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BMT/3/5 Add.
ORIGINAL : English
DATE : September 25, 1995

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA-PROFILING IN PARTICULAR

Third Session

Wageningen, Netherlands, September 19 to 21, 1995

A MODEL STUDY IN TOMATO TO ASSESS THE USABILITY OF MOLECULAR MARKER IN DETERMINING ESSENTIAL DERIVATION

Addendum prepared by the Tomato-EDV Study Group of ASSINSEL

EDV and the impact of molecular markers

Assumption :

- Molecular Marker technology can be used to estimate genetic distance, thereby enabling to determine whether predefined threshold for derivation has been crossed

Advantage of molecular markers:

- Independent of environmental conditions
- Inherited as single Mendelian traits
- Determinable at all developmental stages
- Availability of a wide range of markers

Disadvantage of molecular markers

- Anonymous, therefore relation to phenotype unclear
- Availability dependent of crop
- Specific investments required

Core question : which molecular technique is best suited for measuring genetic distance ?

Main objective of this study

Which molecular technologies are usable ?

- Amount of markers to be measured for statistical reliability?
- Estimation of genetic conformity should be independent of technology and cluster tested
- Determine amount of markers needed for reliable estimation of genetic conformity
- Determine reliability of each technology (experimental errors, reproducibility)

Challenge to investigate a number of potential bottlenecks :

- Tomato has limited level of polymorphism for studied molecular markers
- Most polymorphisms are associated with introgressed characters
- Most horticultural characters (except resistances) are polygenic

Project outline:

Material

Three well known clusters were selected :

- French Montfavet H63-5 types
  (fresh market, Mediterranean area)

- Dutch Moneymaker types
  (fresh market, heated greenhouse segment)

- American Mechanical harvest processor types

Each cluster contained 15 varieties representing the breeding history in that segment

Include replicates to control experimental errors

Project outline :

Technology
Collect phenotypical data from PBR registration files and field trials

Molecular marker technologies selected where :

- Random Amplified Polymorphic DNA technology (RAPD)
- Micro-satellite DNA fingerprinting technology
- Amplified Fragment Length Polymorphism technology (AFLP)

	RAPD	AFLP	Micro-satellites	RFLP
Genetic basis	dominant marker	dominant marker	dominant marker	co-dominant
Datapoints per reaction	± 3 - 6 (12)	± 10 - 20 (8)	± 10 - 20 (29)	1 - 3
Cost per reaction	± ƒ 4,50	± ƒ 40,--	± ƒ 15,--	± ƒ 15,--
Cost per datapoint	± ƒ 0,90 = ƒ 2,25	± ƒ 2,-- = ƒ 5,-	± ƒ 0,90 ƒ 0,50	± ƒ 7,--
Polymorphism level in cultivated tomato	medium	medium	medium - high	low

Project team :

Molecular analysis :

Dr. Marc Zabeau	Keygene N.V. Wageningen, the Netherlands	AFLP technology
Prof. Steve Tanksley	Cornell University, Ithaca, U.S.A.	RAPD and micro-satellite
Prof. James Nienhuis	Univ. of Wisconsin, Madison, U.S.A.	RAPD

Phenotypical analysis:

US breeders	for field evaluation of US processor type cluster
GEVES	for field evaluation of European types

Statistical analysis:

Prof. James Nienhuis	Univ. of Wisconsin, Madison, U.S.A.
Dr. Fred van Eeuwijk	C.P.R.O., Wageningen, the Netherlands

Steering committee

Dr. Alan Steven (chair)	ASTA, USA
Mr. Yves Gonon	FNPSP, France
Dr. Mart van Grinsven	NVZP, the Netherlands

Results:

Molecular analysis

AFLP technology : 325 datapoints from 41 reactions :
± 8 datapoints per reaction ~~fs.~~ per dp

RAPD technology : 82 datapoints from 40 reactions : ~~f 2.25~~ per dp
± 2 datapoints per reaction

Micro-satellites : 29 datapoints from 1 reaction ~~f 0.50~~ per dp

Phenotypic data : 50 datapoints from 50 evaluations: ~~± f1.00~~ per dp
1 datapoint per "reaction"

Statistical analysis

Redundancy in collected datapoints varies between technologies:

- AFLP ± 40 %
- RAPD ± 50 %
- Micro-satellites 0 %
- Phenotype 0 %

Results:

Experimental errors:
Reproducibility of technology:

- AFLP ± 1 %
- Microsatellites < 1 %
- RAPD ?

Inconsistency between replicates:

- AFLP ± 6 % (n=5)
- Microsatellites ± 4 % (n=5)
- RAPD ± 8 % (n=5)

Conclusions :

- Discriminatory power is very comparable between all molecular technologies ànd phenotypical data
- At least 200 datapoints should be collected for determining genetic conformity
- It is possible to combine technologies with similar discriminative power to reach sufficient number of datapoints.
- Varieties are not homogeneous for their DNA "fingerprint", (observed variation: from 2 to 12 %) !
- Statistical methods to deal with this variety-variation are not available (yet)
- It is not know what the genome coverage is of the markers used. The effect of using carefully selected markers well spread over the genome should be tested.
- The effect of using markers in well -introgressed regions versus non-expressed regions of the genome on the estimated genetic conformity could not be studied in this dataset
- The lack of clear breeding genealogy prohibited the analysis of the correlation of estimated genetic distance measured by molecular technologies and breeding efforts.

Fig. 4. MDS plot for two dimensions of California processing tomato cultivars derived from a combined data set of independent bands among AFLP, RAPD, and microsatellite molecular marker techniques. Replicate pairs are indicated with a filled in circle.

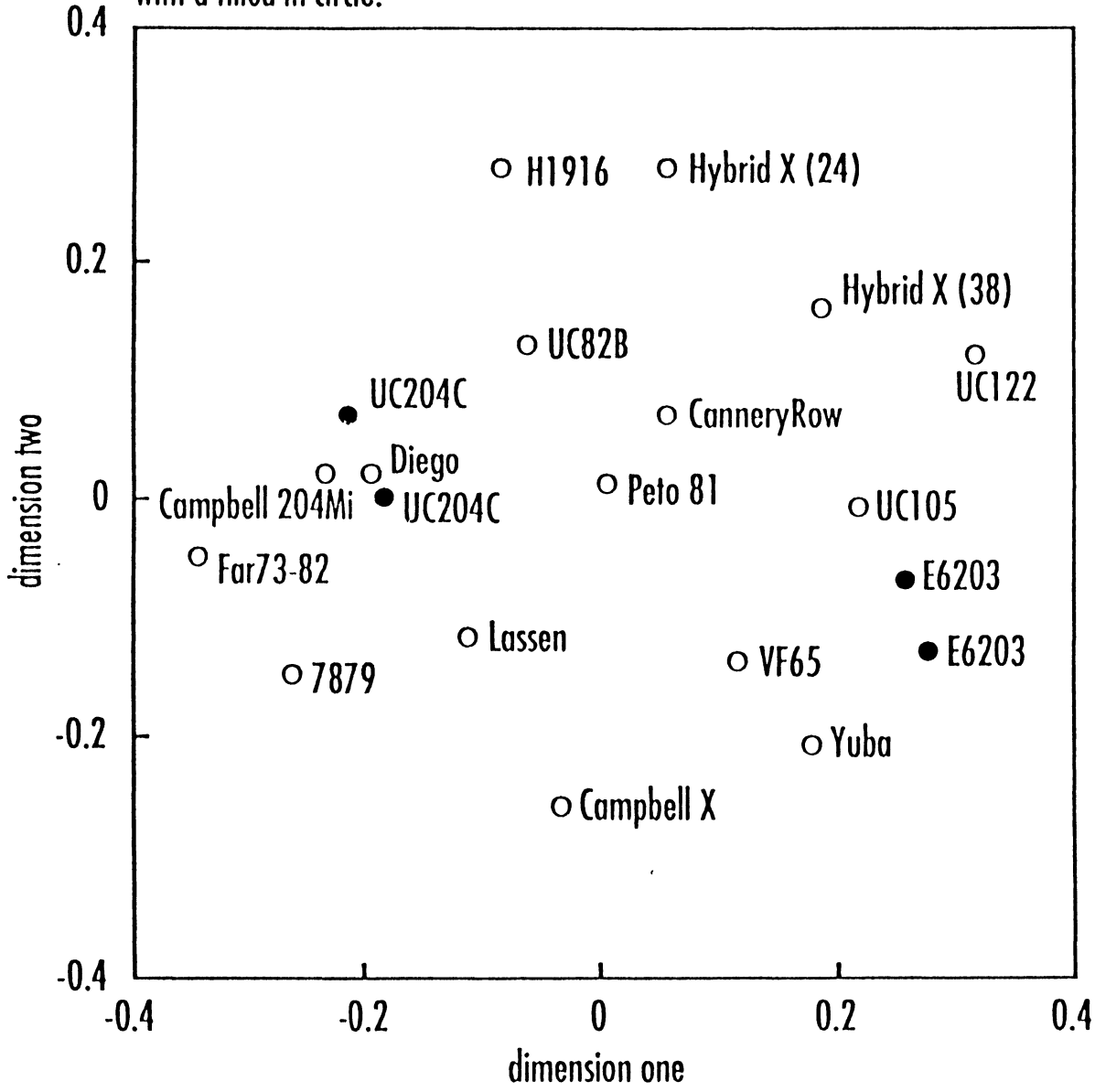

Fig. 5. MDS plot for two dimensions of California processing tomato cultivars derived from a phenotypic data set. Replicate pairs are indicated with a filled in circle.

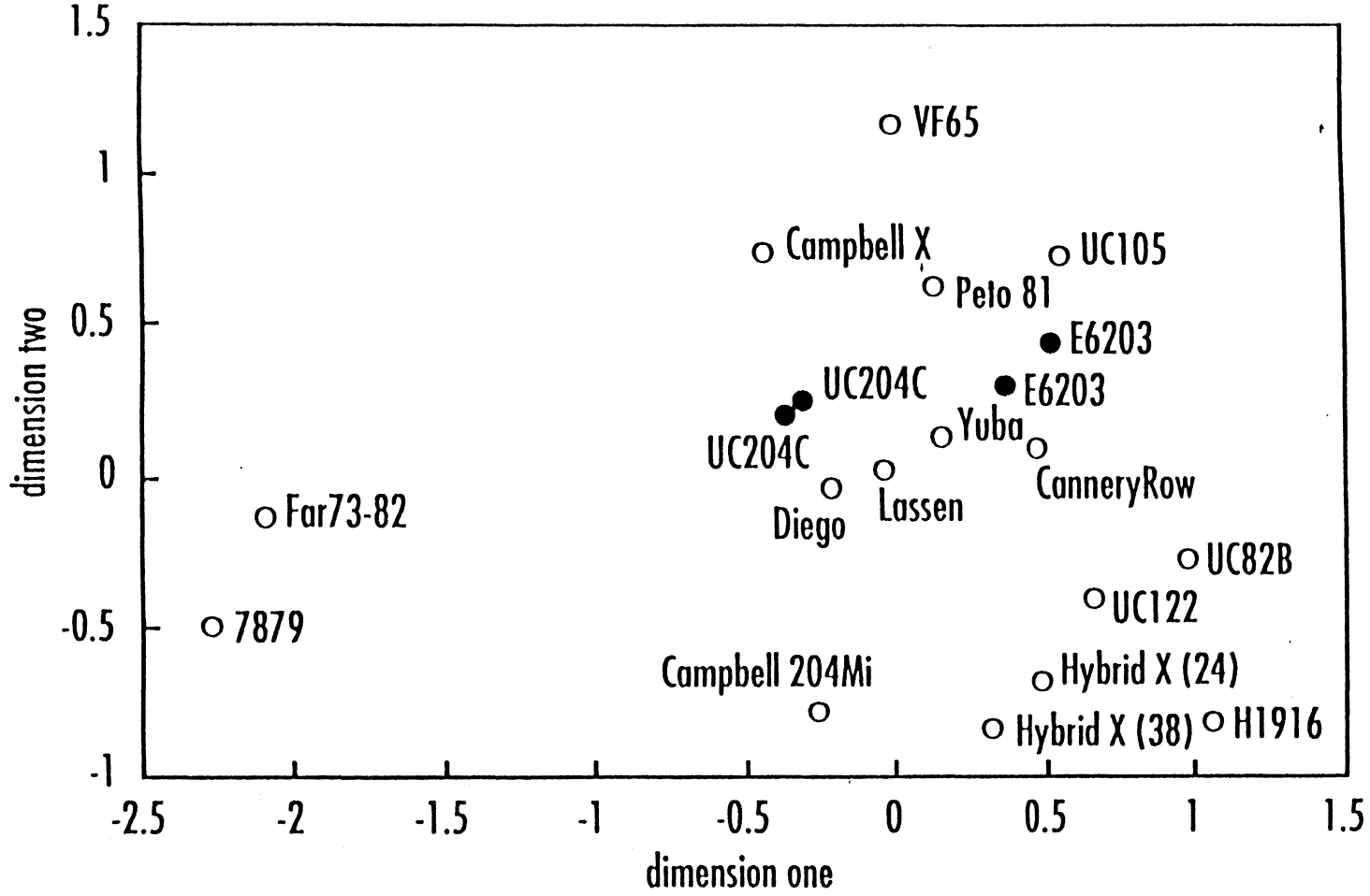

Fig. 1. MDS plot for the genetic distance among bands used in 4 different molecular marker data collections. The number of bands which plot to identical coordinates are indicated next to the symbol for a correlated band.

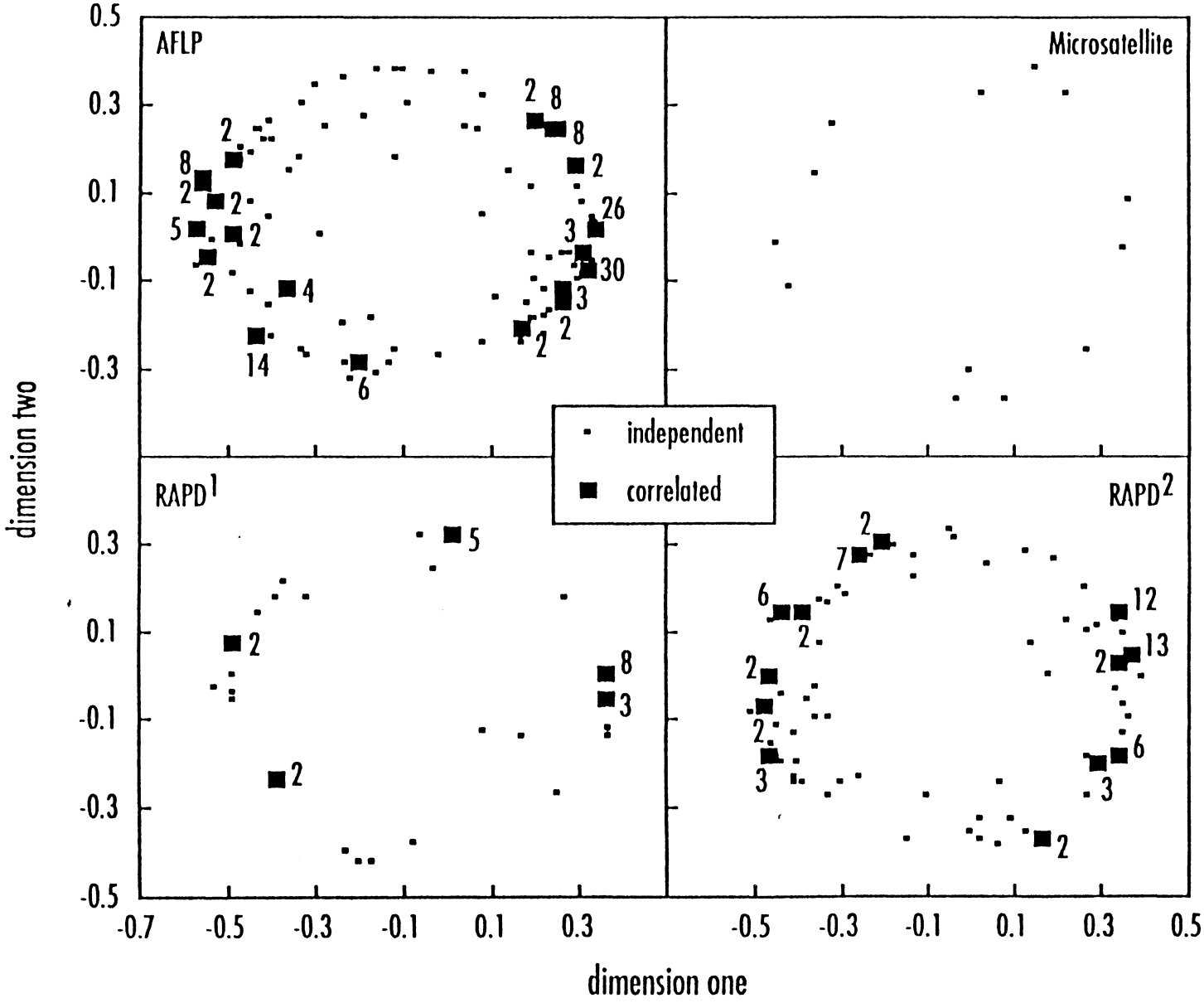

Fig. 2. Linear regressions of coefficients of variance on bootstrap samples for 5 molecular marker data sets used to analyze 20 California processing tomato cultivars.

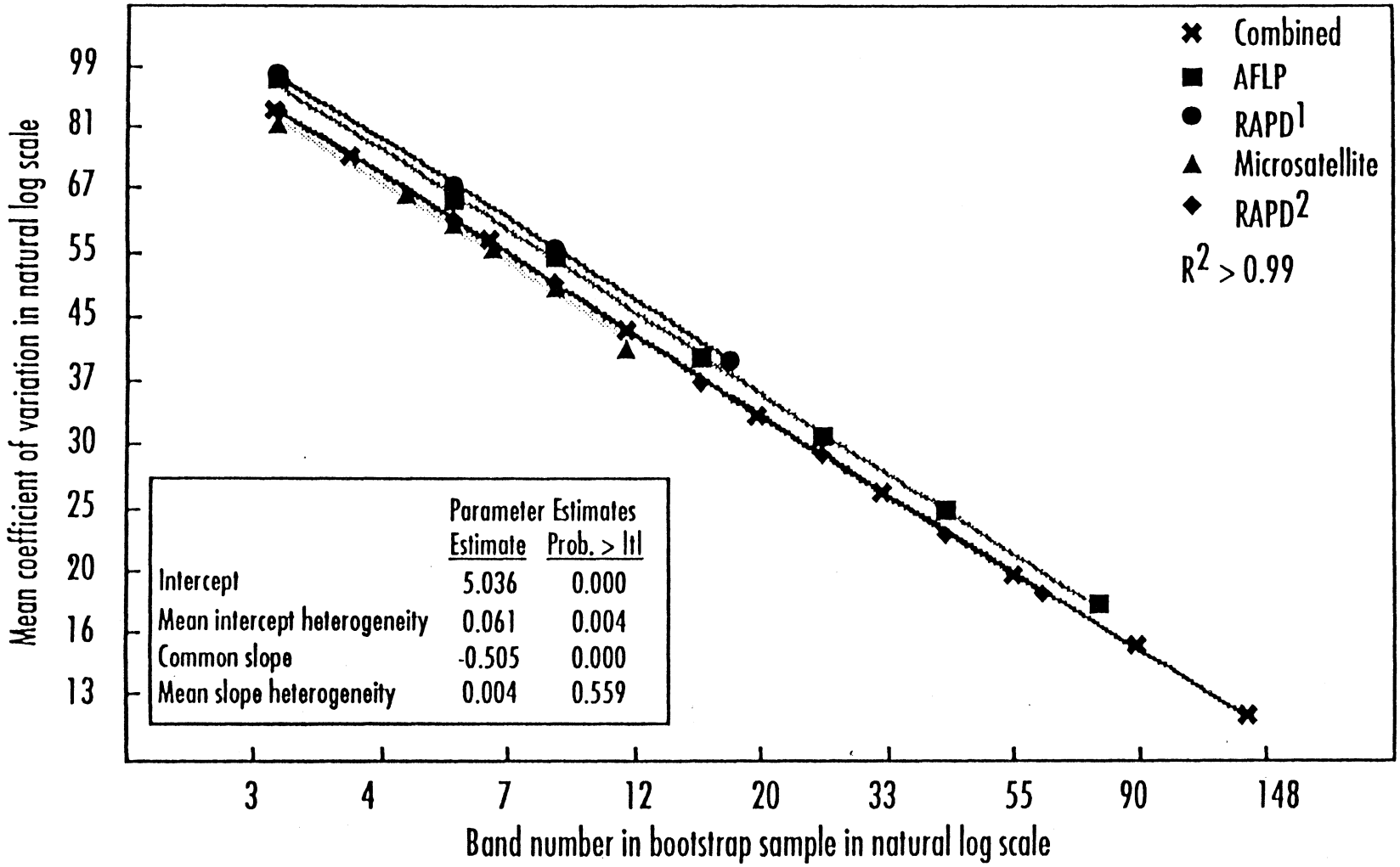

Table 6. Experimental error measured by the number of inconsistently scored bands over replicate entries divided by band number.

California processing cultivars						
	band #	replcate entries	GD estimate	inconsistency #	% inconsistency #	mean % error
Combined	139	11 - 34	0.123	17	12.23	10.07
		22 - 31	0.083	11	7.91	
AFLP	77	11 - 34	0.079	6	7.79	7.14
		22 - 31	0.071	5	6.49	
RAPD1	18	11 - 34	0.111	2	11.11	8.33
		22 - 31	0.056	1	5.56	
Microsatellite	13	11 - 34	0.077	1	7.69	3.85
		22 - 31	0.000	0	0.00	
RAPD2	62	11 - 34	0.145	9	14.52	11.29
		22 - 31	0.081	5	8.06	
European fresh market cultivars						
	band #	replcate entries	GD estimate	inconsistency #	% inconsistency #	mean % error
Combined	170	6 - 9	0.018	3	1.76	4.90
		10 - 15	0.067	11	6.47	
		20 - 53	0.066	11	6.47	
AFLP	127	6 - 9	0.016	2	1.57	4.20
		10 - 15	0.049	6	4.72	
		20 - 53	0.065	8	6.30	
RAPD1	35	6 - 9	0.029	1	2.86	6.67
		10 - 15	0.147	5	14.29	
		20 - 53	0.029	1	2.86	
Microsatellite	23	6 - 9	0.00	0	0.00	4.35
		10 - 15	0.043	1	4.35	
		20 - 53	0.087	2	8.70	

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