Additional report for the CPVO project **"Effect of seed priming on vegetable DUS tests"** on the germination tests for all samples 6 years after receipt of the samples.

Naktuinbouw carried out additional germination tests six years after the start of the first germination tests that were carried out for the CPVO project **"CPVO effect of seed priming on vegetable DUS tests"** in order to determine if problems can be expected if the DUS samples are needed for further examinations (e.g. for maintenance control or use as similar variety for later applications).

Method

The germination tests were carried out by the Naktuinbouw Seed Analysis laboratory. The first and second tests (respectively, in April and October 2014) were performed on 200 seeds. The third test (May 2016) was performed on 100 seeds only. The lower number of seeds was necessary to ensure that there would be enough seeds for later tests. The forth test (November 2020) was performed again on 200 seeds. The first tests were carried out on the received samples. The second, third and fourth germination tests were carried out on seeds from the original re-sealed bags, which were kept in the storage facilities at Naktuinbouw with a temperature of 16 °C (+/- 2°C) and a humidity of 50% (+/- 10 % below and+/- 20 above %). The bags were not re-sealed again but placed in the so called "long term storage". The conditions in this facility are: a temperature of 4 to 5 °C (+/- 3,5 °C below and 0,5 °C above) and a humidity of 25 to 30 % (+/- 10% below and 5% above).

All germination tests were performed on sand with a germination at alternating temperatures of 16 hours at 20°C and 8 hours at 30°C. This method is according to the International Seed Testing Association (ISTA) methodology for these crops. The duration of the germination tests depended on the speed of germination. The test was finished when it could be determined for al seeds if they produced normal or abnormal seedlings or if seeds were dead.

Results

For the three eggplant varieties the percentage of normal seedlings could be calculated after 10 days for all samples. There were no remarkable differences in the results of the primed and non-primed samples nor were there any remarkable differences between the first, second, third and fourth germination test. See table 1 below.

species	variety	primed sample	% of normal seedlings after 10 days			
			1st germination test (started 30-04-2014)	2nd germination test (started on 17-10-2014)	3rd germination test (started on 02-05-2016)	4th germination test (started on 04-11-2020)
Solanum melongena L.	Adele	No	97	98	99	99
	Adele	Yes	99	100	100	99
	Brigitte	No	99	99	99	100
	Brigitte	Yes	98	99	100	98
	Dalia	No	100	100	99	99
	Dalia	yes	100	99	100	100

Table 1: Results of the germination tests of the primed and non-primed seed samples of the eggplant varieties

For the tomato rootstock varieties, the number of days after which the final percentage of normal seedlings could be calculated differed per sample due to differences in germination. See Table 2 below.

species	variety	primed	percentage of												nr of days till the final counting			
			normal seedlings				abnormal seedlings				dead seeds							
			1st test 30-04-2014	2nd test 16-10-2014	3th test 04-05-2016	4th test 04-11-2020	1st test 30-04-2014	2nd test 16-10-2014	3th test 04-05-2016	4th test 04-11-2020	1st test 30-04-2014	2nd test 16-10-2014	3rd test 04-05-2016	4th test 04-11-2020	1st test 30-04-2014	2nd test 16-10-2014	3rd test 04-05-2016	4th test 04-11-2020
Solanum lycopersicum L. x *Solanum habrochaites* S. Knapp & D.M. Spooner	He-Man	no	94	85	86	92	4	8	6	2	2	7	8	6	28	18	21	14
	He-Man	yes	95	93	90	94	3	2	5	3	2	2	5	3	9	14	9	14
	Protector	no	100	95	94	98	-	1	4	2	-	1	2	0	9	8	14	9
	Protector	yes	99	98	96	98	1	-	3	2	-	-	1	0	6	6	6	6
Solanum lycopersicum L. x *Solanum pimpinellifolium* L.	Unifort	no	98	98	99	99	2	-	1	1	-	-	-	0	9	8	9	9
	Unifort	yes	99	97	94	99	1	1	2	1	-	1	4	0	6	6	9	9

Table 2: Totals of the germination tests of the primed and non-primed seed samples of the tomato rootstock varieties divided in normal and abnormal seedlings and dead seeds.

In general the non-primed samples of the *Solanum lycopersicum* L. x *Solanum habrochaites* S. Knapp & D.M. Spooner varieties are slower in germination then the primed samples. This is also the reason for priming the seeds. For the *Solanum lycopersicum* L. x *Solanum pimpinellifolium* L. variety Unifort there is almost no difference between the primed and non-primed sample. In general there are no real differences in results between the four germination tests for the percentages in the three categories normal seedlings, abnormal seedlings and dead seeds.

To be able to compare the percentage of normal seedlings, the percentages after 6 and 9 days are given in Table 3. If the total percentage of normal seedlings, abnormal seedlings and dead seeds could already be calculated after 6 days, “not applicable” is mentioned in the table at 9 days. For the 2020 test for He-Man no data are available for the 9 days count.

species	variety	primed	% of normal seedlings after 6 days				% of normal seedlings after 9 days			
			1st germination test (started 30-04-2014)	2nd germination test (started 16-10-2014)	3rd germination test (started 04-05-2016)	4th germination test (started 04-11-2020)	1st germination test (started 30-04-2014)	2nd germination test (started 16-10-2014)	3rd germination test (started 04-05-2016)	4th germination test (started 04-11-2020)
Solanum lycopersicum L. x *Solanum habrochaites* S. Knapp & D.M. Spooner	He-Man	no	6	8	10	3	35	27	31	Not available
	He-Man	yes	83	70	76	60	95	83	90	Not available
	Protector	no	66	43	23	8	100	95	88	98
	Protector	yes	99	98	96	98	not applicable	not applicable	not applicable	not applicable
Solanum lycopersicum L. x *Solanum pimpinellifolium* L.	Unifort	no	94	84	96	57	98	98	99	99
	Unifort	yes	99	97	85	83	not applicable	not applicable	94	99

Table 3: Results of the germination tests of the primed and non-primed seed samples of the tomato rootstock varieties.

Between the tomato rootstock varieties of the crossing *Solanum lycopersicum* L. x *Solanum habrochaites* S. Knapp & D.M. Spooner there were large differences between the results of the primed and non-primed seed samples in the first, second, third and fourth germination tests. Both varieties, He-man and Protector had a clearly higher percentage of normal seedlings after 6 days for the primed sample compared to the non-primed sample. For He-man this was also the case after 9 days. This is in fact the reason why the seeds are primed.

It is notable that the difference of percentage of normal seedlings after 6 days decreases with almost 20 percent per test for the first, second, third and fourth germination test for the non-primed sample of Protector, while the percentage of normal seedlings for the non-primed sample of He-Man seems to be quite stable for the first, second and third germination test and slightly lower for the fourth germination test. Also for the primed sample of He-Man there is a decrease in percentage of normal seedlings of the fourth germination test compared to the first, second and third test. While for the primed sample of Protector there are no differences in percentage of normal seedlings between the results of the four germination tests after 6 days.

For the *Solanum lycopersicum* L. x *Solanum pimpinellifolium* L. Unifort, the non-primed sample had a lower percentage of normal seedlings in the first, second and fourth test compared to the primed sample. Only in the third test the percentage of normal seedlings of the non-primed sample was higher than for the primed sample which could not be explained..

Conclusions and recommendations

1. In view of a higher and more uniform germination percentage for normal seedlings it is recommended that for the three species in this project Solanum melongena, *Solanum lycopersicum* L. x *Solanum habrochaites* S. Knapp & D.M. Spooner and *Solanum lycopersicum* L. x *Solanum pimpinellifolium* L. primed seeds are used.
2. The results of the first three germination tests showed that the percentage of normal seedlings of primed samples is sufficient to carry out a DUS test after two years when the samples are stored at the following conditions: a temperature of 4 to 5 °C and a humidity of 25 to 30 %.
3. The results of fourth germination test after six years showed that the percentage of normal seedlings of primed samples is sufficient to use the primed samples after six years for maintenance control or use as similar variety for later applications when the samples are stored at the following conditions: a temperature of 4 to 5 °C and a humidity of 25 to 30 %.