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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

**TECHNICAL WORKING PARTY
FOR
AGRICULTURAL CROPS**

**Thirty-Fourth Session
Christchurch, New Zealand, October 31 to November 4, 2005**

ADDENDUM TO DOCUMENT TWA/34/2

REPORT ON AN INTERNATIONAL SEED FEDERATION SPONSORED STUDY
ASSESSING POSSIBLE ESSENTIAL DERIVATION RELATIONSHIPS BETWEEN
RYEGRASS VARIETIES

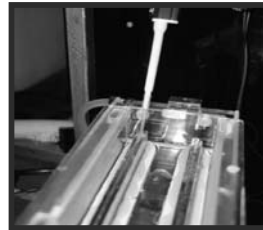
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At the thirty-fourth session of the Technical Working Party for Agricultural Crops held in Christchurch, New Zealand, from October 31 to November 4, 2005, under the agenda item concerning molecular techniques, Mr. Michael Camlin (United Kingdom) made a presentation on an International Seed Federation sponsored study assessing possible essential derivation relationships between ryegrass varieties. A copy of that presentation is provided as an Annex to this document.

[Annex follows]

**Assessing Possible Essential Derivation Relationships Between
Ryegrass Varieties**

Progress Report by M. S. Camlin & T. J. Gilliland



Preliminary ASSINSEL Project 1997-99

ASSINSEL/ISF sponsored study on perennial ryegrass varieties

Project Manager:

Bernard Le Buanec (Secretary General ISF)

Research Team:

Department Plant Genetics and Breeding, Belgium (I. Roldán-Ruiz)

- Project coordination
- AFLP marker analysis

Plant Testing Station, Northern Ireland (T. J. Gilliland)

- Morphological analysis

Keygene, The Netherlands (M. J. T. Van Eijk)

- Technical support for generation of AFLP fingerprints

Objectives of EDV Study 1997- 99

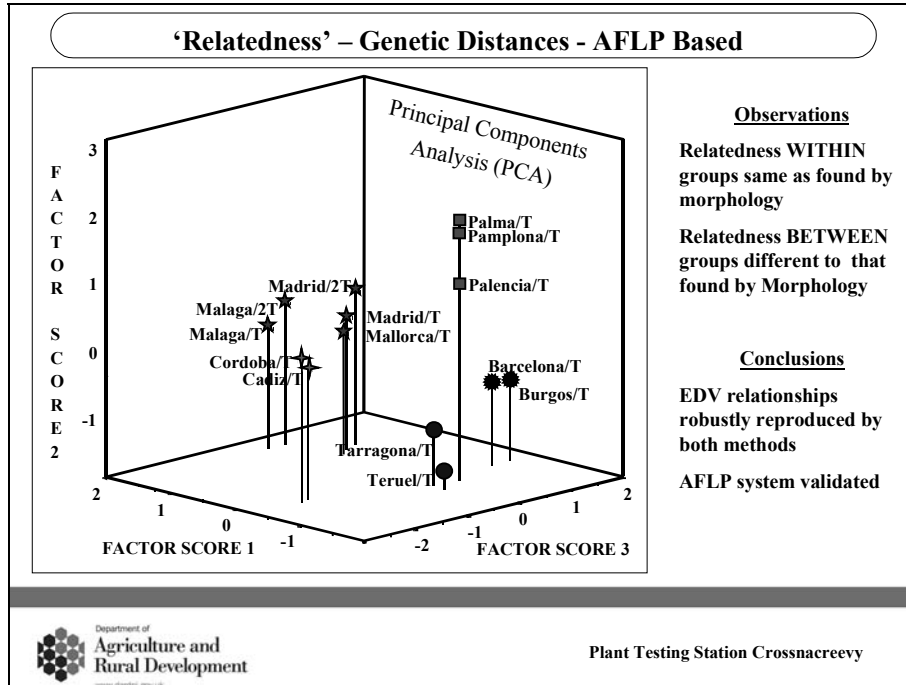
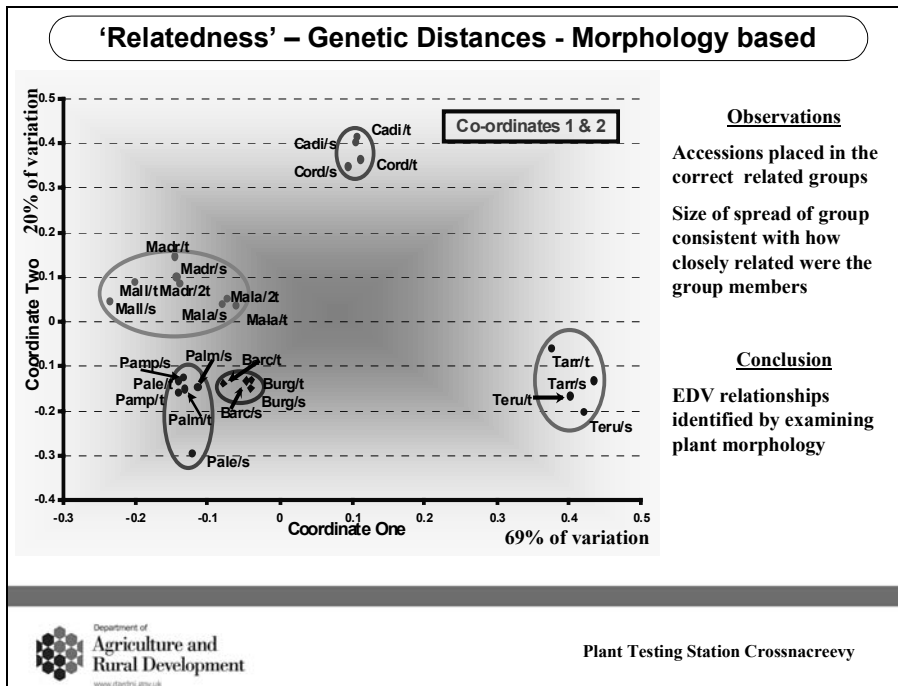
- Generate **morphological** and **DNA-marker** data for a core set of selected ryegrass accessions with known genetic relationships
- Compare the **relationships** between the genetic similarities estimated from the molecular and morphological studies
- Provide a scientifically based **interpretation of the data** from which ASSINSEL may start to develop a future protocol to follow in cases of putative essential derivation

Plant Material Used

Group	Description	Accession code name		
		Seed	Common Tillers	
M	IV EDV Selection (not EDV)	Madrid/S Málaga/S Mallorca/S	Madrid/T Málaga/T Mallorca/T	Madrid2/T Málaga2/T
B	IV EDV	Barcelona/S Burgos/S	Barcelona/T Burgos/T	
C	IV EDV	Córdoba/S Cádiz/S	Córdoba/T Cádiz/T	
P	Polycross 11 (Extreme EDV examples) 7 6	Palencia/S Palma/S Pamplona/S	Palencia/T Palma/T Pamplona/T	
T	Common Background	Tarragona/S Teruel/S	Tarragona/T Teruel/T	

'T' = same tillers of 60 seedlings used for Morphology and AFLP tests.

'S' = second sample from seed



Genetic distances based on AFLP frequencies											
	Barcelona	Burgos	Cádiz	Córdoba	Madrid	Málaga	Mallorca	Palencia	Palma	Pamplona	Tarragona
Barcelona											
Burgos	4.6										
Cádiz	12.6	12.2									
Córdoba	12.9	12.3	2.0								
Madrid	12.0	11.3	10.0	10.6							
Madrid2	12.6	11.5	11.3	11.8	1.4						
Málaga	16.6	15.9	11.8	12.1	5.3						
Málaga2	16.9	15.6	12.1	13.2	5.6	2.5					
Mallorca	17.5	15.8	16.7	15.9	10.4	11.7					
Palencia	9.7	9.7	10.2	10.0	10.0	14.8	16.3				
Palma	12.5	12.0	12.7	11.7	12.6	17.5	17.5	3.6			
Pamplona	13.3	12.9	12.4	11.7	13.4	17.4	18.4	4.0	2.3		
Tarragona	10.7	10.8	10.2	9.6	10.6	14.1	16.7	9.1	13.3	12.2	
Teruel	11.5	10.6	12.3	11.8	12.0	14.9	17.3	10.2	14.4	13.5	3.6

Squared Euclidean Distances
 Red = essentially derived pairing
 Green = close but not essentially derived pair
 Black = unrelated pairs

Genetic distances between all accessions calculated by comparing the presence and absence of markers using squared euclidean distance analysis.

A threshold distance of '5' was proposed as a trigger for an EDV investigation as all essentially derived comparisons fell around or below this level and more distant pairs had greater values

Department of Agriculture and Rural Development
 Plant Testing Station Crossnacreevy

Progress with Adoption of EDV Code of Conduct	
1999-2002	ASSINSEL herbage members' opinions surveyed on using the EDV study and Code of conduct adopted during the ISF Congress in Chicago. Ring test proposed to check technical details of protocol European breeders gave positive response but US breeders had concerns with both technical & legal aspects
Feb 2005	B. Le Buanec, the research team and US breeders held discussions in Oregon, USA and a validation study was proposed
Oct 2005	Protocol for validation study finalised by EU/US Working Group Molecular: SSR markers (30-50) Material: 50 accessions to include some original accessions plus putative EDV pairs from US genepools Phase 1: Compare genetic distances of 15 varieties (30 plants/variety) with bulks of 10, 20 & 30 plants Phase 2: Use bulks (if validated) to assess distances between all 50 accessions and validate or modify '5' threshold then coordinate in a multi-lab ring test.

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