CPVO · OCVV
Community Plant Variety Office
Office Communautaire des Variétés Végétales

Final report R&D project: "*Effect of seed priming on vegetable DUS tests*"

Executive summary

A collaborative project coordinated by the CPVO, with Naktuinbouw (NL), OEVV/INIA (ES), GEVES (FR) as participating examination offices, and the European Seed Association (ESA) took place in 2014 to investigate the effect which seed priming may have on samples submitted for DUS testing for varieties of tomato rootstock and eggplant. The study was conducted on primed and un-primed samples submitted by seed companies for three varieties in each crop. The study was conducted for one growing period according to the normal DUS test procedure outlined in the corresponding CPVO protocols for tomato rootstock and eggplant protocols currently in force. Naktuinbouw and OEVV/INIA conducted the studies on tomato rootstock, whilst Naktuinbouw and GEVES conducted the studies on eggplant. The results from the three participating examination offices correlated with each other. There had been no undue influence on the expression of the characteristics for any of the varieties under study as a result of the seed priming technique; therefore none would have been declared distinct from each other in a side by side comparison in a DUS test. The primed seed samples of all the varieties in the study germinated earlier and more evenly than their non-primed equivalents. A germination test conducted by each project partner half a year later on each of the seed samples they had received, demonstrated that there had been no loss in germination. However, this was not indicative on whether a primed seed sample would rapidly start losing its germination capacity after a few years compared to a non-primed seed sample. Possible further research could be envisaged to investigate a) the long-term lifespan of primed seed samples, and b) to evaluate whether there would be any advantages to be gained by examination offices if they were to accept the use of primed seed samples for DUS test and variety collection purposes.

Background

In relation to seed treatment, the current CPVO vegetable protocols mention the following statement regarding plant material to be sent for DUS testing:

"The plant material must not have undergone any treatment unless the CPVO and the Examination Office allow or request such treatment. If it has been treated, full details of the treatment must be given."

Following a survey carried out in 2011 regarding the possible use of primed or treated seed for DUS testing, several vegetable companies indicated in 2012 to be in favour of having this possibility, as priming of seed is used in several crops to break dormancy. Seed companies have argued that the use of non-primed seed results in poor and uneven germination; furthermore, for numerous varieties all their seed is systematically primed, so it is difficult for the seed companies to sort untreated seed just for the DUS test.

The VEM12 meeting in December 2012 discussed the matter and concluded that it might be interesting to formulate an R&D project on the effect of seed priming on vegetable DUS tests, using a few pilot species which are prone to this phenomenon.

The VEM13 meeting on 2-3 December 2013 looked at the draft proposal, and made a few recommendations for improvements. These have been integrated into the present text. The VEM13 meeting subsequently gave its recommendation that the proposed R&D project go ahead in 2014. The project proposal was subsequently given the blessing of the R&D Advisory Committee and the project was formally approved by the President of the CPVO on 30 January 2014.

Aim

The project analysed the effect of seed priming on the outcome of the DUS test (in particular those characteristics that might be influenced by the germination capacity of the seed sample) for a limited number of vegetable species in nominated examination offices entrusted for those species. After consultation by ESA amongst its vegetable members, it was proposed that the two pilot species should be eggplant and tomato rootstocks. Although these are not amongst the most important vegetable species applications-wise in the Community plant variety rights system, commercially they are mostly primed.

The studies carried out under the R&D project were undertaken for one growing period on the basis of the DUS test for those species. In order to make the project cost effective, short in duration, whilst at the same time meaningful in the representation of results, the varieties were observed over just one growing period in a selection of entrusted examination offices that carry out the most DUS tests for the CPVO in those species.

The project coordinator was the CPVO, with other project partners being ESA and the selected entrusted examination offices Naktuinbouw (NL) for both tomato rootstock and eggplant, OEVV/INIA (ES) for tomato rootstock, and GEVES (FR) for eggplant. The project commenced in March 2014 with the delivery of seed samples by ESA members to the three aforesaid examination offices. The final observations were made in November 2014.

Cost indications

The cost estimated for testing each sample of a variety is 2600 Euros. This is the combination of the standard DUS cost for one growing period in eggplant and tomato rootstock , plus the additional costs of two germination tests per variety and variable costs associated with the R&D project. The table below illustrates the total costs:

Pilot species	Examination office	Varieties	Samples per variety
Eggplant	Naktinuinbouw	3	2 (one primed, one non primed)
	GEVES	3	2 (one primed, one non primed)
Tomato rootstock	NAktuinbouw	3	2 (one primed, one non primed)
	OEVV	3	2 (one primed, one non primed)
Total samples: 24 = € 62,400			

Methodology

The R&D project will work on two sets of vegetable species, whose applicable CPVO protocols and entrusted examination offices are the following:

- Eggplant (*Solanum melongena* L.):
    - Protocol CPVO/TP/117/1
    - Entrusted examination offices: GEVES (FR), Naktuinbouw (NL), OEVV (ES), UKSUP (SK).
- Tomato rootstock (*Solanum lycopersicum L., S. lycopersicum x S. pimpinellifolium, S. lycopersicum x S. habrochaites*)
    - Two different CPVO protocols according to type of rootstock: CPVO/TP/44/4 for *Solanum lycopersicum* and *S. lycopersicum x S. pimpinellifolium* ; future CPVO/TP/294/1 for Solanum lycopersicum x Solanum habrochaites
    - Entrusted examination offices: GEVES (FR), Naktuinbouw (NL), DGAV (PT), OEVV (ES), NFCSO (HU), COBORU (PL)

In order to have a meaningful R&D results, it was decided to have the varieties of each pilot species grown in the main two entrusted examination offices for those species. According to the number of Community plant variety rights applications received, the entrusted examination office taking part in the project will be:

- ✓ Eggplant: Naktuinbouw, GEVES
- ✓ Tomato rootstocks: Naktuinbouw, OEVV

For each pilot species three varieties were studied under the R&D project. Each variety was sent as a primed and as a non-primed sample from the same seed lot; the quantity of seed per sample was half the quantity compared to the normal plant material requirements for the DUS test to be found in the S2 Gazette. In order to have representative samples from the industry, where different seed priming techniques may be utilised, ESA requested that for each pilot species, the three varieties come from different companies, although this was not fully achieved. The varieties used for the project were the following:

Tomato rootstock:

- *S. lycopersicum x S. habrochaites* 'He-Man' (Syngenta)
- *S. lycopersicum x S. habrochaites* 'Protector' (Clause)
- *S. lycopersicum x S. pimpinellifolium* 'Unifort' (Monsanto)

Eggplant:

- 'Adele' (Rijk Zwaan)
- 'Brigitte' (Rijk Zwaan)
- 'Dalia' (Monsanto)

The primed and the non-primed samples of the three varieties per pilot species were sown at the same time in the individual examination offices, although the sowing dates between examination offices varied slightly according to local conditions. A common set of un-primed comparison/control varieties was also sown at the same time. The examination office transplanted a random sample of young plants from each sown seed sample whilst avoiding making any selection during this stage. Each of the characteristics in the protocol were observed for one growing period according to the normal observation time outlined in the CPVO protocol. This included the observation of the asterisked disease resistance characteristics in tomato rootstock, except the test for *Pyrenochaeta lycopersci* (PI), for which the methodology on the CPVO protocol has proven to be unreliable. Special attention was paid to those characteristics, such as vigour, time of harvest maturity, etc. which may have been influenced by an earlier (or later), or uneven germination of the seed sample.

The participating examination offices for each pilot species drew up separate variety descriptions for the primed and non-primed samples of the three varieties under study. A theoretical DUS declaration was also made for each sample, to establish whether the effect of seed priming could have had an influence on the outcome of the technical examination and the possible registering of the same variety as two different varieties.

The precise methodology used by each of the three project partners for the tests it undertook on the tomato rootstock and/or eggplant samples is outlined in the annexes to the present report.

Related Study Forming Part of the Project

Entrusted examination offices have expressed concern that seed priming may also have the undesirable effect of dramatically reducing the germination rate over time. This would have negative consequences on the storage in the reference collection of the standard sample of varieties submitted for DUS testing as seed primed. Therefore the R&D project also investigated this related aspect of the effect of seed priming on the DUS test.

Participating entrusted examination offices opened the seed packets of primed and non-primed seed varieties at the same time and recorded this date. On the same day, small sub-samples from each packet were taken and a germination test carried out on each of these. The packets were re-sealed and stored as per normal DUS test procedures for future use as a standard sample. Once the "DUS test" forming part of the R&D project was concluded after one growing period (including drawing up of DUS declaration and variety description), the seed packets of the primed and non-primed seed were re-opened at a recorded date and a further germination test carried out. The results of the two germination tests were compared to each other to assess a possible drop in the germination rate over time of the primed and non-primed samples.

The outcome of these germination rate test results were analysed in order to assess whether it could have an influence on the storage and use of standard samples in the reference collection of entrusted examination offices. At the concept stage of the project it was recognised that the 6-9 month time period between the initial opening of the seed packet and its subsequent re-opening at the end of the growing period may yield limited information. Ideally germination tests would need to be done over a longer period up to 5-10 years, but this was beyond the duration over the period of 6-9 months, but this was beyond the duration of the R&D project.

Results

The detailed results obtained by each of the three project partners for the tests it undertook on the tomato rootstock and/or eggplant samples is outlined in the annexes to the present report.

Tomato rootstock

Naktuinbouw

The three samples of the primed seed germinated earlier and more evenly than their non-primed equivalents. Over time though, the non-primed samples caught up, so that by the time the observations were made on characteristics, there were no notable differences in the plant development between both sets of samples.

For the three varieties, there were no significant differences in the expression of the morphological characteristics between the primed and the non-primed samples of the same variety. The only exception was in the disease resistances, where a different note was given to 'He-Man' in the test for *Meloidogyne incognita*; however, since this was a quantitative characteristic and the primed and non-primed seed still expressed resistance, Naktuinbouw explained that it would have given the same outcome in a DUS test.

Five and a half months after the initial germination test, a second germination test was carried out. There were the same rates of emergence for both sets of samples as in the first test, and there was no loss in germination capacity for either the primed or the un-primed sample of the three varieties being studied. It is the intention of Naktuinbouw to continue making germination capacity tests at regular yearly intervals, even if this is beyond the scope of the R&D project.

OEVV/INIA

The primed samples of 'He-Man' and 'Protector' germinated earlier and more evenly than their non-primed equaivalents. Over time though, the non-primed samples of these two varieties caught up, so that by the time the observations were made on characteristics, there no notable differences in the plant development between both sets of samples.

For 'Unifort' though, there was no apparent difference in the time and development of seedling emergence between the primed and un-primed samples (see page 7 of Spanish annex). This did not correlate with the Naktuinbouw results for 'Unifort', and raises the question as to whether there could have been an error with the delivery of either two primed, or two un-primed samples of this variety.

For the three varieties, there were no significant differences in the expression of the morphological characteristics between the primed and the non-primed samples of the same variety. The only exception was earlier flowering of the primed sample of 'Protector' (6 days), which had a note 4 attributed to it, whilst the non-primed sample was attributed a 5; however, INIA explained that his small difference would not have made the plants of both samples distinct from each other in a DUS test. There were question marks or whether both samples of 'Unifort' were sufficiently uniform for the disease resistance *Fusarium oxsyporum* f. sp. *lycopersic*i (Fol) Race 2 (ex 3).

Six months after the initial germination test, a second germination test was carried out. There were the same rates of emergence for both sets of samples as in the first test, and there was no loss in germination capacity for either the primed or the un-primed sample of the three varieties being studied

Eggplant

Naktuinbouw

The three samples of the primed seed germinated earlier and more evenly than their non-primed equivalents. Over time though, the non-primed samples caught up, so that by the time the observations were made on characteristics, there no notable differences in the plant development between both sets of samples.

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For 'Brigitte' and 'Dalia', there were no significant differences in the expression of the morphological characteristics between the primed and the non-primed samples of the same variety. For 'Adele', statistical analysis seemed to demonstrate slight variations between the plants of the primed and non-primed samples for the quantitative characteristics "Fruit: length", "Fruit: diameter" and "Time of flowering"; however, these were not apparent visually, and would not have led to a different note being attributed in a DUS test.

Five months after the initial germination test, a second germination test was carried out. There were the same rates of emergence for the primed samples and the un-prime sample of 'Dalia'; however, there was slower and more irregular germination for the un-primed 'Brigitte', and even more so for the un-primed 'Adele'. What was surprising was a 12% drop in germination for the un-primed sample of 'Adele' compared to the germination test undertaken five months earlier.

Conclusions and discussion

The results from the three project partners for the two pilot species correlate with each other in general. This proves that the methodology and trial set up of the R&D project was robust and reliable. As is likely with any ring trial, some minor differences did occur between the project partners according to the sample they were testing. These anomalies could be attributed to things like the specifics of the individual trial set up at each project partner, the providence and maintenance of the seed sample, etc.

It can be stated that under the 2014 growing conditions of the R&D project, the seed priming process does not affect the expression of characteristics of any of the three tomato rootstock varieties and three eggplant varieties under trial. In a few cases, a one note difference was attributed by the examination office to a couple of varieties, but this was not sufficient to render the plants from the primed seed sample distinct from the plants of the non-primed seed sample. Therefore irrespective of whether seed was submitted as primed or un-primed, the variety would give the same outcome in a regular DUS test.

As was to be expected, all the seedlings from the primed seed samples germinated earlier and more evenly compared to their equivalent non-primed samples. There was the subsequent possibility that the plants emanating from the primed seed would maintain their head start throughout the duration of the DUS test, meaning that this could have a corresponding impact on characteristics such as "Plant: height" and "Time of flowering"; however, this did not prove to be the case. By the time the first observations on characteristics were made, the plants from the un-primed seed lots had caught up with their primed seed equivalents, so both sets of plants for the same variety were at the same stage of development. Even though seed priming did not exert any influence on the long term development of plants for tomato rootstock and eggplant, it could be that in some short season crops like radish, cornsalad, or even lettuce, some quantitative characteristics may indeed be affected by the primed seed germinating earlier.

A major concern expressed from the outset of the R&D by the three examination offices was the issue of the longevity of the primed seed samples. The technique of seed priming is used by seed companies to provide earlier germination of seedlings over a shorter time period for commercial growers and plant producers, compared to non-primed seed of the same variety. This is of outmost importance to such customers, which have to sow several thousand seed at the same time. If the subsequent seedlings then needs to be transplanted, it is imperative that this is done as quickly as possible after sowing and that the seedlings are at the same stage of development, since the transplanting process is often automised. According to the scientific literature and the recommendations of the seed companies themselves, the downside to this technique is that the lifespan of a primed seed sample is shorter than a non-primed one. Whereas this may not be an issue for commercial growers, who may wish to change growing a variety in the subsequent seasons, or even to seed companies who may consider a variety obsolete after a few years, this issue of great importance to examination office in the maintenance of their variety collections. Once a variety becomes part of common knowledge, then its availability as a comparison variety for future DUS trials has to be ensured as long as the variety remains in existence.

The problem for examination offices is knowing when they would need to renew a seed sample if this had been originally submitted as being primed. The duration of the R&D project (one year) does not allow to investigate how long a primed seed sample can be kept in the long term, although Naktuinbouw have expressed that they will investigate this aspect regularly in the coming years with the primed seed samples they received for the R&D

project. A possibility could therefore be that sub-projects to the present R&D project are requested to analyses this particular aspect in more detail.

According to the seed technology experts of certain vegetable seed companies, a primed seed sample will not experience any loss in germination for up to five years, as long as this is maintained under optimal conditions. This means storage at ca. 5°C and ca. 20% relative humidity in a hermitically sealed and foil lined seed packet. However, examination offices often need to re-open a seed packet, take out the required amount of seed in higher temperatures and relative humidities, and then re-close the seed packet afterwards; all these actions wil have adverse effects on the longevity of the remaining sample. Procedures could be implemented to mitigate these practical difficulties, such as requesting the seed sample to be submitted as several sealed seed packets rather than one individual one, thereby avoiding the need to open and reseal one packet continuously. The storage and data logging of such samples would be more complicated for examination offices though, which would probably also have to invest in larger and more sophisticated climate controlled seed storage facilities.

As a consequence of the extra work and costs which handling primed seed samples would imply, all three examination offices have expressed their reticence in working with these, if the priming technique itself does not influence the expression of characteristics and the outcome of a DUS test. The benefits of submitting primed seed would at face value only seem to benefit breeders and seed companies, since for them it means easier handling of stocks; rather than having separate large commercial primed seed batches, and small batches of un-primed seed of the same variety for DUS purposes, they could work with just one type.

Further benefits therefore need to be found for the examination offices if they are to be persuaded to accept primed seed of candidate and reference varieties for DUS purposes. Representatives of seed companies have argued that for some crops there is an inherent problem for seed dormancy to be broken, and this is where seed priming plays an essential role. Communications emanating from one or two examination offices have expressed that in tomato rootstock varieties, which is a fairly recent commercially exploitable crop, difficulties have indeed been encountered in producing an evenly germinated sample for the DUS test. In order to circumvent this problem, higher number of seed than is required has tended to be sown in order to ensure a sufficient number of plants for the DUS test. There is however the risk that a subconscious selection is made by the examiner of the most vigorous seedlings to be transplanted for the DUS test; caution must be taken that a consequent seedling selection is not made which would disregard possible off-type plants, thereby influencing the declaration on uniformity. If seed priming can effectively be used to promote even germination of a DUS candidate variety, then this could indeed provide a practical benefit to examination offices compared to current practices.

There remain some open questions after the conclusion of this R&D project, in particular the practical implications of the maintenance of primed seed sample in variety collections. Since this is an issue which has not been fully investigated in the project due to the length of time which it requires, any possible adoption on the use of primed seed for DUS testing purposes will have to be discussed with the stakeholders in the forthcoming CPVO vegetable expert meeting.