

**Technical Working Party on Testing Methods and Techniques****TWM/1/23****First Session****Virtual meeting, September 19 to 23, 2022****Original:** English**Date:** September 15, 2022

---

**ISTA REPORT ON THE USE OF MOLECULAR TECHNIQUES***Document prepared by an expert from the International Seed Testing Association (ISTA)**Disclaimer: this document does not represent UPOV policies or guidance*

The Annex to this document contains a copy of a presentation on “ISTA report on the use of molecular techniques”, prepared by an expert from the International Seed Testing Association (ISTA), to be made at the first session of the TWM.

[Annex follows]

# ISTA report on the use of molecular techniques

**Presenter:** Ana Vicario  
**Location:** TWM - UPOV  
**Date:** September, 2022

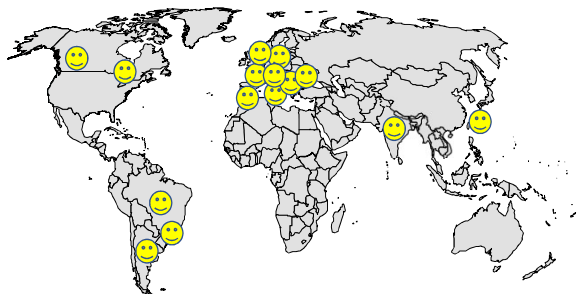
## ISTA VARCOM members



1. Chair: Ana Laura Vicario
2. Vice-Chair: Marie Claude Gagnon
3. Anne Bernole
4. Berta Killermann
5. Chiara Delogu
6. Hedwich Teunissen
7. Kae-Kang Hwu
8. Ksenija Markovic
9. KsenijaTaski-Ajdukovic
10. Keshavulu Kunusoth
11. Mariana Menoni
12. Ana Patricia Fernández Getino
13. Sean Walkowiak
14. .
15. .

Member Since

2007  
 2020  
 2016  
 2004  
 2007  
 2007  
 2007  
 2013  
 2010  
 2010  
 2021  
 2021  
 2022



## Agenda



How ISTA included DNA-based markers in the Rules

Proficiency Test (wheat) PT “0” – Results

Pea and Oat to the Rules  
Progress with Barley

Update on the development on new markers for  
detection of annual types in perennial rye grass varieties

Next Triennium

## How ISTA included DNA-based markers in the Rules



2016  
position paper

The **objective**:  
to have laboratories accredited to  
perform DNA-based variety verification  
testing by means of molecular markers.

**semi-performance-based approach - SPBA**

**various aspects of the laboratory are**  
performance- based

the **markers sets to be used are “prescribed”**

## How ISTA included DNA-based markers in the Rules



Validation of the marker set will follow the **ISTA Method Validation for Seed Testing** procedure



### Identify informative, repeatable and reproducible markers

Markers should be **selected based on the literature and the knowledge and experience** of the crop group regarding marker quality and levels of polymorphism.



Selected molecular markers **must be publicly available** such that primer sequences could be disclosed to laboratories seeking accreditation.

### ISTA Method Validation for Seed Testing

Varieties used **should represent as much as possible the global variability of the cultivated species**.

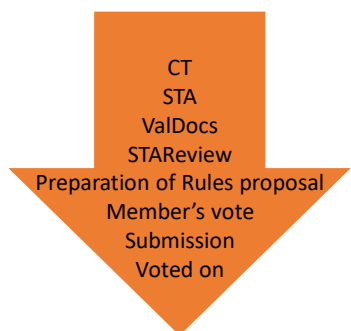
Note: Any electronic or hard copies of this document are uncontrolled copies. Confirm validity before use.

INTERNATIONAL SEED TESTING ASSOCIATION 1000 BRUSSELS, BELGIUM

## How ISTA included DNA-based markers in the Rules



### 1- Validation Procedure



If positive, published on ISTA Rules

### 2- Guarantee future PT

Request reference varieties from breeders and find a place to keep them for a long period so they could be available for future PT.

Obtain the matrix of reference profiles.

Guarantee sample preparation and shipment.



Specially for DNA-based tests

1. Prepare the PT (select samples and references)
2. Prepare and ship samples
3. Evaluation of results (using the new spreadsheet)
4. Reports for laboratories
5. General report for ISTA

**Rating for microsatellite analysis P Ts**

Change any values in a yellow cell

Number of alleles: 33

Number of varieties: 6

Minimum requirements for A rating:

% of varieties wrongly rated: 0% and % of alleles with a wrong result: 0%

Minimum requirements for B rating:

% of varieties wrongly rated: 20% and % of alleles with a wrong result: 10%

Minimum requirements for C rating:

% of varieties wrongly rated: 40% and % of alleles with a wrong result: 20%

Rating	Lab	# of varieties wrongly rated	P(%)	# of alleles with a wrong result	P(%)
A	1	0	0.0%	0	0.0%
B	2	1	16.7%		
C	3	2	33.3%		
BMP	4	0	0.0%		
A	5	5	83.3%		
C	6	0	0.0%		
	7	2	33.3%		

Reference A  
Reference B

Lab 1

Lab 2

Lab 3

Lab 5

Lab 6

Lab 7

Lab 8

Lab 9

Lab 10

Lab 11

Lab 12

Lab 13

Lab 14

Lab 15

Lab 16

Lab 17

Lab 18

Lab 19

Lab 20

Lab 21

Lab 22

Lab 23

Lab 24

Lab 25

Lab 26

Lab 27

Lab 28

Lab 29

Lab 30

Lab 31

Lab 32

Lab 33

Lab 34

Lab 35

Lab 36

Lab 37

Lab 38

Lab 39

Lab 40

Lab 41

Lab 42

Lab 43

Lab 44

Lab 45

Lab 46

Lab 47

Lab 48

Lab 49

Lab 50

Lab 51

Lab 52

Lab 53

Lab 54

Lab 55

Lab 56

Lab 57

Lab 58

Lab 59

Lab 60

Lab 61

Lab 62

Lab 63

Lab 64

Lab 65

Lab 66

Lab 67

Lab 68

Lab 69

Lab 70

Lab 71

Lab 72

Lab 73

Lab 74

Lab 75

Lab 76

Lab 77

Lab 78

Lab 79

Lab 80

Lab 81

Lab 82

Lab 83

Lab 84

Lab 85

Lab 86

Lab 87

Lab 88

Lab 89

Lab 90

Lab 91

Lab 92

Lab 93

Lab 94

Lab 95

Lab 96

Lab 97

Lab 98

Lab 99

Lab 100

Lab 101

Lab 102

Lab 103

Lab 104

Lab 105

Lab 106

Lab 107

Lab 108

Lab 109

Lab 110

Lab 111

Lab 112

Lab 113

Lab 114

Lab 115

Lab 116

Lab 117

Lab 118

Lab 119

Lab 120

Lab 121

Lab 122

Lab 123

Lab 124

Lab 125

Lab 126

Lab 127

Lab 128

Lab 129

Lab 130

Lab 131

Lab 132

Lab 133

Lab 134

Lab 135

Lab 136

Lab 137

Lab 138

Lab 139

Lab 140

Lab 141

Lab 142

Lab 143

Lab 144

Lab 145

Lab 146

Lab 147

Lab 148

Lab 149

Lab 150

Lab 151

Lab 152

Lab 153

Lab 154

Lab 155

Lab 156

Lab 157

Lab 158

Lab 159

Lab 160

Lab 161

Lab 162

Lab 163

Lab 164

Lab 165

Lab 166

Lab 167

Lab 168

Lab 169

Lab 170

Lab 171

Lab 172

Lab 173

Lab 174

Lab 175

Lab 176

Lab 177

Lab 178

Lab 179

Lab 180

Lab 181

Lab 182

Lab 183

Lab 184

Lab 185

Lab 186

Lab 187

Lab 188

Lab 189

Lab 190

Lab 191

Lab 192

Lab 193

Lab 194

Lab 195

Lab 196

Lab 197

Lab 198

Lab 199

Lab 200

Lab 201

Lab 202

Lab 203

Lab 204

Lab 205

Lab 206

Lab 207

Lab 208

Lab 209

Lab 210

Lab 211

Lab 212

Lab 213

Lab 214

Lab 215

Lab 216

Lab 217

Lab 218

Lab 219

Lab 220

Lab 221

Lab 222

Lab 223

Lab 224

Lab 225

Lab 226

Lab 227

Lab 228

Lab 229

Lab 230

Lab 231

Lab 232

Lab 233

Lab 234

Lab 235

Lab 236

Lab 237

Lab 238

Lab 239

Lab 240

Lab 241

Lab 242

Lab 243

Lab 244

Lab 245

Lab 246

Lab 247

Lab 248

Lab 249

Lab 250

Lab 251

Lab 252

Lab 253

Lab 254

Lab 255

Lab 256

Lab 257

Lab 258

Lab 259

Lab 260

Lab 261

Lab 262

Lab 263

Lab 264

Lab 265

Lab 266

Lab 267

Lab 268

Lab 269

Lab 270

Lab 271

Lab 272

Lab 273

Lab 274

Lab 275

Lab 276

Lab 277

Lab 278

Lab 279

Lab 280

Lab 281

Lab 282

Lab 283

Lab 284

Lab 285

Lab 286

Lab 287

Lab 288

Lab 289

Lab 290

Lab 291

Lab 292

Lab 293

Lab 294

Lab 295

Lab 296

Lab 297

Lab 298

Lab 299

Lab 300

Lab 301

Lab 302

Lab 303

Lab 304

Lab 305

Lab 306

Lab 307

Lab 308

Lab 309

Lab 310

Lab 311

Lab 312

Lab 313

Lab 314

Lab 315

Lab 316

Lab 317

Lab 318

Lab 319

Lab 320

Lab 321

Lab 322

Lab 323

Lab 324

Lab 325

Lab 326

Lab

## The logo of the International Seed Testing Association (ISTA) is a circular emblem. It features a central balance scale, symbolizing uniformity and justice. The words "UNIFORMITY" and "IN SEED TESTING" are inscribed within the upper part of the circle, while "INTERNATIONAL SEED TESTING ASSOCIATION" forms the outer ring.

6 participating laboratories: 3 accredited and 3 willing to evaluate their performance.  
Mandatory for accredited laboratories.  
Set of 6 varieties, 8 SSR wheat markers, 2 varieties sent as control and also their SSR profiles.  
Final report sent on October 2021.  
4 laboratories performed with an A and 2 with a B.

Samples are kept and prepared and shipped for each PT by CGC.

		Sample 1							
		Xgwm003	Xgwm413	Xgwm155	DuPw205	DuPw115	DuPw004	DuPw217	DuPw167
Reference A		96	109	166	189	209	311	240	262
		94	121	164	184	206	213	231	248
Reference B	1	102	109	164	183	209	311	0	264
	2	102	109	164	184	209	311	0	264
	3	102	109	164	184	209	311	0	264
	4	102	109	164	184	209	311	0	264
	5	102	109	164	184	209	311	0	264
	6	102	109	164	184	209	311	0	264
	7	102	109	164	184	209	311	0	264
	8	102	109	164	184	209	311	0	264



## Pea and Oat to the Rules – Progress with Barley

### Part C. Rules changes and new methods requiring a vote

#### Chapter 8

##### C.8.10.4. New method on *Pisum*

##### C.8.10.5. New method on *Avena sativa*

Identification and verification of varieties or testing genetic purity by means of DNA-based techniques is of extended use in many laboratories nowadays. In 2017, the first DNA-based method was included in the Rules for testing wheat. The interest for including such a technical procedure increased over time. The Variety Committee propose the inclusion of a DNA-based test for testing *Pisum* and *Avena* varieties.

The following proposal has been developed by a working group of the ISTA Variety Committee and approved by majority of votes by committee members.

### For barley

The statistical analysis is finalized. Leaders are writing the validation documents and Rules proposal for this year.

## Update on the development of new markers for detection of annual types in perennial rye grass varieties



Project leader: Giovanni López

Collaboration with Shaun Bushman from USDA who is developing the markers and Daniel Curry from Oregon State University who is providing seeds samples and technical support.

1. Next-Generation sequencing of the genomes
2. Selection of a set of 10 markers.
3. Development of the primers and probes.
4. Testing 5 markers on seed samples of Perennial and Annual *Lolium* varieties using two different testing strategies.
5. First tests are finished and now we are ready to assess the selected markers in more laboratories (ISTA validation procedure).



### Plans for next triennium

New crops and marker type.

Work with ATC in optical markers validation.

Set up a working group to continue writing the DNA handbook.

Define a strategy for setting up and organizing PT on new crops.

Experts invited to collaborate

Thank you!

Follow us on social media:

