

ISTA Variety Committee

Chair: Rainer Knoblauch

Vice Chair: Kae-Kang Hwu

Report at the Ordinary Meeting 2005

Bangkok, Thailand



- 1. Family name: Knoblauch
- 2. First name: Rainer
- 3. Date of birth: 22. August 1955
- 4. Nationality: German
- 5. Civil status: Not married
- 6. Education: Technician for seed testing and applied botany



Since 1971 working at the State Agricultural Testing and Research Station, LUFA-Augustenberg as Senior Analyst, trained in all areas of seed testing.

Actual responsibilities: Variety testing, seed sampling, working safety

Key qualifications: 19 years experience on variety testing with electrophoretical methods (IEF, PAGE, SDS)

Lecturer for students, trainees, seed testers national and international

ISTA Workshops:	1997, 2001	Argentina
	2003	South Africa, Thailand
	2004	Slovenia, Egypt
	2005	China, Jamaica



Committee Membership List

Chair:	Rainer Knoblauch	Germany
Vice-chair:	Kae-Kang Hwu	Taiwan
Members:	Robert John Cooke	United Kingdom
	Kalyn Brix-Davis	United States
	Emanuela Casarini	Italy
	C. C. Debashree	India
	W. Drost M. Sc	Canada
	Jerzy Drzewiecki	Poland
	Berta Killermann	Germany
	Paul Koranyi	Hungary
	Uri Kushnir	Israel
	Anne Middleton	Canada
	Chandgi Ram	India
	Amar Tahiri	Morocco
	David Zhang	France



A Rules Development



Introduction of Rules Changes 2005

Chapter 8 will be re-designed in the new format and actually incorporate:

- a. bio-molecular tests for varietal purity
- b. the testing for specified impurities (e.g. GMO's)

Acceptance of the Chapter

completed 2005

Rules Proposal

1. Amendments to the IEF methods:

- IEF of *Zea mays* give alternative distilled water as extraction buffer for the protein extraction
- IEF of *Zea mays* for the alternative use, of pH 2-9 (ampholyte range), give the possibility to use also the pH range 5-8+2-11+ 4 mol Urea + Taurine

2. IEF for *Triticum* and *Avena* as an alternative Method to PAGE

- Comparative test for validation 2005

completed 2006



B Workshops and Seminars

2004 Slovenia, Egypt

Variety testing, together with GMO TF

2005 China, Jamaica

Variety testing, together with GMO TF

2005

Seminar on progress in Variety Committee

planned

2006 Germany

Workshop on Protein Electrophoresis

planned

2007

Workshop on non electrophoretical or DNA based
Methods of Variety Testing

planned



Special Projects

Reorganise the Variety Committee and find adequate members
Install Working Groups on the different types of Variety Testing
(technique, crop or region specific)

Methods:

- **Morphology**
- **Anatomy**
- **Chemistry**
- **Physics / fluorescence optic**
- **Cytology**
- **Biochemistry**



completed 2005

to complete 2005



METHODS FOR VERIFICATION OF SPECIES AND CULTIVAR

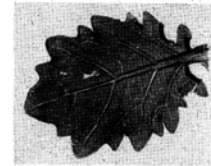


Abb. 7 Primärblatt von Raps

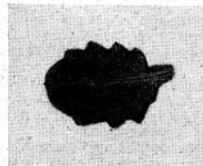


Abb. 8 Primärblatt von Rüben

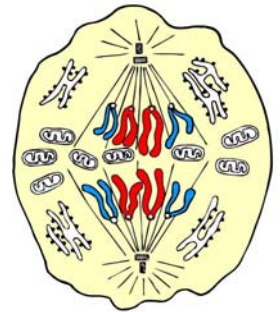
- | | | |
|----------------------|---|---|
| Morphological | <ul style="list-style-type: none">- ripe plant- seedling | <ul style="list-style-type: none">- <i>Brassica, Raphanus</i>- <i>Lactuca, Cichorium</i>, winter-summer cereals |
| Anatomical | <ul style="list-style-type: none">- seed | <ul style="list-style-type: none">- <i>Brassica</i> species (testa) |
| Chemical | <ul style="list-style-type: none">- Phenol- Formic acid- Potash solution- Lugol solution- Hydrochloric acid | <ul style="list-style-type: none">- <i>Triticum</i> varieties- <i>Sinapis arvensis</i> in <i>Brassica</i>- <i>Sinapis arvensis</i> in <i>Brassica</i>- <i>Lupinus</i> with / without alkaloids- <i>Vicia sativa / villosa</i> |
| Fluorescence | <ul style="list-style-type: none">- floret- seedling | <ul style="list-style-type: none">- <i>Avena</i> white / yellow var.- <i>Trifolium</i> species- <i>Melilotus</i> species- <i>Lolium perenne / multiflorum</i>- <i>Festuca rubra / ovina</i>- <i>Allium</i> species |



METHODS FOR VERIFICATION OF SPECIES AND CULTIVAR

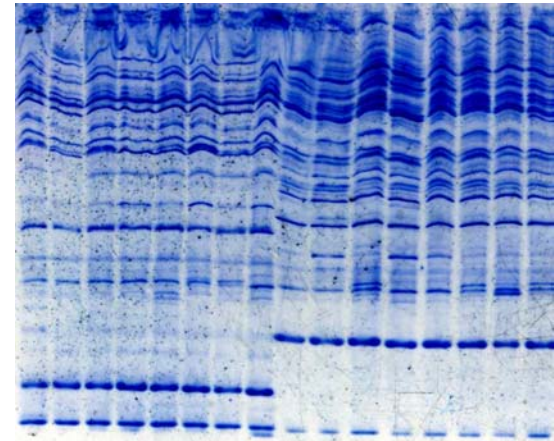
5. Cytology

- seedling
 - *Beta* species
 - *Triticum durum / aestivum*
 - *Poa* species, var.
 - *Phleum* species, var.
 - *Trifolium pratense*, var.



6. Biochemical

- seed
 - *Triticum, Hordeum* (acid-PAGE)
 - *Lolium, Pisum* (SDS-PAGE)
 - *Zea* (UTLIEF)
 - *Helianthus* (UTLIEF)



Special Projects

Identify 'white spots' and find sponsors for research work

Check the need of variety test methods for tropical and medical plant species

Do a survey on methods to find

specialists/institutions that have methods available

specialists /institutions that are prepared to collaborate with the committee

Send a questionnaire to all ISTA - Stations world wide

Design a searchable database from all data obtained from the survey

Make the database available to all interested

a. via ISTA-website, and

b. through a hardcopy version



to complete 2006

to complete 2006

to start 2005

to complete 2007



Questionnaire on survey of methods

Questionnaire to 15 Committee members, 8 answers

Samples analysed by Morphology (incl. Field trials), ~ 2100
Anatomy, Chemistry, Physics/fluorescence optic, Cytology

Samples analysed by electrophoresis ~ 5850

Isozymes	32 %
PAGE	29 %
SDS	27 %
IEF	12 %

- More information about methods used in ISTA labs worldwide, knowledge of hot spots of research needed

Installation of working groups !

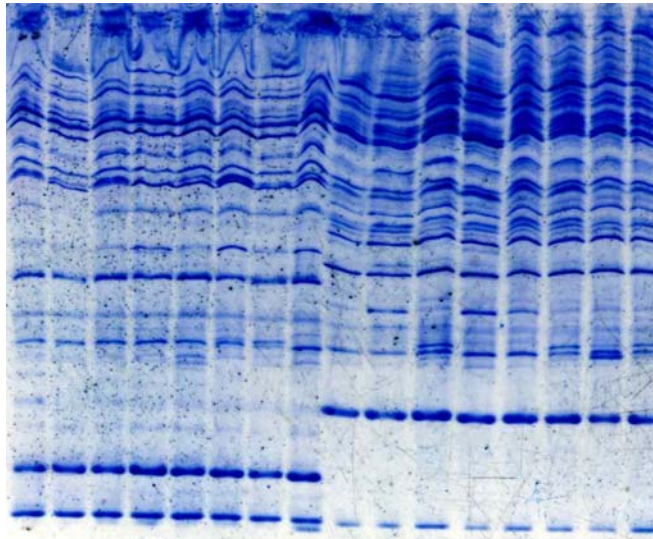
Species incl. Varietis	Extraction solutions 225 µl	Running conditions Program 1/2	Urea g	AB-Solution ml	Ampholyte 4.4 ml	TEMED-Solution µl	APS-Solution µl/20%	°C Cooling Temperature	Focussing single/double
Avena sativa	30 % CleOH	P 2	16	50	2-9	50	350	5	d
Cucurbita sp	30 % CleOH 250 µ	P 1	16	50	2-9	50	350	10	d
Helianthus annuus	CleOH 30 %	P 2	16	50	2-9 5-8+2-11	50	350	10	S/d
Hordeum vulgare	PO ₄ buffer	P 2	16	50	5-8 4-6 5-8+2-11	50	350	5	d/S
Lycopersicon sp.	4 mmol NaCl 60 µl	P 1	/	50	4-7+5-8	40	30	10	d
Pisum sativa	30 % CleOH	P 2	16	50	2-9	50	350	5	d
Poa sp.	30 % CleOH 12,5 µl	P 2	16	50	2-9	50	350	5	S
Secale cereale	PO ₄ 500 µl	P 2	/	50	4-5+3-10	120	210	5	S
Trifolium sp.	30 % CleOH 100 µl	P 2	16	50	2-9	50	350	7	d
Triticum sp.	PO ₄	P 2	/	50	4-5+3-10	120	210	5	S
Triticosecale	PO ₄	P 2	/	50	4-5+3-10	120	210	5	S
Triticum sp.	30 % CleOH H ₂ O	P 1	16	50	5-8 5-8+2-11	50	350	10	d

Species incl. Varietis	Separations distance (cm) from Anode to Cathode or from Cathode to Anode	Electrode distance from the Anode/Cathode mm	Applicator – Strips blue/bright = light	Additions	Sample Application (ammont µl)	Application-Side Anode/Cathode, + / -	Special's	Single/Bulk
Avena sativa	9/7	15	l	/	10	⊕	/	S
Cucurbita sp	7	2	b	/	22	⊕	/	S
Helianthus annuus	9	15 2	b b	/	17 8	⊕	/	S
Hordeum vulgare	7	10	l	/	7	⊖	/	S
Lycopersicon sp.	7	5	l	/	21	⊕	/	S
Pisum sativa	7	15	l	/	10	⊕	/	S
Poa sp.	9	15	b	/	12	⊖	/	S
Secale cereale	9	15	l	/	7	⊖	Sample 100 mg	B
Trifolium sp.	7	2	b	/	15	⊕		S
Triticum sp.	9	15	l	/	8	⊖		S
Triticosecale	9	15	l	/	8	⊖		B/S
Zea mays	7	2	b	/	22 21	⊕	/	S



C Special Projects

6. Initiate proficiency tests, together with Proficiency Test Committee proposed species *Triticum*, *Zea* to start 2005
7. Design of a new Variety Handbook completed 2005
8. Preparation for integration of the GMO Task Force into the Variety Committee





**My thanks go to all of you
who brought input in the committee work.**

**I am expecting a fruitful teamwork,
to satisfy the increasing demand for variety determination.**



Thank you

