert'	England	6
R8	204	'Sutton'	England	2
R9	205	'Victoria'	England	2
R10	209	'Champion Red'	England	2
R11	221	'Merton Foremost'	England	2
R12	222	'Reeds Early'	England	5
R13	223	'Timperley Early'	England	6
R14	224	'Cawood Delight'	England	2
R16	226	'Stockbridge Harbinger'	England	4
R17	227	'Stockbringe Cropper'	England	6
R18		'Julita'	Sweden	2
R19		'Glasrabarber'	Sweden	2
R20		'Hallonrabarber'	Sweden	1

DNA marker analysis

Young leaves were freeze-dryed in 2 ml Eppendorf tubes. After freeze-drying DNA was extracted using NordGen standard exstraction method.
Four ISSR (inter simple sequence repeat) primers were used for analysis of all samples: nr 888, 890, 891 and 841. PCR reaction was carried out as follows: 23 µl of master mix, containing PCR buffer, 0.25 mM/µL dNTPs, 0.4 U of Taq polymerase (New England Biolabs) and 750 ηmol of primer was added to 2µl DNA, 20ηg/µL. The amplification conditions: 95°C for 30 sec, 20 cycles of 95°C for 50 sec, (52+ 0.1)°C in each cycle for 50 sec, 72°C for 50 sec; 25 cycles of 95°C for 50 sec, 54°C for 50 sec, 72°C for 50 sec; 72°C for 4 min.
The analysis of amplified DNA was carried out on a 10% ready-made PAAG gel from ETC Elektrophorese-Technik. Data were scored as presence and absence of band. Shannon-Weaver index (I) was calculated: $I = -(p_i \ln[p_i] + q_i \ln[q_i])$, where p is frequency for presence of band,

whereas q frequence of absence of band (Hutchenson, 1970; King and Schaal, 1989).
Average Shannon-Weaver index for each primer product was calculated.
Jaccard similarity values (Jaccard 1908) were calculated and a clustering analysis was performed using the NTSYS-pc statistical package (Rohlf 1998).

Results

Amplification of 4 ISSR primers yielded 19 polymorphic bands (table 2). The highest number of polymorphic bands was detected in products from primer 891, wheras highest value for Shannon-Weaver index resulted for primer 841.

Table 2 ISSR markers used in the analyses, Shannon- Weaver divesity index and number of polymorphic bands

Primer	Number of polymorph bands	Shannon-Weaver index	Size of polymorph bands (base pairs)
888	5	0.516	700, 750, 760, 770, 800,
890	4	0.339	650, 700, 751, 771
891	8	0.512	350, 480, 500, 580, 620, 650, 680, 705
841	2	0.651	504,512

The similarity matrix between individuals was calculated and as shown in the dendrogram in Figure 1 there were differences not only between the different clones but also between individual samples.
When each clone was analysed separately one could detect a number of different genotypes within clone, which means that a number of clonal accessions are not pure. With the 90 % marker sensitivity, one to five genotypes per clone were detected (table 3).

Table 3 Number of genotypes detected for each clone using ISSR markers

Emk Nr	Liberts Nr	Clone variety	Number of samples	Number of genotypes
R1	202	'Elmblitz'	2	1
R3	218	'Purpurat'	6	4
R4	219	'Vierländer Blut'	5	3
R5	215	'Karpow Liepshiego'	2	2
R6	216	'Wszesny Horsera'	2	1
R7	203	'Prince Albert'	6	4
R8	204	'Sutton'	2	2
R9	205	'Victoria'	2	2
R10	209	'Champion Red'	2	1
R11	221	'Merton Foremost'	2	2
R12	222	'Reeds Early'	5	3
R13	223	'Timperley Early'	6	5
R14	224	'Cawood Delight'	2	2
R16	226	'Stockbridge Harbinger'	4	3
R17	227	'Stockbringe Cropper'	6	3
R18		'Julita'	2	1

R19		'Glasrabarber'	2	2
R20		'Hallonrabarber'	1	1

Conclusions and Recommendations
According to the results of this study analysed clones are not pure, i.e. there is variation within the clone.
The clone samples should be compared with samples from reference plants which are known to be true-to-variety. The most probable cause to the impurity of the clones is that the plants have been allowed to flower and set seeds, and seedlings have established in the individual plants.
The collection should be replanted with tested and true-to-type plants. In the management instructions for the rhubarb collection the importance should be stressed of removing the flowers to avoid seed production.
Today, no material from this collection should be distributed representing the supposed varieties.

References

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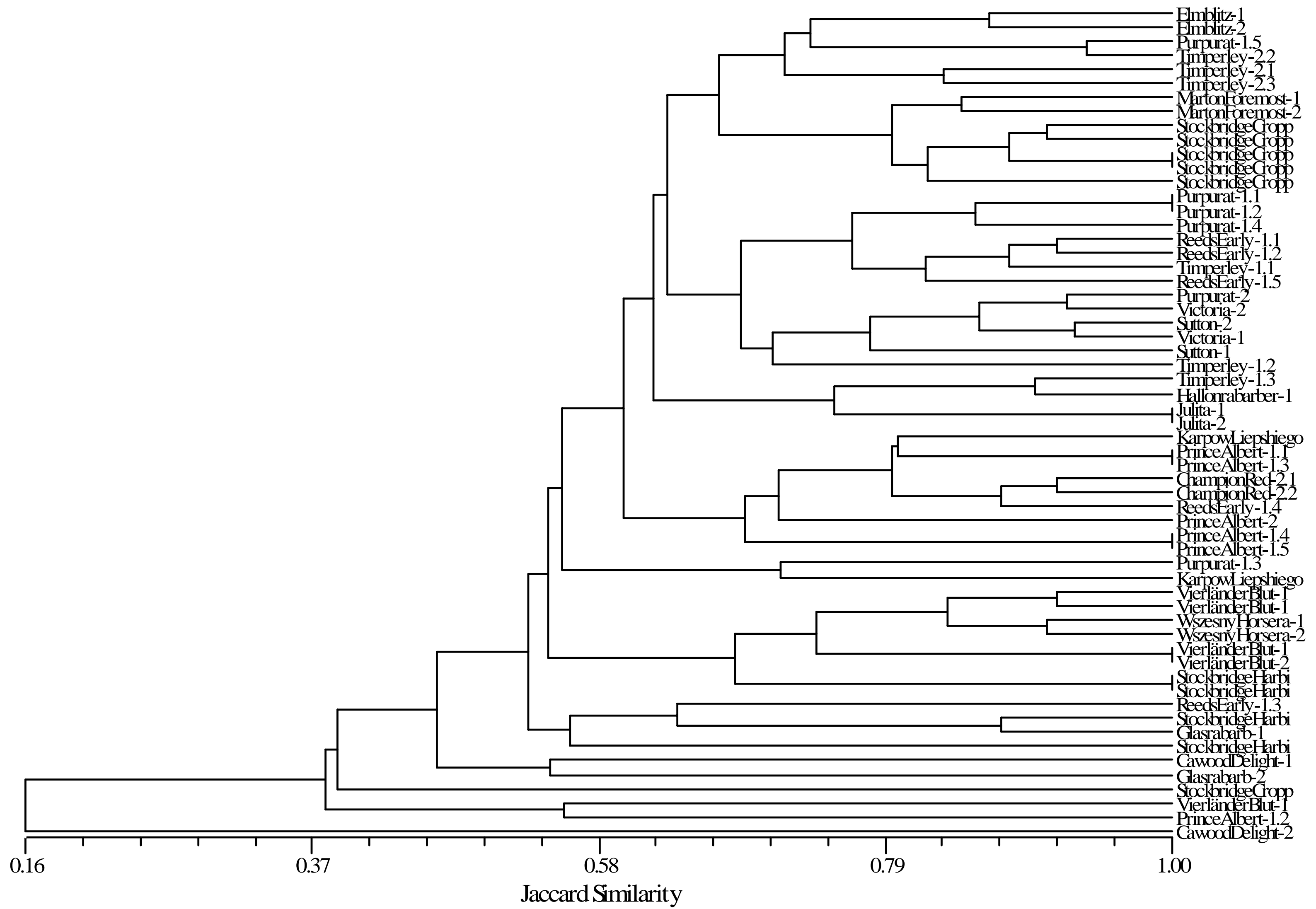

Figure 1 UPGMA dendrogram based on ISSR data showing the genetic relationships among individual rhubarb samples.