# ENSCONET germination database report

***Produced by Ruth Eastwood, January 2009 – Version 1.2***

## Introduction

The European Native Seed Conservation Network (ENSCONET) is funded by the European Community's Sixth Framework Programme as an Integrated Activity implemented as a Co-ordination Action. Task 1.3 of Activity N3 (Curation) is to obtain and collate data on successful seed germination conditions that overcome problems of seed dormancy.

To achieve this task network partners submit collection and germination test data to a combined database. This database is currently accessible to ENSCONET members and unpublished tests are only visible to the institute which submitted them. The data investigated in this report were taken from March 2008. Since then 181 germination records have been added but these contain insufficient data to be included in analysis. As requested by some partners, 750 germination tests are hidden for publication purposes. As partners contributing germination data are diverse with different policies, research agendas and lab capabilities the data shows some heterogeneity. The minimum required data is species name, collection country and germination result. Successful tests are classified as those with germination percentages of 70 or more.

Based on the European Environment Agency (EEA) bioregions (2005 boundaries) ENSCONET divides Europe into 11 diverse habitat and climatic zones (Appendix 1). The EEA Anatolian bioregion is not considered under ENSCONET as it is geographically outside of Europe and the Mediterranean bioregion is split into two parts, East and West, for logistic purposes. Seed collecting activity has been focused on ten bioregions. The Steppic bioregion has not been considered to date. Bioregion origin is recorded at the point of collection. This study investigated, using the ENSCONET germination tests data, whether any trends in germination tests were apparent between collections from different bioregions. No clear trends were apparent yet some differences are discussed.

This report collates the data within the ENSCONET database and pays particular attention to germination test conditions. Subjects which would benefit from future research are given in italics.

Since by their nature wild species are very varied it is difficult to make specific germination recommendations in this report. A team at the Millennium Seed Bank, RBG Kew, are working on a germination predictor using taxonomic and geographical affinities in an attempt to make specific predictions for germination conditions. General recommendations in this report should not be taken as specific for any germination test and are based only on data within the ENSCONET database.

## General and Institute Statistics

ENSCONET partners hold 37,780 different accessions (March 2008 - Table 1) of 9684 taxa (7903 species). The number of accessions (collections) per taxa ranged from 1 (3463 taxa) to 359 (1 taxon). The ENSCONET germination table contains records of 17207 germination tests from 5583 accessions. The number of tests per accession ranged from 1 (401 taxa) to 231 (1 taxon). The accessions were collected by 26 Institutions in 41 countries covering 9 European bioregions. Collections came from 96 different families and 609 genera. Ten Institutes have contributed germination test data to the ENSCONET database. 89% of the test data was generated by RBGK and 43% of these accessions came from the Atlantic Bioregion.

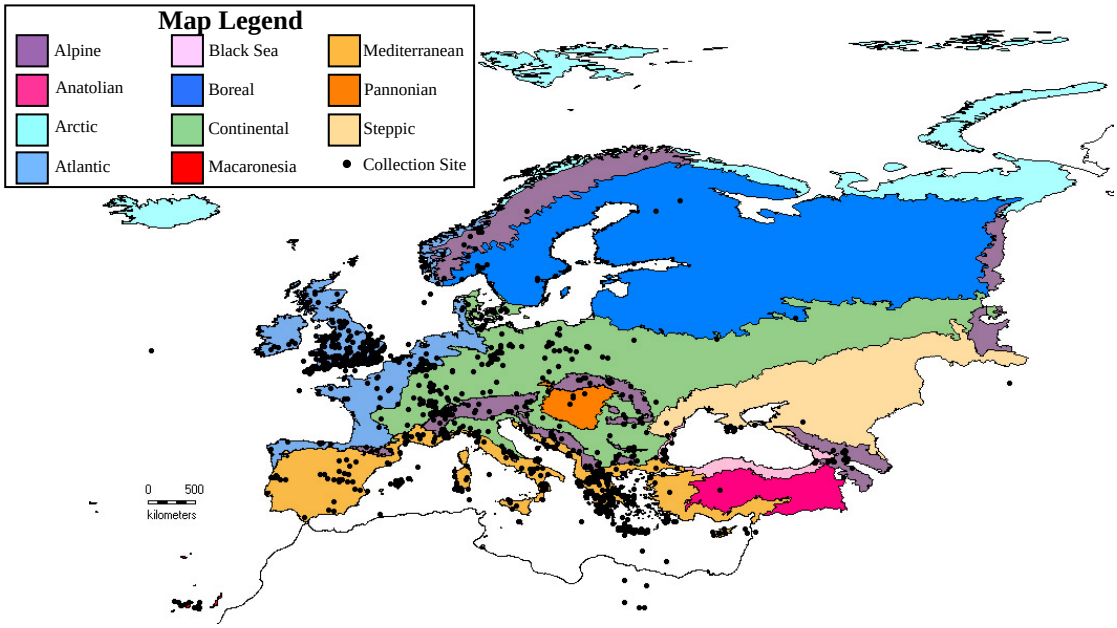
**Table 1: Distribution of collected seed accessions and numbers of germination tests carried out.**
*See Appendix 2 for acronym list.*

| Institute | Number of Accessions collected | Number of Tests | Number of Accessions tested | % Accessions tested |
|---|---|---|---|---|
| **RBGK** | 7972 | 15255 | 4727 | 59% |
| **NKUA** | 444 | 326 | 82 | 18% |
| **MAICh** | 617 | 634 | 114 | 18% |
| **UPM** | 5917 | 104 | 71 | 1% |
| **NBGB** | 607 | 84 | 81 | 13% |
| **PAV-UNI-CFA** | 1099 | 197 | 102 | 9% |
| **JB Soller** | 640 | 50 | 38 | 6% |
| **UVEG** | 1877 | 201 | 71 | 4% |
| **BG-CBDC-PAS** | 276 | 276 | 276 | 100% |
| **FUL** | 2556 | 80 | 21 | 1% |
| | | 17207 | 5583 | 14.8% |
| **JB Cordoba** | 6270 | 0 | | |
| **TCD** | 165 | 0 | | |
| **Jardin Canario** | 535 | 0 | | |
| **CYARI** | 174 | 0 | | |
| **MNHN** | 3966 | 0 | | |
| **Pisa BG** | 363 | 0 | | |
| **FUB-BGBM** | 325 | 0 | | |
| **NHMOSLO** | 40 | 0 | | |
| **Geneva*** | 386 | 0 | | |
| **Luxembourg*** | 107 | 0 | | |
| **ATHD†** | 363 | 0 | | |
| **BBGK†** | 854 | 0 | | |
| **Perugia†** | 1305 | 0 | | |
| **SARC-RIPP†** | 66 | 0 | | |
| **SVGB†** | 768 | 0 | | |
| **UPOL†** | 88 | 0 | | |
| | 37780 | | | |
| * Associate Member | | † non ENSCONET | | |

**Bioregions and geographical data**

ENSCONET partners have verified the viability of seed accessions from the Canary Islands to Turkey, Norway to Italy, by germination testing. Coverage is more extensive than shown on Map 1 where only accessions with lat/long data (3486 out of 5583 successfully tested collections) have been plotted.

Map 1 highlights the **need for verification of geographic data** within the ENSCONET database, collection points are visible not only outside of Europe but within the oceans.




**Map 1: Distribution of collected and tested seed accessions across Europe.** Bioregions are shaded following EEA recommendations. Black circles represent collection sites for tested accessions. Only 11 % tested accessions had associated lat/long data.

*A further utility of these data, if more were available, would be to look for climatic differences in germination temperature through mapping.* This would require greater resolution than currently available. With the implementation of the ENSCONET Seed Collecting Manual in partner institutes lat/long data should be recorded routinely for new collections.

Some species have been collected from multiple bioregions or countries and by different Institutes. Analysis shows that there are 1717 species with untested and successfully tested accessions in the database. Thus the database is an **important resource for germination conditions** for many untested accessions.

By March 2008 the bioregion with the most collections was the West Mediterranean (Table 2 and Fig. 1). However, a greater percentage of accessions have been tested and passed tests in the Atlantic bioregion. *Future work could address the paucity of collections in the Black Sea and Boreal bioregions and start collecting in the Arctic and Steppic bioregions.*

Data were partitioned into bioregions to investigate significant differences between germination conditions. There were no tests for Black Sea accessions. No clear differences became apparent during analysis. Similarly multiple accessions of the same species collected in different bioregions showed no clear differences in the germination conditions used. However, sample sizes were too small to identify significant trends.

**Table 2: Breakdown of collected accessions, species and numbers of tests carried out and their success**

Data in italics used in Fig 1.

| | | | | **Test Result** | | |
|---|---|---|---|---|---|---|
| **Bioregion** | **No. Species Collected (*No. Accessions Collected*)** | **% Species Tested (*% Accessions Tested*)** | **No. Germ. Tests** | **Data unavailable** | **0-69% Fail** | ***70-100% Pass*** |
| **Alpine** | 198 (*403*) | 33 % (*63* %) | 265 | 6 % | 45 % | *49 %* |
| **Anatolian** | 0 | | | | | |
| **Arctic** | 0 | | | | | |
| **Atlantic** | 2508 (*6406*) | 47 % (*37* %) | 6577 | 0 % | 31 % | *50 %* |
| **Black Sea** | 1 (*1*) | 0 % (*0* %) | 0 | - | - | - |
| **Boreal** | 46 (*64*) | 22 % (*20* %) | 82 | 0 % | 30 % | *70 %* |
| **Continental** | 492 (*799*) | 26 % (*24* %) | 526 | 0 % | 25 % | *75%* |
| **East-Med.** | 1737 (*4125*) | 29 % (*25* %) | 3974 | 0 % | 16 % | *84 %* |
| **Macronesian** | 625 (*1479*) | 7 % (*4* %) | 236 | 0 % | 13 % | *87 %* |
| **Pannonian** | 98 (*126*) | 14 % (13 %) | 68 | 0 % | 13 % | *87 %* |
| **Steppic** | 0 | | | | | |
| **West-Med.** | 3699 (*14424*) | 7 (*2*) | 912 | 0 % | 36 % | *64 %* |

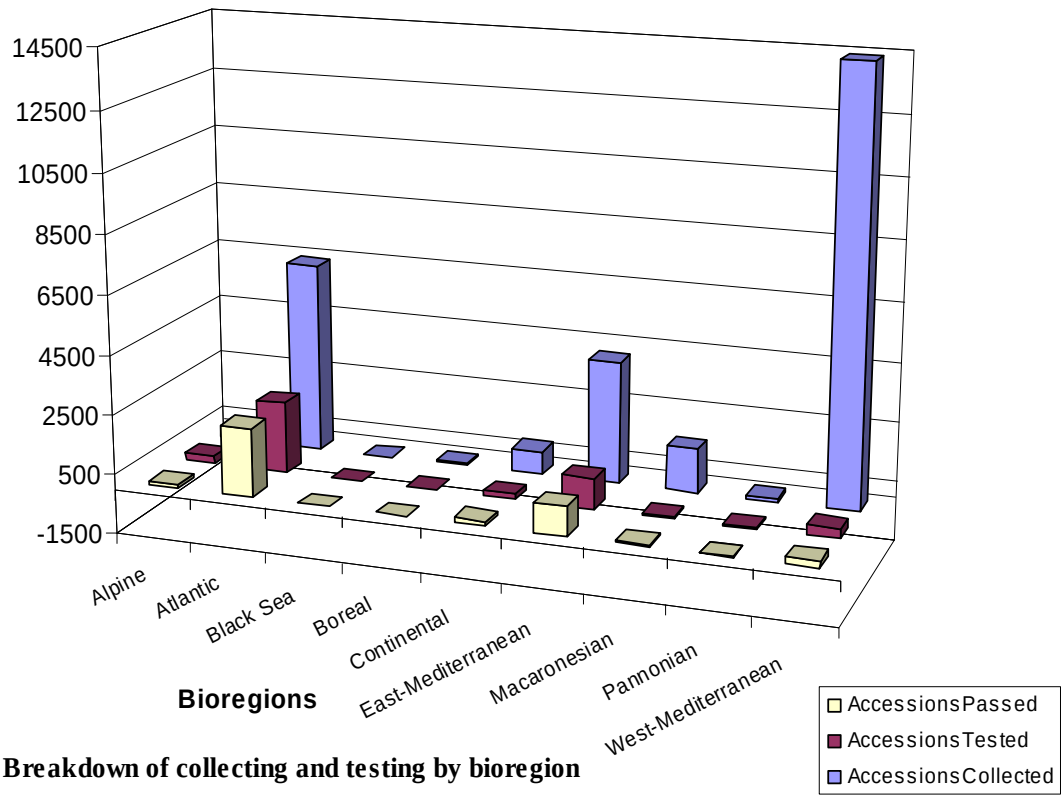



**Fig. 1: Breakdown of collecting and testing by bioregion**

## Germination Tests

The tests discussed in the following section are only those which were successful.

Pre-treatment

55% of tests had germination rates >70% without any pre-treatment. No pre-treatment was the mode in all bioregions except Alpine where the mode was stratification and only 12% tests had no pre-treatment.

Pre-Treatment Scarification, Chemicals and Conditions

Mechanical, chemical and thermal scarifications were used. 24% tests involved some scarification. Mechanical scarification was the most frequently used with 95% of scarified tests using this method (Table 3). Mechanical scarification was the most frequently applied scarification in all bioregions.

A number of other pre-treatment conditions have been utilised: cold and hot stratification, chemical application, imbibition (specially mentioned as a treatment not comment on seed behaviour), high humidity, darkness and alternating temperatures. Only 18% of tests utilised one or more of these treatments. The three most common treatments were imbibition, high humidity (but this is specified as a condition of the artificial aging process carried out in research tests) and cold stratification.

Chemical treatment included the use of Gibberellic Acid (GA3), Potassium Nitrate ($KNO_3$), smoke and Ethephon. Only 9% of successful tests included a chemical component in the germination test, this may not have been as a pre-treatment.

The two most common (19%) pre-germination actions were mechanical stratification and chemical treatment (6%). Mechanical scarification was the most common in five regions (Table 3). Stratification and chemical treatment together was the most common pre-treatment to Alpine accessions (See further notes on the Alpine Bioregion). High humidity was most prevalent in the Boreal and Macronesian bioregions reflecting a high number of research tests. However, mechanical scarification was the second most common treatment for both of these bioregions.

For the majority of families no application of a particular method was obvious from the germination data. However, notable exceptions were the Leguminosae and Cistaceae where mechanical scarification was by far the most common pre-treatment and Ranunculaceae and Papaveraceae where chemical treatment was most frequent. It is known that related groups often have similar germination requirements but it was difficult to extract these from the dataset.

Pre-treatment Temperature

Pre-treatment temperatures ranged from 0˚C to 35/20˚C with 20˚C being the overall mode for all tests (Table 3). When bioregions were compared the Atlantic bioregion was notably different having a modal pre-treatment temperature of 6˚C.

*Further work on the subject of pre-treatment temperatures would be interesting to investigate whether the low pre-treatment temperature commonly used for accessions from the Atlantic bioregion are a product of the testing process or necessary for successful germination.*

Only 142 successful tests were noted as stratified including 130 tests at a temperature of 20˚C. A comparison of the first pre-treatment temperature and germination temperature showed that 1198 successful tests had lower pre-treatment temperatures (785 with a difference greater than 10˚C), 226 successful tests had higher pre-treatment temperatures (106 with a difference greater than 10˚C), and 428 had a constant germination temperature. *These data indicate the use of the term stratification needs to be clarified and standardised across institutions.*

Comparison of the modal temperatures used in pre-treatment and germination show these are very similar for Boreal, Continental, East Mediterranean Macronesian, Pannonian and West Mediterranean bioregions i.e. temperature pre-treatments are not being applied. The Atlantic bioregion had a much lower modal pre-treatment and the largest temperature difference between pre-germination and germination temperatures.

Pre-Treatment Duration

Pre-treatment duration ranged from 1 to 252 days with the mode being 56 days (Table 3). However the modal values for bioregions, except Atlantic, when treated separately are less than 56 days (Table 3). These results indicate that for most tests a two-week period under pre-treatment conditions is sufficient.

## Germination

### Germination Temperature

Germination temperature range from 2˚C to 40˚C but 21˚C is the modal value. All bioregions except Alpine have a modal value of 21˚C (Table 3).

Min-Max successful germination temperatures were calculated for each species. These data show only a small range (50% species no range or 1˚C, 70% species range of 5˚C or less) for the majority of species. The largest range is 30˚C (three accessions show this, two with 5˚C–35˚C and one 10˚C-40˚C). 688 (33%) species are recorded at only one temperature. These results are most likely to be more of an artefact of how test conditions are set up than biological differences. *Further research would be needed to accurately gain an idea of temperature ranges for successful germination, to see whether some species have very narrow germination temperatures or if all have similar range lengths but just centre around different temperatures.*

In order to look for bioregion or latitudinal gradients accessions with germination data were plotted using different colours to represent different temperature groups. No pattern was observable.

### Germination Light:Dark Conditions

The most utilised (54% tests) light:dark regime was 12:12 (Table 3). The second was 8:16, 38% tests. Modal light:dark regime was 12:12 for each bioregion except Alpine where 8:16 was more commonly used.

### Germination Results

The germination test data indicates 75 % of tests in the database have successful (i.e. >70%) germination results. This figure does not account for any dead or dormant seeds within the tested batches. Pass rates can not be accurately calculated with the current data as not all institutes submitted failed tests.

## Discussion of why the Alpine bioregion results appear different to the other bioregions

All but sixteen of the tests in the Alpine bioregion were carried out by Botanical Garden – Center for Biological Diversity Conservation of the Polish Academy of Sciences. They were carried out during a high through-put period of testing when the effect of other germination conditions such as media and chemical treatment where being investigated. Thus the differences seen between the germination tests of this bioregion and the other bioregions are due mainly to the types of test set up rather than reflecting a biological difference.

When comparing germination test conditions of all accessions (regardless of bioregion) of species in the alpine list there is no better performance of the “Warsaw test conditions”. This is additional data to indicate that the difference highlighted between the Alpine and all other bioregions is an artefact of the testing process rather than a reflection of a biological difference. It must be noted however that the sample of species in this comparison is low and additionally Warsaw set up 500 seeds in each germination test whereas RBG Kew uses only 20.

**Table 3. Bioregion modal germination conditions for successful tests**

| Bioregion | Modal treatment | Modal pre-treatment when pre-treatment is applied | Modal pre-treatment temperature (˚C) | Modal duration of pre-treatment (days) | Modal germination temperature (˚C) | Modal light:dark regime |
|---|---|---|---|---|---|---|
| **All** | No treatment | Mechanical scarification | 20 | 56[5] | 21 | 12:12 |
| **Alpine** | Stratification and chemical use[1] | Stratification and chemical use[1] | 20 | 30[1] | 25/15[1] | 16:8[1] |
| **Atlantic** | No treatment | Mechanical scarification | 6[4] | 56[6] | 21 | 12:12 |
| **Boreal** | No treatment | High humidity[2] | 20 | 14 | 20/21 | 12:12 |
| **Continental** | No treatment | Mechanical scarification | 20 | 30 | 21 | 12:12 |
| **East Mediterranean** | No treatment | Mechanical scarification | 20 | 14 | 21 | 12:12 |
| **Macronesian** | No treatment | High humidity [3] | 20 | 14 | 21 | 12:12 |
| **Pannonian** | No treatment | Mechanical scarification | 20 and 45 | 14 | 21 | 12:12 |
| **West Mediterranean** | No treatment | Mechanical scarification | 20 | 14 | 21 | 12:12 |

Differences are highlighted.

[1] See discussion on the Alpine region.
[2] High humidity is used in artificial aging tests. Once these research tests are excluded the modal pre-treatment for the Boreal bioregion becomes mechanical scarification.
[3] High humidity has only one test more than mechanical scarification.
[4] 6˚C modal temperature with 267 tests but closely followed by 20˚C (250 tests).
[5] Biased by the number of Atlantic tests with a pre-treatment duration of 56. 14 days was the second most common pre-treatment duration.
[6] 14 days was the second most common pre-treatment duration.

## Conclusions

The ENSCONET database is an **important resource** for germination data. This value will grow as the number of accessible holdings increase. Additionally, the more comprehensive the records the greater the potential for observing and extracting patterns of germination. This is particularly relevant to test conditions and geographic data.

ENSCONET partners have made **good progress** collecting and testing seed accessions. The collections in ENSCONET are not only broad with regard to species but also in relation to interspecific genetic diversity illustrated by multiple collections of 6221 taxa.

It is positive to know that many **species can be regenerated** from seed at moderate temperatures using simple germination conditions (no pre-treatment, temperature change or chemical application). It was also found that treatment type is more influential than treatment duration. These finding are important as they means the seeds held in ENSCONET seed banks have the potential to be used in reintroduction programmes or for scientific research which requires growing plants and can be germinated by potential users without complicated laboratory facilities.

Germination data collected as part of routine Seed Bank testing is not easily amenable to analytic study due to test heterogeneity. Tests are set up to verify seed quality not to provide data for comparative study. These data are very useful to return germination conditions if a species match occurs but are **limited in the generation of germination predictions** unless part of a wider project. Although the way germination test data are collected in the ENSCONET project will not change it can be enhanced by specific research projects when necessary.

### Suggestions for Further Work

- Research into germination requirements of the same species collected in different bioregions.
- Increased collecting in Boreal and Black Sea bioregions. Collecting programmes to be started in the Arctic, and Steppic bioregions.
- If more data could be plotted it may be possible to map these against climatic data to look for trends in germination conditions.
- Investigations into temperature ranges for germination.

## RECOMMENDATIONS

- Profitable for ENSCONET members to search ENSCONET germination database and Kew's Seed Information Database (http://data.kew.org/sid/index.html) when deciding on germination conditions to set up as species may well have already been tested.
- Important that ENSCONET members continue to submit germination data with as much associated data as possible.
- Choose treatment type carefully as this is more influential on germination success than treatment duration.
- It is important to record lat/long data when collecting accessions.
- Verification of geographic data is important and needed.
- Use of the term stratification needs to be clarified and standardised across institutions.
- Use simple test conditions unless prior knowledge indicates otherwise.

**Appendix 1 – Composition of European Bioregions[1]**

| | Alpine | Anatolian[2] | Arctic | Atlantic | Black Sea | Boreal | Continental | Macronesian | Pannonian | Steppic | East-Med.[3] | West-Med.[3] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Area (Km2)** | 780,000 | 450,000 | 670,000 | 830,000 | 120,000 | 2,900,000 | 2,700,000 | 10,372 | 133,000 | 1,150,000 | 1,200,000 | |
| Albania | | | | | | | | | | | | |
| Austria | | | | | | | | | | | | |
| Azerbaijan | | | | | | | | | | | | |
| Belarus | | | | | | | | | | | | |
| Belgium | | | | | | | | | | | | |
| Bosnia-Herzegovina | | | | | | | | | | | | |
| Bulgaria | | | | | | | | | | | | |
| Croatia | | | | | | | | | | | | |
| Cyprus | | | | | | | | | | | | |
| Czech Republic | | | | | | | | | | | | |
| Denmark | | | | | | | | | | | | |
| Estonia | | | | | | | | | | | | |
| Finland | | | | | | | | | | | | |
| France | | | | | | | | | | | | |
| Greece | | | | | | | | | | | | |
| Georgia | | | | | | | | | | | | |
| Germany | | | | | | | | | | | | |
| Hungary | | | | | | | | | | | | |
| Iceland | | | | | | | | | | | | |
| Ireland | | | | | | | | | | | | |
| Italy | | | | | | | | | | | | |
| Latvia | | | | | | | | | | | | |
| Lithuania | | | | | | | | | | | | |
| Luxembourg | | | | | | | | | | | | |
| FYR Macedonia | | | | | | | | | | | | |
| Malta | | | | | | | | | | | | |
| Moldova | | | | | | | | | | | | |
| Montenegro | | | | | | | | | | | | |

| | Alpine | Anatolian[2] | Arctic | Atlantic | Black Sea | Boreal | Continental | Macronesian | Pannonian | Steppic | East-Med. | West-Med. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Netherlands | | | | | | | | | | | | |
| Norway | | | | | | | | | | | | |
| Poland | | | | | | | | | | | | |
| Portugal | | | | | | | | | | | | |
| Romania | | | | | | | | | | | | |
| Russia | | | | | | | | | | | | |
| Serbia | | | | | | | | | | | | |
| Slovak Republic | | | | | | | | | | | | |
| Slovenia | | | | | | | | | | | | |
| Spain | | | | | | | | | | | | |
| Sweden | | | | | | | | | | | | |
| Switzerland | | | | | | | | | | | | |
| Turkey | | | | | | | | | | | | |
| UK | | | | | | | | | | | | |
| Ukraine | | | | | | | | | | | | |
| Kazakhstan | | | | | | | | | | | | |

[1] As defined by the European Environment Agency, 2005 (http://dataservice.eea.europa.eu/dataservice/metadetails.asp?id=839&i=1)
[2] ENSCONET subdivides the species rich Mediterranean bioregion into East and West for logistic purposes.
[3] The Anatolian bioregion is not considered under ENSCONET as it is geographically outside of Europe.

**Appendix 2 – Institute Abbreviations**

| Abbreviation | Institute |
|---|---|
| RBGK | Royal Botanic Gardens, Kew |
| NKUA | National and Kapodistrian University, Athens |
| MAICh | Mediterranean Agronomic Institute Chania (Crete) |
| JB Cordoba | IMGEMA-Jardín Botánico de Córdoba |
| TCD | Trinity College Dublin |
| Jardin Canario | Jardín Botánico Gran Canaria |
| CYARI | Agricultural Research Institute Cyprus |
| UPM | Universidad Politecnica de Madrid |
| NBGB | National Botanic Garden Belgium |
| MNHN | Museum National d'Histoire Naturelle Paris |
| PAV-UNI-CFA | Università di Pavia / Centro Flora Autoctona della Lombardia |
| Pisa Botanic Garden | Università di Pisa, Orto Botanico |
| JB Soller | Jardi Botanic de Soller (Mallorca) |
| UVEG | Universitat de València |
| BG-CBDC- PAS | Botanical Garden Polish Academy of Sciences Warsaw |
| FUB-BGBM | Botanischer Garten und Botanisches Museum Berlin-Dahlem, FU Berlin |
| FUL | Jardim Botânico - Fundação da Universidade de Lisboa |
| NHMOSLO | Botanical Garden, Natural History Museum, University of Oslo, Norway |
| Luxembourg – *Associate Member* | Musée national d'histoire naturelle Luxembourg |
| Geneva – *Associate Member* | Conservatoire et Jardin botaniques Genève |
| ATHD – *non ENSCONET* | Julia & Alexander N. Diomides Botanic Garden |
| BBGK – *non ENSCONET* | The Balkan Botanic Garden at Kroussia Mountains |
| Perugia – *non ENSCONET* | University of Perugia, Italy |
| SARC-RIPP – *non ENSCONET* | RIPP Piešťany Slovak Republic |
| SVGB – *non ENSCONET* | Suceava Genebank , Romania |
| UPOL – *non ENSCONET* | University of Olomouc, Czech Republic |