

## Introduction

Maintenance protocols are part of the AEGIS quality system (AQUAS), aimed at ensuring that AEGIS accessions are properly maintained in their respective gene banks. It is a prerequisite for establishing AEGIS that AEGIS partners can trust each other in that they follow agreed-upon standards in their gene bank operations. The other element of the AEGIS quality system is an operational gene bank manual developed by participating gene banks (associated members of AEGIS member countries) and is available at the AEGIS website (<a href="http://aegis.cgiar.org/">http://aegis.cgiar.org/</a>). The specific procedures (minimum and recommended standards) for crop species collection management are developed by ECPGR working groups. Since there is no ECPGR working group on rye an *ad hoc* group was gathered in the frames of the ECPGR-funded project 'Improving the prerequisites for a European rye collection'. This document is one of the outcomes from the project .

In the current document, the specific maintenance procedures for accessions of cultivated rye and its wild relatives preserved at European gene banks are described. The list of procedures and recommendations is based on results from questionnaire on current maintenance practices in Europe and advices from experts working on conservation and utilisation of this crop.

## **Methods**

A discussion on good maintenance practice for rye was carried out at the workshop 'Improving the prerequisites for a European rye collections'

(http://www.ihar.edu.pl/gene\_bank/improving\_the\_prerequisites\_for\_a\_european\_rye\_collection.php) During this meeting a draft for the maintenance procedures was developed and it was agreed to send out a questionnaire to gain better overview of current practices for rye accession

maintenance in Europe. After the survey was conducted the necessary modifications in the draft were made, and the document was sent out for the final comments to the project group, the comments were incorporated and procedures presented at the ECPGR European Secale Database webpage (<a href="http://www.ihar.edu.pl/gene\_bank/europejska\_baza\_zyta\_en.php">http://www.ihar.edu.pl/gene\_bank/europejska\_baza\_zyta\_en.php</a>). Deviations from suggested procedures may take place when dealing with specific research material and genetic stocks.

## **Maintenance procedures**

The maintenance procedures are subdivided in conservation and regeneration sub-tasks, and a minimum and recommended requirements as well as current practices for gene bank procedures are listed in the tables (table 1 for cultivated rye & table 2 wild *Secale* accessions).

Table 1. The recommendations and current practices/requirements for maintenance of cultivated rye accessions

Procedure Procedure	<b>Current practice</b>	Minimum requirement	Recommended		
1. Conservation					
1.1. The lowest germination rate accepted for long-term conservation	70 - 90%	70%	80%		
1.2. The accepted seeds moisture content for long-term conservation	4 - 8%	8%	4 - 6%		
1.3.  The accepted seeds moisture content for medium/short- term conservation	4 - 15%	below 15%	4 - 6%		
1.4. The storage temperature for the long-term storage	-30°C to -18°C	-20°C to -18°C	-20°C to -18°C		
1.5. The storage temperature for medium/short- term conservation	-20°C to -18°C or +4°C to +8°C	+4°C to +8°C	-20°C to -18°C		
1.6. Containers/packages for long-term conservation	Sealed (laminated) aluminium- polyethylene bags	Sealed (laminated) aluminium-polyethylene bags	Sealed (laminated) aluminium-polyethylene bags		

Procedure	<b>Current practice</b>	Minimum requirement	Recommended
1.7.  Containers/packages for medium/short- term conservation	Sealed (laminated) aluminium- polyethylene bags, paper bags, jars (glass or plastic) with twist- off lid	Jars (glass or plastic) with twist-off lid	Sealed (laminated) aluminium- polyethylene bags
1.8.  Labelling of accessions for long- term conservation	Water- proof label (and frost tolerant) label on the containers/packages; some gene banks put also one copy of such a label in the container/package	Water -proof and frost tolerant label on the containers/packages and one copy of such a label in the container/package	Water-proof and frost tolerant label on the containers/packages and one copy of such a label in the container/package
1.9.  Labelling accessions for medium/short term conservation	Water-proof (and frost tolerant) label on the containers/packages and/or one copy of such a label in the container/package, hand written number with water prove marker	Hand written number on the container/package with water-proof marker	Water-proof label on the containers/packages and one copy of such a label in the container/package
1.10.  Minimum number of seeds for long-term conservation (including safety back up)	2000 - 10 000 seeds	2000 seeds	6000 seeds
1.11. Use of vacuum packaging for long- term conservation	'yes' and 'no'	'no'	'yes'
1.12. Frequency of germination tests	5 - 10 years	10	5
2. Regeneration			
2.1.  The number of seeds per accession sown for regeneration	100 - 500	100	500
2.2.  The minimum number of plants harvested for regeneration to be approved	80 - 300 (all plants from isolated accession plot harvested)	80* (all plants from isolated accession plot harvested) *In case lower number of plants are harvested and there is no available seeds in order to repeat the regeneration a note in the data base should be made to indicate the risk for reduced diversity	250 (all plants from isolated accession plot harvested)

Procedure	<b>Current practice</b>	Minimum	Recommended
		requirement	
2.3. The isolation method	<ul> <li>a) Distance 120 - 500m</li> <li>b) Isolators (cotton, linen or tomofan isolation tents) or separated greenhouse chambers</li> </ul>	<ul> <li>a) Distance 120m if plots also separated by hedges, if not – 300m</li> <li>b) Isolators (cotton, linen or tomofan isolation tents) or separate greenhouse chambers</li> </ul>	a) Distance more than 300m b) Isolators (cotton, linen isolation tents) or separate greenhouse chambers
2.4. The harvesting method	Manual harvesting	Manual harvesting	Manual harvesting
2.5. Cleaning harvested seeds	Manual cleaning or use of laboratory thresher	Manual cleaning or use of laboratory thresher	Manual cleaning or use of laboratory thresher

Table 2. The recommendations and current practices/requirements for maintenance of wild *Secale* accessions, only procedures deviating from maintenance or cultivated rye are included

Procedure	<b>Current practice</b>	Minimum requirement	Recommended		
1. Conservation					
1.1 The lowest germination rate accepted for long- term conservation	50 - 80%	50%	65%		
2. Regeneration	T.	T	T		
2.1.  The number of seeds per accession sown for regeneration	10 - 500	50	250		
2.2.  The minimum number of plants harvested for regeneration to be approved	10 - 200 (all plants from isolated accession plot harvested)	50* (all plants from isolated accession plot harvested) *In case lower number of plants are harvested and there is no available seeds in order to repeat the regeneration a note in the data base should be made to indicate the risk for reduced diversity	100 (all plants from isolated accession plot harvested)		
2.5. Cleaning harvested seeds	Manual cleaning or use of specific cleaning equipment	Manual cleaning or use of specific cleaning equipment	Manual cleaning or use of specific cleaning equipment		