BRESOV - Breeding for Resilient, Efficient and Sustainable Organic Vegetable production

PROTOCOLS AND GUIDELINES TO MAXIMIZE ORGANIC SEED PRODUCTION FOR BROCCOLI, TOMATO AND SNAP BEAN

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EU H2020 RESEARCH PROJECT BRESOV: SHAPING THE FUTURE OF ORGANIC BREEDING & FARMING

The BRESOV project (www.bresov.eu), that ran from May 2018 until April 2023, aimed to tackle the nutritional challenges of a growing world population and changing climatic conditions by enhancing productivity of different vegetable crops in an organic and sustainable farming infrastructure. BRESOV has worked on broccoli, snap bean and tomato as those staple vegetable crops have significant roles in meeting our global food and nutritional security goal. Under organic conditions they can also contribute to storing carbon and introducing nitrogen for improved organic soil quality. The BRESOV partners have used modern techniques to explore the natural diversity within each crop and then use this to expand the breeding base to select and improve resilience traits in organic farming systems using annual crop rotation schemes. The new breeding lines have been tested for efficiency when grown under water, temperature and nitrogen stresses as well as for resistance to pests and diseases. In addition, desirable traits such as taste, visual appearance and post-harvest performance have been assessed.

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PREFACE

With the goal to increase organically managed farmland to 25% by 2030 together with the upcoming requirement to use exclusively organic seeds for organic farming, more seeds produced under organic conditions are needed on the market. Seed production under organic conditions is even more challenging than in conventional production systems, mainly due to the restricted access to regular products for fertilization and plant protection. To increase the number of healthy seeds, we tested different agronomic factors which could influence the number of seeds produced in several organic farms in Europe and provide recommendations on relevant factors in these guidelines.

1. INTRODUCTION

The objective of the protocols and guidelines are to maximise organic seed production for tomato, *brassica* and snap bean by providing seed producers with protocols and recommendations for relevant agronomic factors, which can influence the amount and quality of their seed production.

Seed producers aim to produce a high quantity and quality of seeds. This includes to produce a high number of seeds with high germination rates. In farming for seed production, production cycles are often longer than for production for feed or food. This increases the risk of production losses due to abiotic and biotic stresses. Especially in organic production systems, where the use of conventional pesticides and fungicides is prohibited, the challenges to produce large amounts of high-quality seeds are even higher.

Based on the input of stakeholders, the following six factors were identified and tested in three different climatic regions throughout Europe:

- Plant density (Tomato, *brassica* and snap bean)
- Use of microorganisms in combination with amino acids and foliar application of nutrients (Tomato, *brassica* and snap bean)
- Fruit harvesting regime (Tomato)
- Grafting on rootstocks (Tomato)
- *Rhizobium* symbiosis (Snap bean)
- Transplantation time (*Brassica*)

We assessed plant growth, appearance of abiotic and biotic stresses and measured seed number, Thousand Seed Weight (TSW) and seed germination rates of different varieties to determine the effect of these different agronomic practices on seed production.

Photo credit: OBS, 2021

2. HOW TO ASSESS THE IMPACT OF AGRONOMIC FACTORS ON SEED YIELD AND QUALITY?

2.1. GENERAL METHODOLOGY FOR FIELD EXPERIMENTS ON SEED PRODUCTION

Photo credit: ITAKA, 2019

The goal of the BRESOV seed production field trials was to assess the effect of different agronomic factors on seed production in *brassica* (mainly broccoli and cauliflower), tomato and snap bean. We aimed at increasing the number of seeds produced, while maintaining a good quality and high level of seed germination rate.

The BRESOV trials were conducted in open field trials (all three crops), tunnel (*brassica*, tomato) and greenhouse (tomato and *brassica*) in three different climatic regions in Europe: in Brittany, France, in Aargau, Switzerland, and in Sicily, Italy.

In general, the seedling or seeds were planted or sown in a randomized split plot design (Fig. 1 - see next page). This means that the fields were divided into three blocks representing three repetitions. Within each block, the field was divided in three main plots representing the nutrition protocols (e.g., control, treatment 1 with a specific dose of microorganisms and treatment 2 with another dose of microorganisms = **main effect**).

Within each main plot, we tested different genotypes (4-8 depending on the crop and trial = **split effect**). However, the genotypes within each repetition were randomized, which means that they were randomly mixed to reduce potential neighbouring effects. For example, the tomato trial conducted by P6-FiBL in Switzerland followed a randomized split block design with 3 repetitions (blocks) per genotype and treatment. Each repetition consisted of 12 plants per genotype, of which each 6 plants were assigned to the first treatment (frequently harvested) and 6 to the second treatment (less frequency harvested).

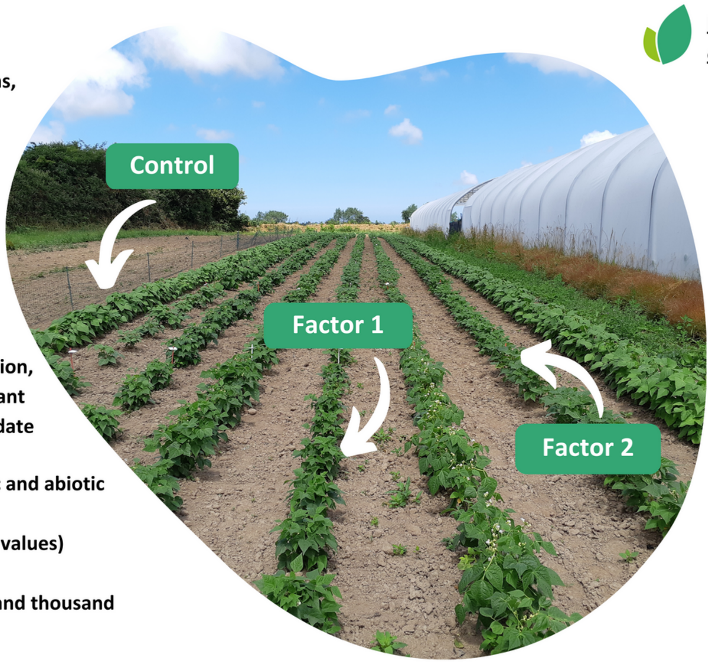
With this experimental setup in different regions, we were able to better distinguish the variation in seed production introduced by the agronomic practices, the different repetitions (which may be introduced by micro-climatic or different soil conditions within the field), the different experimental locations/years, the genotypes and their interaction effects.

Which factors can influence seed production?

- Location (e.g., climatic conditions, soil properties)
- Trial year
- Genotype
- Agronomic practices
- Interaction effects

Which data should be gathered during a seed production trial?

- Farming practices (e.g., fertilisation, soil preparation,sowing date, plant protection, watering) including date of application
- Plant health: occurence of biotic and abiotic stresses
- Number of lost plants (=missing values)
- Plant growth and fruit yield
- Seed production: seed number and thousand seed weight



How to assess the effect of different factors on seed production?

- Experimental setup, e.g. split plot design
- Experimental factors to test: control plot, factor 1, factor 2
- At least 3 repetitions per plot

How to analyse the data and identify relevant factors influencing seed production?

- To assess the different contributions of the different experimental factors to the total assessed variation , use e.g., ANOVA with post hoc test Tukey's HSD
- If you conducted field trials with different genotypes, in different locations and years you can also assess the variation introduced by these factors and their interaction effects

Photo credit: OBS - 2019 snap bean field trials

Fig. 1 General experimental setup and data assessment for seed production field trials.

2.2 SEED QUANTITY ASSESSMENT

To calculate the effect of different factors on seed production, it is important to assess the number of plants per plot (including e.g., the number of plants lost or not yielding seeds) and to collect dry pods (snap bean), siliques (broccoli) or ripe tomato fruits for seed extraction.

In the seed tomato trials, a subsample of about 1 kg of fruits was extracted by smashing the fruits and mixing with approximately 10% water. Afterwards, the mixture was left for fermentation at 25°C for three days, before the fruit flesh and other residues were washed off the seeds in a sieve. The seeds were dried on filter paper before counting (detailed method of tomato seed extraction and Schwitter *et al.* 2022).

For *brassica* and snap bean, the seeds were extracted from the dry pods and counted. Next to seed number, the Thousand Seed Weight (TSW) was measured. With the number of harvested plants, seed number and TSW, we extrapolated the medium number of seeds per plant (*brassica* and snap bean) and the medium number of seeds of the first two bunches of tomato or over a specific harvest period.

2.3. SEED GERMINATION PROTOCOLS FOR SMALL SEED LOTS ON PAPER OR ON SOIL

High germination rates and normal development of germinated plants are criteria to assess the quality of seeds. The seed quality can be evaluated by seed germination tests. In the Council Directive 2002/55/EC of 13 June 2002 on the marketing of vegetable seed it is defined that cauliflower seeds being sold must exhibit at least 70% germination rate, and at least 75% for broccoli, tomato and snap bean seeds.

There are two different ways of assessing seed germination at small scale, depending on availabilities of a growth chamber or greenhouse: on paper and on soil.

2.3.1. SEED GERMINATION PROTOCOL ON PAPER (BRESOV PRACTICE ABSTRACT 5)

If it is possible to control the temperature and light in a growth chamber, seed testing on paper is ideal. 150 seeds were divided into three lots of fifty seeds each. Seeds were placed in three aluminum containers containing absorbent paper moistened with distilled water (Fig. 2). Seeds were covered by absorbent paper and the aluminum containers were placed in the dark at optimal temperature (~20°C for *brassica* and snap bean, ~25°C for tomato). Seedling assessment took place when the cotyledons appeared and one true leaf was developed. The number of normal and non-germinated plantlets were scored. Following standards of the International Seed Testing Association (ISTA), for tomato the final counting took place fourteen days after sowing, eight days for snap bean and ten days for *brassica*. Out of the three replicates, the average number of normal seedlings was calculated by adding the number of normal seedlings (NNSs) of each replicate and by dividing by three:

Average number of normal seedlings = (NNSRep1+NNSRep2+NNSRep3)/3

The germination rate was calculated as follows:

Germination rate [%] = (Average number of normal seedlings/ Number of seeds sown) x 100

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Fig. 2 Seed germination assessment on wet absorbent paper and broccoli germinated seedlings.

2.3.2. SEED GERMINATION PROTOCOL ON SOIL (BRESOV PRACTICE ABSTRACT 13)

To test the seed germination rate of seed lots, a simple soil-based method can be performed as an alternative to the paper-based method (see practice abstract 5). Seeds were sown in 150 cell seedling starters with 50 seeds per replicate having three replicates per seed lot. To test different seed lots, the different replicates of each lot were placed in different seedling starters. The seedling starters were filled in with a soil suitable for small pots and with high drainage ability. One seed was placed in each pot and covered with soil. The seedling starters were watered by avoiding to float the seeds up. The temperature was kept at around 18 °C for *brassica*, 25 °C for tomatoes and snap beans and the soil moist. Seedling assessment and calculation of germination rates took place when the cotyledons appeared and one true leaf was developed as described in 2.3.1 (Fig. 3). Classification of germinated seedlings into the different category was done by following the protocol of the ISTA handbook of seedling evaluation specific to each crop type (ISTA, 2006).

Fig. 3 Exemplary pictures for normal (left plantlet in each picture) and abnormal (right plantlet in each picture) cauliflower seedlings.

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3. SUMMARY OF THE SEED PRODUCTION TRIALS

3.1. SNAP BEAN

Photo credit: OBS, 2021

The trials on snap bean were conducted over four years in two different climatic regions, Brittany in France and Sicily in Italy, on eight different genotypes. Agronomic factors tested were plant density (both sites), plant nutrition (both sites), *Rhizobium* inoculation (Brittany) and plant nutrition with additional foliar application (Sicily). TSW was highly influenced by the cultivar chosen, contributing to 71% to the total variation assessed in the trials, while the agronomic factor contributed to 12% of the variation. The seed number per sqm was mainly influenced by the agronomic factor tested, which contributed to 20% of the total variation.

3.1.1. MULTI-SITE TRIALS: PLANT DENSITY AND PLANT NUTRITION

In Brittany and Sicily, three different plant densities and different doses of microorganism application in combination with nutrient were tested.

Seed production was significantly higher per sqm for plants with the highest chosen plant density of 23.8 plants/sqm (Brittany) and with the medium density in Sicily (Rizzo *et al.*, 2023). Therefore, we recommend to transplant snap beans for seed production in Brittany in the following planting scheme: 5 plantlets per spot (bulk) with distance within rows of 0.30 m and distance between rows of 0.70 m, which allows mechanical weed control. Under the climatic and soil conditions of Sicily, plants grown with 14.3 plants/sqm yielded best results, also with regard to germination rate. With 14.3. plants/sqm, 5 plantlets were sown in bulks with a distance within rows of 0.5 m and between rows of 0.7 m.

Treatment with microorganisms and micronutrients did significantly increase snap bean seed production by application of D50%: Product applications were done at indicated BBCH stages for snap bean (BBCH stages snap bean, Feller *et al.*, 1995). We applied two different products of ITAKA Crop Solutions: 3KO® containing *Trichodermus* (*T. Arzianum, T. asperellum, T. atroviride*) and *mycorrhiza* (*Glomus mosseae, G. intraradices*) and ACE®, which supplied nutrients and amino acids to the plants (Tab. 1).

Tab. 1 Application scheme for application of microorganisms and amino acids in snap bean.

Snap Bean		Product application and doses (ITAKA Crop Solutions)	
BBCH stage	Number of Applications	Product name: 3KO	Product name: ACE
9	1	2 kg/ha	32 kg/ha
15 to 19	1	1 kg/ha	16 kg/ha
25 to 29	1	1 kg/ha	16 kg/ha
51 to 59	1	0.5 kg/ha	8 kg/ha
71 to 75	1	0.5 kg/ha	8 kg/ha
	Total:	5 kg/ha	80 kg/ha

3.1.2. SINGLE-SITE TRIAL: RHIZOBIUM INOCULATION

Seed production was significantly increased when seeds were inoculated with Rhizobium prior sowing in Brittany. Legumes benefit from a symbiosis with naturally occurring soil-borne bacteria, which fix nitrogen present in the air for plants to use. Plant aerial dry weight of inoculated plants was significantly higher compared to the treatments without inoculation. With seed inoculation, an increase in seed production by about 40% was achieved. We tested RhizoFix® RF-60, Feldsaaten Freudenberger on snap beans.

The product is ready to use and should be applied right before sowing. The product was evenly poured (or sprayed) onto the seed and the treated seeds were well mixed before sowing (BRESOV Practice abstract 10 and BRESOV Practice Abstract 11).

3.2. TOMATO

Photo credit: SECL, 2021

Photo credit: ITAKA, 2021

The trials on tomato were conducted over four years in three different climatic regions - France, Switzerland and Italy - on four to eight different genotypes. Determinate plants were grown in the field in Sicily, while indeterminate plants were grown in the greenhouse in all three different sites. Agronomic factors tested were plant density (France and Italy), plant nutrition (France and Italy), different harvesting regimes (Switzerland), grafting (France) and plant nutrition with foliar application (Italy). In the French and Italian trials, cultivars contributed to 37% variation of TSW taken trials from the two regions together. The agronomic factor tested contributed over all to 5% of the total variation. The seed number per sqm was mainly influenced by the cultivar, which contributed with 36%. The agronomic factor contributed with 10% and the interaction effect between cultivars and agronomic factor with 23%. The detailed results of the Swiss trial are available in the annexed publication (Schwitter *et al.*, 2022), and similarly to the other trials, genotypes were a significant factor for total fruit yield and TSW. Frequent harvest resulted in 25% more fruit and therefore total seed yield.

3.2.1. MULTI-SITE TRIALS: PLANT DENSITY AND PLANT NUTRITION

In indeterminate tomatoes grown in greenhouse, higher plant densities showed to decrease the number of seeds harvested per plant, but taken the higher number of plants per sqm into account, increased total seed number per sqm. This is why we recommend to increase the plant density to 5 plants/sqm. We transplanted the plants with a distance between rows of 0.5 m and within rows of 0.2 m. Each plant was grown with a single stem.

Although the total contribution of the agronomic factor to the variation of TSW was low, the application of D100%+F increased TSW in comparison to D50% (Malgioglio *et al*., 2021) .

3.2.2. SINGLE-SITE TRIALS: GRAFTING AND HARVESTING REGIME

Grafting increased yield and seed production in our trial in France, regardless of variety and rootstock tested (Floury *et al*., 2022). No difference was established between rootstocks. Grafting increased seed production by about 60%. Increase in seed production by grafting was related to the increase in yield, i.e., number of fruit and fruit weight, rather than the number of seeds per kg of fruit which was reduced by grafting (BRESOV practice abstract 9).

Different fruit harvesting frequencies were applied to test their effect on seed quality and germination rate. We applied two different harvesting regimes: In freq 1, mature fruits were regularly harvested twice a week, counted and weighed to assess fruit production. After three weeks, seeds from mature fruits harvested on extraction day were extracted. In freq 2, fruits with a mixed ripe maturity level were harvested after three weeks on extraction day only, counted, weighed and seeds were extracted. Seed production differed between genotypes and extraction time-points. Different harvesting procedures, and with that different fruit maturity levels, did not affect TSW and seed germination. Additionally, cool storage of tomato fruits prior to seed extraction was tested. Cooling fruits for 2-3 weeks before extraction did not affect germination rate negatively. The findings allow seed producers to choose based on their needs to rather harvest the fruits frequently or in bulk without compromising seed quantity or quality (for detailed information see the publication on the effect of tomato harvesting frequency, Schwitter *et al.* 2022, BRESOV Practice abstract 14, and Herforth-Rahmé, Joelle & Patricia Schwitter (2023) as well as Schwitter P. & Herforth-Rahmé J. (2023) publication in farmers' and Swiss agricultural research).

3.3. BRASSICA

Photo credit: OBS, 2019

Photo credit: ITAKA, 2019

The trials on *brassica* were conducted over four years in two different climatic regions, Brittany in France and Sicily in Italy, on four to eight different genotypes. Agronomic factors tested were plant density (Brittany and Sicily), plant nutrition (Brittany and Sicily) and plant nutrition with foliar application (Sicily). The cultivar contributed to 45% variation of TSW taken all experiments together. In addition, the cultivar x treatment 10% cultivar x year 6%, agronomic factor tested contributed over all to 6% and the location to 6% of the total variation. In contrast to snap bean and tomato, the seed number per sqm was also mainly influenced by the cultivar x year interaction (42%) and the cultivar chosen, which contributed by 39%. The agronomic factor tested contributed with 4% and cultivar x treatment with 7%. A considerable contribution to the total variation was not represented by the other factors taken into consideration (Year, location and their interaction effects with cultivars and agronomic factor tested). Therefore, cultivar choice is a highly relevant factor for increasing seed production especially for *brassica*.

3.3.1. MULTI-SITE TRIAL: PLANT DENSITY AND PLANT NUTRITION

In Sicily the experimental trials conducted during the five years, aimed at individuating the best sowing date or transplantation date. Especially for the sprouting type and for the biannual landraces /cultivars of broccoli, the sowing or the trasplantation from the end of June to the beginning of August contributes to a good vegetative growth of the plant and then a good establishment of the inflorescence and consequently a good seed production. In relation to the best plant crop density varying from 4 plants per sqm for sprouting types and Mediterrean landraces and 6 plants per sqm for the apical dominance types, the nutrition protocols by ammino acids and microbial consortia improve the seed yield and germinalbility.

In Brittany, the highest plant density with 6 plants per sqm yielded the best results. Therefore, we recommend a planting density of 0.5 m distance between rows and 0.3 m distance within rows. The application of microorganisms did not yield a significant difference in seed production in Brittany as for the other crops.

Similarly, to the results of other crops, the application of microorganisms showed significant differences in the trials conducted in Sicily (Detterbeck *et al.*, 2021).

3.3.2. SINGLE-SITE TRIAL: PLANT NUTRITION AND FOLIAR APPLICATION AND TRANSPLANTATION TIME

Application of microorganisms and micronutrients in combination with foliar nutrient application increased plant vigour seed production in trials in Sicily (Treccarichi *et al.*, 2022). Plants were grown at a density of 4 plants/sqm with a basic organic fertilization of 140 kg/ha N, 123 kg/ha P, 105 kg/ha K. Product applications were done at indicated BBCH stages for *brassica* ([BBCH stages *brassica*](), Feller *et al.*, 1995). Four different products from ITAKA Crop Solutions were applied: 3KO® containing *Trichodermus (T. Arzianum, T. asperellum, T. atroviride*) and *mycorrhiza (Glomus mosseae, G. intraradices*), ACE®, which supplies nutrients and amino acids to the plants, ST02213® (foliar amino acids and plant extracts) and Micro7213® (microelements) (Tab. 2).

The trial conducted in Brittany with different transplantation time-points (3 in total, each two weeks apart in autumn) did not significantly affect seed production.

Brassica		**Product application and doses (ITAKA Crop Solutions)**			
BBCH stage	N° Applications	3KO	ACE	ST02213	Micro7213
12 to 19 (transplantation)	1	3 kg/ha	48 kg/ha	/	/
39	1	3 kg/ha	48 kg/ha	5 kg/ha	3 kg/ha
49	1	2 kg/ha	32 kg/ha	5 kg/ha	4 kg/ha
59	1	1 kg/ha	16 kg/ha	5 kg/ha	4 kg/ha
69	1	1 kg/ha	16 kg/ha	5 kg/ha	4 kg/ha
	TOTAL:	10 kg/ha	160 kg/ha	20 kg/ha	15 kg/ha

Tab. 2 Application scheme for application of microorganisms, amino acids and foliar nutrients in *brassica*.

4. GERMINATION RESULTS

Taking all snap bean trials together, the most prevalent factor contributing to 30% of total assessed variation in germination rate was the interaction between genotype and agronomic factor. The agronomic practice tested contributed with 19%. Overall, the germination rates in snap bean trials were sufficiently high to meet the germination rates for marketing. However, in the trial with the highest plant density led to lower seed germination rates in Sicily. We therefore recommend the medium density for seed production in Sicily. In addition, the treatment with microorganisms and foliar application could have a beneficial effect on germination rates based on our data.

In tomato, due to the assessed data, the variation introduced by the location and/or the agronomic factor (40% in total) could not be distinguished. However, we hypothesize that the high temperatures present in the Sicilian trials could have had a negative effect on seed germination rate, why it is recommended to prevent high temperatures in tomato seed production.

Regarding contribution to total assessed variation in germination rate in *brassica*, the cultivar x location contributed with 24% and the agronomic factor chosen with 22%. Overall, seed germination rate was sufficiently high in all *brassica* trials conducted and met 75% of germination rate, which is needed for marketing of seeds in the EU.

5. RECOMMENDED AGRONOMIC FACTORS TO INCREASE SEED PRODUCTION IN ORGANIC AGRICULTURE

To increase seed production in *brassic*a, tomato and snap bean we identified several influencing factors. In our trials, the TSW was mainly determined by the choice of cultivar in all the three crops. In tomato and *brassica*, variation in seed number per sqm was also mainly influenced by genotype and interaction effects between the genotype and agronomic practice (tomato) and year (*brassica*). In snap bean, the variation was evenly distributed between different factors analysed. Therefore, we recommend to choose cultivars suited to the specific climatic conditions for ideal seed production.

Overall, the agronomic practices contributed to around 4-22% of the total variation, depending on crop and characters identified. In all three crops, different planting densities had a significant effect on seed production (Tab. 3). Here, we can recommend the medium to highest densities that were tested for the three crops. Although the seed production per plant was reduced for some crops at the highest densities, the seed production per sqm was nevertheless significantly higher compared to the lower densities.

Tab 3. Recommended agronomic factors to increase seed production of the snap bean, tomato and *brassica*.

Crop	Recommended agronomic factor	Best results within BRESOV experiments
	Plant density	Brittany: 23.8 plants/sqm (5 plantlets per spot (bulk) with distance within rows of 0.30 m and distance between rows of 0.70 m) Sicily: 14.3 plants/sqm (5 plantlets are sown in bulks with a distance within rows of 0.5 m and between rows of 0.7 m) CAVE: Seed germination rates (nutrition protocol and *Rhizobium* inoculation can have beneficial effect)
	Microorganism application in combination with amino acids	Sicily: the total amount of each product applied (3KO, ACE, ITAKA srl)
	Rhizobium symbiosis	Seed treatment with RhizoFix® RF-60, Feldsaaten Freudenberger
	Plant density	Greenhouse, indeterminate production: 5 plants/sqm (distance between rows of 0.5 m and within rows of 0.2 m. Each plant was grown with a single stem).
	Microorganism application in combination with amino acids and foliar nutrition	Sicily: the total amount of each product applied (3KO, ACE, ST02213, Micro7213, ITAKA srl)
	Different harvesting regimes	Regular harvesting or bulk harvests every three weeks do not influence TSW or seed germination. Both can be used based on seed producer's needs.
	Cooling fruit before seed extraction	Cooling tomato fruits for 2-3 weeks before seed extraction did not affect germination rate negatively and can save on number of extractions by pooling several harvests together.
	Grafting	Grafting increases fruit production and with that seed production. Commercially available rootstock comparable to tested BRESOV rootstock.
	Plant density	6 plants/sqm (0.5 m distance between rows and 0.3 m distance within rows).
	Microorganism application in combination with amino acids and foliar nutrition	Sicily: the total amount of each product applied (3KO, ACE, ST02213, Micro7213, ITAKA srl)

In tomato, harvesting regime as well as fruit cooling had no influence on seed production and quality except for the fact that regularly harvested plants produce more fruits. We therefore recommend to follow the strategy that suits best to the producer needs: small seed production of different varieties can benefit from regular harvest and storage of fruits for a pooled extraction to save time and resources, while larger producers can harvest every three weeks and extract the seeds after removing obviously bad fruits.

Some agronomic factors were tested at a single location only, but positively influenced seed production. This included inoculation with *Rhizobium* in snap bean and grafting in tomato. In general, we recommend to increase the productivity and vigour of the plants. Healthier, more vigorous plants produce more fruits and with that seed production is increased.

Application of microorganisms, amino acids and foliar application showed positive effects in Sicily in contrast to the trials conducted in France. We hypothesize that this could either be due to the different climatic conditions, due to different soil conditions (marginal soil in Sicily in comparison to long cultivated organic, well-nourished soils in Brittany) or due to a combination of these effects. Another hypothesis is that the strains used in ITAKA products are adapted to the Sicilian region and are therefore more effective there.

Photo credit: SECL, 2020

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