

## Final report

# **Modification of the cultivation scheme and the plant material requirements for *Helleborus*.**



**Administrative coordinator:** CPVO

**Technical coordinator:** Naktuinbouw

**Name of the contact persons:** Kees Grashoff (Naktuinbouw)  
Laetitia Denecheau (CPVO)

**Authors:** Willem Wietsma  
Laetitia Denecheau

**Duration of the project:** 18 months, from April 2013 to September 2014

**Total estimated cost:** 30 600€

## Summary

In 2013-2014 Naktuinbouw investigated, in a project co-financed by Naktuinbouw and CPVO, a proposal of the applicants to modify the current cultivation scheme of *Helleborus* for DUS testing. It was concluded that a cultivation in pots with a delivery in April, starting in the open and transferred to a cold greenhouse end of November (thereafter Treatment 2), provides a culture more comparable with the common practise. It has also been proposed to reduce the number of requested plants from 24 to 15 in order to ensure a minimum number of 10 plants on which observations should be made in the DUS test.

The intention is to treat the living reference collection in the same way, to optimize the analyses of difference. However special attention should be brought to the transfer of the collection since plants in pots maybe more difficult to maintain for a long period.

## Introduction

The CPVO was approached by breeders/applicants/procedural representatives of *Helleborus* with a concrete proposal to modify the current cultivation scheme and plant material submission requirements for *Helleborus* varieties with the objective to be able to conduct and conclude the DUS technical examination within one year with more certainty.

The number of CPVO applications received (from 1995 to 2013) for *Helleborus* varieties is presented in the table:

Species Name	Number applications
<i>Helleborus</i> L.	39
<i>Helleborus orientalis</i> Lam.	11
<i>Helleborus niger</i> L.	29
<i>Helleborus</i> x <i>nigercors</i> J. T. Wall	16
<i>Helleborus</i> x <i>ericsmithii</i> B.Mathew	5
<i>Helleborus</i> x <i>ballardiae</i> B.Mathew	6

Currently, the DUS testing of *Helleborus* varieties in the frame of an application for Community plant variety rights is centralised at Naktuinbouw, where plants are cultivated outdoor in the full soil. This cultivation scheme does not seem to be optimal as, in the past, the weak drainage of the soil caused some damages. Some cold winters or bad weather conditions could also damage the candidate varieties and the reference collection. Moreover the commercial standard way of production seems to be more in ad equation with a pot culture rather than ground cultivation.

The current submission requirements for PBR-examination are 24 plants submitted in April. Observations are carried out outdoor until varieties start to flower as from December. At that moment, five plants are transferred into a cold greenhouse in pots. The flower characteristics are described in the cold greenhouse on the five plants,

from January till March. The leave characteristics are described on the remaining 19 plants in the field in June of the subsequent year. If the plants perform correctly, the duration of the test is one year. The uniformity is firstly assessed in the field on the 24 plants and later on, on the 5 greenhouses plants and 19 remaining plants on the field in the spring.

The breeders/applicants/procedural representatives of *Helleborus* were consulted as regards these modifications, 3 options were proposed to them:

Option 1: Unchanged situation.

Leave the DUS test procedure as it is now. In the trial report, it is referred to this option as *Treatment 1*

Option 2: Move to a cultivation in pots with a delivery in April.

The cultivation would no longer take place in the plain soil but in pots, which would be moved to a cold greenhouse over the winter, where observations will be carried out. This cultivation scheme would avoid the influence of bad weather conditions on the trial and seemingly match better modern practices of commercial *Helleborus* cultivation. Young plants would still need to be delivered in April. In this report it is referred to this Option as *Treatment 2*.

Option 3: Move to a cultivation in pots and a delivery of fully developed plants in September.

In addition to the option 2 above, young plants would no longer be delivered in April but fully developed plants (not flowering) would be delivered in pot in September and would be observed in a cold greenhouse until they flower.

Out of 7 answers received, the majority of the stakeholders (6) were in favour of the option 2. Some of them made some reservations as regards the option 3, mentioning the possible effect of the growing conditions at the applicant's premises until September on the expression of the observed characteristics. 1 applicant was in favour of the option 3.

The purpose of the project was to investigate the suitability and the implementation of the option 2 taking into account the outcome of the inquiry.

### **Objectives addressed:**

Therefore the Office and Naktuinbouw have investigated the possibility to implement the cultivation scheme of the option 2 "Move to cultivation in pots with a delivery in April" for *Helleborus* at Roelofarendsveen.

Have been investigated:

- the implementation of the pot cultivation for the DUS examination indoor (in cold greenhouse), including the living reference collection
- the implementation of a lower number of plants observed: 10 plants instead of 24 and the consequence on the uniformity judgement
- the consequence of the pot cultivation on variety descriptions and on DUS reports
- the evolution of the cost for the technical examination for a *Helleborus* candidate variety

## **Material and methods**

Design of the trial:

The selected varieties were only varieties granted plant variety rights.

The test included 12 varieties, selected in the following species :

Species Name	Number of varieties included
<i>Helleborus orientalis</i> Lam.	2
<i>Helleborus niger</i> L.	4*
<i>Helleborus</i> x <i>nigercors</i> J. T. Wall	4
<i>Helleborus</i> x <i>ericsmithii</i> B.Mathew	1
<i>Helleborus</i> x <i>ballardiae</i> B.Mathew	1

\* Four white varieties have been included in the test, taking into account the difficulties to establish the distinctness in this group.

For each variety, the test included two treatments:

- Treatment 1: 12 plants submitted in April and planted in the plain soil beginning of May. 10 plants out of these 12 have been later on potted in the greenhouse in November ('plain soil treatment').

- Treatment 2: 10 plants submitted in April and planted in pots outdoor beginning of May. These plants were transferred to the cold greenhouse in November ('pot treatment').

On 21/08/13, 15/11/12 and 15/05/14, the plants in pots and in the field were counted.

In week 47 (18-22 November) the 10 plants in pots were put into a cold greenhouse (Treatment 2).

In week 48, 10 out of the 12 plants grown in the plain ground (treatment 1) have been re-planted in pots and also put in the greenhouse side by side with the pot treatment. The remaining 2 plants on the field have been destroyed

All varieties have been described according the Standard Simplified Protocol: NL/KST/3, d.d. 16-10-2011, available under <http://www.naktuinbouw.nl/onderwerp/nationale-protocollen-ten-behoeve-van-het-dus-onderzoek>

The Standard Simplified Protocol: NL/KST/3, d.d. 16-10-2011 has been developed in a National Protocol: NL/KST/1, d.d. 18-11-2014 meantime. The measurements were made according NP/KST/1 and translated into notes (according to the approach described in UPOV/TGP 13).

## Results

### 1- Implementation of the pot cultivation, (Treatment 2)

The examination office has assessed the influence of the pot cultivation on the expression of the characteristics observed in the DUS test (plant habit, height, colour, according to the standard simplified protocol in vigor). This influence has been evaluated by comparing the variety descriptions of plants grown in pots, outdoor from April to November and then indoor (Treatment 2) with plants grown in the plain soil outdoor from April to November and

then transferred in pots indoor (Treatment 1), for the same variety . The uniformity in the samples in the two different treatments has also been observed. The results are summarised in the attachments table 1 and 2.

Comparison of the variety descriptions.

Based on both treatments and on the 12 varieties, 24 variety descriptions were made according to the protocol. Differences between the two treatments were found only in the following measured characteristics :

Characteristic observed	Number of varieties pairs out the 12 pairs, showing a difference of one note between the two treatments
5. Leaf: petiole: length	3 pairs
13. Leaf: cross section	4 pairs
16. Leaflet: length	4 pairs
17. Leaflet: width	3 pairs
24. Peduncle: length	4 pairs
25. Peduncle: width	1 pair
30. Bract: length	1 pair
31. Bract: width	1 pair
35. Sepal: length	3 pairs
36. Sepal: width	1 pair
41. Nectary: length	2 pairs
43. Filament: length	1 pair
49. Carpel: length	1 pair

Differences of more than 1 note were not observed.

For the other characteristics (leaf colour, flower colour, etc) no differences between the two treatments were found.

A difference in flowering time was also observed between the two treatments for some varieties: for 3 varieties (A. niger 2x; H. orientalis 1x) the flowering in pot culture (Treatment 2) was about 1-2 weeks earlier than for the field treatment (Treatment 1). For one case (H. x ericsmithii) the flowering in pot culture (Treatment 2) was 1-2 weeks later than for the field treatment (Treatment 1). This could certainly lead to different classes of notes. However this characteristic is not yet part of the national protocol.

From a general point of view, during the observations made in January till end of March, the varieties in both treatments were sufficiently uniform according the standards applied; however the pot culture was more uniform.

As expected, the two treatments would lead to minor differences in characteristics; however it could be considered that the differences observed will not cause discrepancies or problems in the reporting. The differences in the observations for a given variety in the 2 treatments are not of such an extent that they would affect the establishment of distinctness between the 12 close references varieties included in the trial. In addition, as there is a living collection, the selection of reference varieties to be grown alongside candidates is not only based on variety descriptions, it could always be double-checked during the trial. In conclusion, the decision on the distinctness should not be affected by the new cultivation scheme.

Development of number of plant surviving during the test period per treatment

From the 120 plants of the pot treatment, 6 plants died and 4 plants were poorly developed (15-11-2013), whereas in the plain soil only one plant died out of the 144 plants.

The plants of the pot treatment were put in a cold greenhouse in week 47. The plants of the plain soil were put in pots in week 48 and transferred into a cold greenhouse, side by side with their corresponding variety in pots.

During the growing period in the cold greenhouse, some plants died also.

On 15<sup>th</sup> May 2014, 26 plants out of 120 pots plants died (22 %), whereas for the plain soil, only 5 plants out of the 120 plants died (4 %).

The high number of plants loss from the pot treatment in the cold greenhouse died in April and May, after the DUS observations (made between January-March). Therefore the loss of the plants was not an impediment for the finalization of the DUS experimentation.

However maintaining these plants in pots for reference collection purposes can be a problem. Some applicants confirmed the difficulty to handle this crop in pots for a long period. Therefore for the maintenance of the living reference collection, one must pay attention to this problem.

Probably the growth circumstances in the pots were not optimal (too hot in summer, too wet after heavy rain), the examination office will have to continue optimising the pot culture in the future.

## **2- Maintenance of the reference collection in pots**

The current living reference collection contains more than 80 varieties.

It is intended to treat the living reference collection in the same way (cultivation in pots) to optimize and keep reliable the analyses of difference. However, there is a risk for the maintenance of the living reference collection.

The examination office proposes to renew the living reference collection in 2015-2016 by making cuttings from the old collection and to transplant them to a new field. In case of missing plants in the current collection, the examination office will have to ask for replacement samples.

The proposal is to maintain 2 plants outside and 2 plants in pots in a cold greenhouse for security reasons for the time being. However the examination office would need more experience and more time to establish with certainty a well adopted cultivation and maintenance scheme for the living reference collection. Then it would perhaps be possible to reduce the number of plants per reference variety in the living reference collection.

For all the reference varieties grown in pots, the quantitative characteristics will be described again in order to update the variety description's database in order to be comparable with the future candidate varieties grown and described in pots.

## **3- Implementation of a lower number of plants: 10 plants instead of 24. Consequence on the uniformity judgement.**

Only few cases of lack of uniformity were observed in the past trials. For the seed propagated applications, only one application showed too many off-types. For the vegetatively propagated varieties only one application with too many off-types was tested so far. No mutant off-types were ever found in the framework of the community plant variety system.

As some plants died during the experimentation in the pot treatment, it is proposed to ask for 15 plants to ensure a minimum of 10 plants at the end of the trial. The 5 extra plants will be destroyed before the plants are moved to the cold greenhouse in December, on a random basis.

## **4- Evolution of the cost for the technical examination for a Helleborus candidate variety**

In the current situation, the technical examinations are carried out in full ground outdoor. This corresponds to the cost group 9: Species with living reference collection, outdoor test, long cultivation, 2300€. The intention is not to change the cost group. The total duration of the test is about 10 months, seven months the plants are grown outside and 3 months in a cold greenhouse. The reduction of plants (from 24 to 15, and then 10 plants in the cold greenhouse) will compensate the costs of the 3 months greenhouse culture.

## **Conclusion**

**Considering the wish of the majority of the applicants to move to cultivation in pots with a delivery in April and based on the facts that:**

**1- plants from the pot cultivation were more uniform**

**2- the change of the treatment has only a limited effect on the expression of the characteristics. In case differences did occurred, only 1 note difference was observed.**

**The examination office is of the opinion to change the examination conditions and the cultivation scheme into a pot culture starting in the open in April and transferred to a cold greenhouse at the end of November. 15 plants will be requested. The initial cost group of the DUS technical examination will be kept.**

**A special attention will be given to the living reference collection in pot.**

#### **5- Follow up**

The CPVO will communicate to the stakeholders the outcome of the project and implement the new scheme as from April 2015 for all active applications which will be tested for the first year in 2015.

## **Cost of the project**

	<b>%</b>	<b>cost</b>
Naktuinbouw	50%	15 300 €
CPVO	50%	15 300 €
Total	100 %	30 600 €

## **Annexes**

- Standard Simplified Protocol *Helleborus*: SSP: NL/KST/3, d.d. 16-10-2011; National Protocol: NL/KST/1, d.d. 18-11-2014
- Table 1. Number of plants at August 21, 2013, November 15, 2013, May 2014, and quality of the plants.
- Table 2. Differences in character per treatment (field and pot), for 12 varieties.