# Selection and breeding of tomato for organic conditions

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# Abstract

One of the main aims of the H2020 Framework Programme BRESOV project is the development of tomato materials specifically adapted to organic agriculture. Germplasm of potential interest for organic agriculture was assembled in a core collection (CC; 233 accessions) for pre-breeding, and in a breeding set (BS; 217 accessions) for selection and breeding. Both the CC and BS have been characterized for multiple morphological, agronomic, and quality traits under organic conditions in Italy and Spain. Also, the CC and BS, and a collection of EMS mutants have been characterized for tolerance to drought and high temperature. A wide diversity was found in the materials for most traits. Most of the cultivated tomato materials of the CC and BS were also genotyped using the high-throughput ddRAD sequencing technique revealing a high genetic diversity in the collection and allowing the detection of candidate genes and associations for relevant traits under organic conditions. The screening of BS for several diseases (*Phytophthora*, *Fusarium*, TSWV, ToMV) has allowed the identification of new sources of tolerance and resistance. A selected set of materials has been screened both in Italy and in Spain under control, drought and low N fertilization conditions, and resilient materials under these conditions have been identified. Hybrids obtained between selected materials has allowed the identification of promising hybrids heterotic for yield and with high fruit quality. In addition, several backcross programmes have been undertaken in order to introduce resistance to several diseases, and advanced backcrosses with introgressed resistances have been obtained. New intraspecific and interspecific hybrids have been developed and tested as rootstocks. Some of them outperformed control rootstocks commonly used for grafting tomato. The new improved tomato materials specifically selected for organic conditions can have a dramatic impact in the production of organically grown tomato.

Keywords: breeding, hybrids, landraces, organic systems, resilience

# **INTRODUCTION**

The development of tomato cultivars specifically adapted to organic agriculture has been recognized as a need for this cultivation system (Lammerts van Bueren et al., 2011). Often farmers do not have available tomato materials specifically selected and suited to organic conditions and very frequently have to make use of commercial cultivars, including  $F_1$  hybrids, mostly developed for conventional agriculture and adapted to high inputs systems.

Breeding tomato for organic conditions takes into account complex traits such as nutrient and water use efficiency, tolerance to abiotic and biotic stresses, so that a high resilience is obtained, and fruit quality (Lammerts van Bueren et al., 2011). Using unexplored germplasm such as local landraces and exploiting genotype × environment effects are key factors for selecting and developing new materials for organic tomato cultivation (Chable et

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Acta Hortic. 1354. ISHS 2022. DOI 10.17660/ActaHortic.2022.1354.13 Proc. III Int. Organic Fruit Symp. and I Int. Organic Vegetable Symp. Eds.: F. Branca et al.

al., 2020). In this respect, some materials of great interest such as the highly resilient and longshelf-life tomatoes of the "da serbo" or "de penjar" type, which are characteristic of the Mediterranean regions of Italy and Spain, have largely been explored from the breeding point of view (Conesa et al., 2020).

Moved by the pressing need of selecting and developing promising cultivars for organic vegetable production, the BRESOV project, a multi-actor research and innovation action from the H2020 programme, is aimed at the selection and breeding of new brassicas, snap beans and tomatoes resilient materials. Here, we present the main activities performed on tomato pre-breeding and breeding with the aim of developing new tomato materials with improved performance under organic conditions.

## **MATERIALS AND METHODS**

#### **Plant materials**

The germplasm accessions and breeding materials available to the BRESOV project partners were assembled to create a core collection (CC) and a breeding set (BS) of tomato. Materials included in the CC were selected so that they were representative of the tomato genepool diversity, including wild species and cultivated tomato materials. Information already available on origin, phenotypic and genotypic data, and potential for organic agriculture, including high resilience, and resistance or tolerance to diseases, as well as on genetic and phenotypic diversity were considered in establishing the CC. The BS mostly contains local cultivars and breeding lines with high perceived potential for direct use in organic agriculture or for the selection and breeding of new materials adapted to organic agriculture. Selection was performed after consultation to stakeholders (societies for organic agriculture, breeding companies, and foundations) and organic farmers and associations, taking also into account key traits such as high resilience, resistance or tolerance to diseases and abiotic stresses, diversity of commercial types, and potentiality for the market. A particular emphasis was performed in selecting accessions of the highly resilient tomatoes of the "da serbo" and "de penjar" types, which have been traditionally grown under Mediterranean conditions during summer with low or no irrigation.

An ethyl methanesulfonate (EMS) mutant collection was developed by University of Almería using 'Moneymaker' as the genetic background. This collection was also made available to the BRESOV project objectives for contributing to the increase of the genetic diversity available in the plant genetic resources (PGR). Two subsets of 4,010 and 3,759  $M_2$  families were established.

## Morphoagronomic characterization of CC and BS

The CC and BS were grown in the open field at two organic fields in Alcàsser (Spain) and Monsampolo del Tronto (Italy) during the spring-summer season of 2019. The cultivation and management of the crop was the usual for organically grown tomato. Details on the cultivation and management of plants can be retrieved from Tripodi et al. (2021). At each site, plants were grown in a randomized complete block design, with three replicates and four plants per replicate. Plants were characterized using the BRESOV phenotyping kit, which contains 21 descriptors of general data about the phenotyping assay, 15 descriptors for morphological traits, and 20 descriptors for agronomic traits, including root traits. This phenotyping kit also incorporates instructions for taking pictures, management of heterogeneous accessions, and a field for observations. ANOVA tests were used to evaluate the significance of differences for pseudo-qualitative and quantitative traits and broad-sense heritability ( $H^2$ ) was estimated as the ratio between phenotypic and genotypic variation (Tripodi et al., 2021).

## Characterization of mutant collection and CC and BS for heat and drought tolerance

From the EMS mutant collection,  $M_2$  families with six or more plants (73.2%) and the CC and BS were transplanted for the phenotype screening and genetic characterization, which in turn were performed in the summer seasons of 2018 and 2019. Up to 10 plants per  $M_2$  family were grown in commercial nursery greenhouses, under natural photoperiod

conditions, but ensuring minimum temperature of 35°C day/23°C night, and a reduction of water availability ranging from 50 to 0% with respect to the irrigation standard conditions used in southwestern Spain. The following traits were weekly scored: leaf senescence, leaf chlorosis, necrosis, severe reduction of growth and decreased leaf expansion or development. Tolerant materials were re-evaluated to check their adaptation to organic production in greenhouse following a random block design with three replications and using 'Moneymaker' as a control. Plant height, leaf senescence and chlorosis, fruit set and fruit weight (second and third truss) were scored.

# High-throughput genotyping of CC and BS and GWAS analysis

A total of 288 cultivated tomato accessions from the CC and BS have been genotyped using double digest restriction-site associated DNA (ddRAD) sequencing using the *Mbo*I and *SphI* enzyme pair. SNP call, filtering and marker classification used are detailed in Esposito et al. (2020). The admixture model was implemented to infer population structure and a functional annotation of the identified variants associated genes was performed. MAF values were computed for SNP loci to identify private SNPs in "da serbo"/"de penjar" materials, and those SNPs with contrasting MAF values were analysed for their biological function. In addition, a mini-core collection based on genotypic data using Core Hunter software (De Beukelaer et al., 2012). Further details on the genotyping and procedures performed can be retrieved from Esposito et al. (2020).

GWAS analysis was performed using a compressed mixed linear model using TASSEL incorporating population structure covariates and the kinship estimated using the centred identity by state distance (Tripodi et al., 2021). Significance of the marker-trait association was performed using the Bonferroni correction at p=0.05. Using the physical mapping of SNPs significantly associated to the trait, a functional annotation of candidate genes near the SNP was performed.

# Screening of CC and BS for tolerance to diseases

The CC and BS have been evaluated against *Phytophthora infestans*, while the BS has also been screened against *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), *Tomato spotted wilt virus* (TSWV), and *Tomato mosaic virus* (ToMV). Screenings have been performed under controlled conditions in climatic chambers using using artificial inoculation on plantlets. For *P. infestans*, inoculation was performed by spraying a sporangia suspension to the leaves and keeping the plants in mini greenhouses with 100% humidity. A tolerance and/or susceptibility percentage compared to the susceptible control was calculated for each accession and the percentage of foliar necrotized area was evaluated. For *Fol*, roots of seedlings were cut and dipped for 24 h in a solution of Fol spores. Symptoms were scored at 7, 15 and 30 days after inoculation (DAI). For TSWV and ToMV, inoculum was prepared from infected plants. Inoculation was performed on 3-4 true leaves plantlets by rubbing the leaf surface with a cotton swab. Symptoms were scored at 7, 15 and 30 DAI, and ELISA tests were also performed to check the virus presence and accumulation.

# Selected set (SS) establishment and characterization

Based on the results of performance of the CC and BS under organic conditions, resilience, tolerance to diseases, and representation of diversity, a selection of 42 outstanding genotypes was performed. The SS was grown in the open field in the spring-summer season in the two same organic sites used in 2019 for the CC and BS. Three treatments were applied: control (supply of N fertilizer and irrigation based on the calculation of crop evapotranspiration), water stress (by reduction of watering to 30% of the control), and nitrogen deficit (0% of N fertilizer). Plants were distributed using a block randomization scheme, with three blocks, and four plants per replicate. Plants were characterized using the BRESOV phenotyping kit. ANOVA tests were performed to evaluate the effects of genotype (G), environment (E) and treatment (T), as well as their double and triple interactions.



## Breeding of new materials for organic conditions

Eight hybridization and breeding programmes have been performed or are underway to develop new materials specifically adapted to organic conditions. The breeding programmes have been the following: a) diallel crossing scheme without reciprocals has been performed between six local tomato cultivars with excellent organoleptic properties in order to obtain 15  $F_1$  hybrids; b) diallel crossing scheme with reciprocals between three local "de penjar" cultivars; c) introgression through backcrossing of ToMV resistance allele  $Tm2^2$  into 'Valenciana' and "de penjar" local cultivars; d) breeding four local cultivars for resistance to ToMV ( $Tm2^2$ ) and Fol (12); e) breeding other four local cultivars, used also for developing hybrids (see point g), for resistance to ToMV (Tm2<sup>2</sup>), TSWV (Sw5), Tomato yellow leaf curl virus (TYLCV, Ty5), Verticillium dahliae (Ve), and Fol (I2) by crossing with one breeding line carrying genes *Tm2<sup>2</sup>*, *Sw5*, *Ve*, and *I2* and another breeding line carrying *Ty5*; f) development of hybrids complementary for agronomic and quality; g) development of hybrids between 4 recipient lines with good agronomic and quality characteristics and 10 lines donor of interesting traits both in Italy and Spain; h) development of interspecific hybrids between 8 cultivated tomato (S. lycopersicum) accessions and 3 wild accessions with high vigour of S. habrochaites, S. lycopersicum var. cerasiforme, and S. pimpinellifolium as potential new rootstocks, which have been evaluated for heat and drought tolerance and also for performance as rootstocks in Almería (Spain) and Sicily (Italy) by grafting a commercial cultivar in each site and compared with the ungrafted cultivar or grafted onto commercial rootstocks.

# **RESULTS AND DISCUSSION**

#### BRESOV core collection (CC) and breeding set (BS) establishment

The BRESOV CC is composed of 233 accessions, of which 28 are wild accessions from 13 different species from *Solanum* section *Lycopersicon*, 54 are Italian landraces, 51 are Spanish landraces, and 100 are cultivars from other parts of the world. Materials of the CC include accessions with different phenotypes for multiple traits of interest in organic conditions, including mutations for plant traits, materials with high resilience (22 accessions of the "da serbo" and "de penjar" types), and resistance or tolerance to diseases. The BS contains 217 materials, of which 109 are Italian and Spanish landraces, including 74 "da serbo" and "de penjar" types, 30 are cultivars from other parts of the world, and 78 are breeding lines and hybrids. In this way, the tomato BS is highly representative of the variation existing in tomato and includes materials with different shape, colour, size and bioactive compounds content, as well as hybrids and breeding lines carrying different combinations of genes for tolerance to diseases. Both sets of materials represent an assembly of tomato genetic resources specifically selected for breeding for organic agriculture and therefore are an important resource for breeders aimed at developing tomato cultivars for organic farming (Lammerts van Bueren et al., 2011).

## Morphoagronomic characterization of CC and BS

A wide diversity was observed in the materials evaluated for morphological characteristics. In particular for qualitative morphological traits, the whole range of states for the descriptors was present in the collection, with a few exceptions, such as the lack of presence of accessions with highly exerted stigma (typical of wild species), and of accessions with brown or green fruits as well as of plants with low vigour and very weak vigour and very soft fruit firmness. Regarding quantitative morphological and agronomic traits, in general, wide ranges of variation were observed as well as high values for the coefficient of variation, indicating the presence of a large genetic variation for the traits evaluated in the BS. Although most accessions were determinate, which is the standard type of plant common for tomato for the fresh market, some were determinate or semi-determinate, which are especially suited for processing tomato cultivation in the open field as this type of trait improves productivity and water use efficiency in this type of tomato. As occurs with most cultivated tomato accessions, the style of most accessions was inserted or had the style at the same level as the stamen, favouring self-pollination. Only 9 accessions had a slightly exerted stamen and none

of them had the highly exerted stamen typical of out-crossing tomato wild relatives.

Variability for fruit morphology in the collection was very high. Only 28.1% of the accessions did not display green shoulder, while, among the rest, most of them had light or medium green shoulders. Most of the accessions had red fruits (>60%), being remarkable that 31% of the accessions were pink, a trait that is increasingly requested by consumers. Few accessions had other colours (yellow, orange, and purple). A wide diversity was observed for the fruit predominant shape, with the 11 categories of fruit shape being represented in the collection. The most common categories were slightly flattened and ovate, while the least frequent were the pear shaped and pepper shaped. Most of the accessions (>80%) had closed blossom end scar and medium firmness with a remarkable percentage of accessions (31%) were classified as hard, all highly desirable traits in tomato for the fresh market. Wide diversity was observed for the ribbing at calvx end, with most accessions (>60%) having very weak or weak ribbing, whereas large variation was found for fruit size, with differences of 500-fold between the accessions with lowest and highest fruit weight. Values of soluble solids content (SSC) were also variable with maximum values achieved of 9.4% SSC in Italy and 7.5% in Spain. Similarly, large variation was found for agronomic traits of interest, such as earliness, ripening uniformity, number of fruits per plant and yield, with some cultivars reaching very high yield values of almost 8 kg plant<sup>-1</sup> and outperforming commercial controls, as well as for root traits. For most traits, the genotype (G) effect was the greatest contributor to the sums of squares, although significant effects of cultivation site (E) and G×E interaction was generally significant. The wide diversity for morphological and agronomic traits shows that both the BRESOV CC and BS are of great interest in the development of different varietal types of tomato for organic conditions (Tripodi et al., 2021).

Several significant correlations were found between quantitative agronomic and morphological traits. In this way, high positive correlations (>0.8) were found between number of locules and fruit weight. High positive correlations were also found between both latter traits and puffiness appearance, radial cracking (but not with concentric cracking), and fruit fasciation. The number of fruits per plant was negatively correlated with the number of locules and fruit weight, as well as traits associated to fruit size. Yield displayed a high positive correlation with fruit size and also with the number of locules and other traits related to fruit size. We also found a positive correlation between concentric cracking and ripening earliness.

## Characterization of mutant collection and CC and BS for heat and drought tolerance

After combined heat and drought stress treatment, 142 of the  $M_2$  families (2.59%) included at least one plant tolerant to stress effects. Among these putative tolerant plants, two kinds of phenotypes were identified: i) plants with green and turgid shoot apices, scarce or absent senescence symptoms, and/or low chlorotic deficiencies in basal leaves, and ii) plants with necrotic shoot apices that produced new vigorous axillary shoots. Tolerant plants were grown to maturity under non-stress conditions to allow M<sub>3</sub> seed production progenies. Genetic analysis indicated that the tolerant phenotype of 9 of the 142 selected M<sub>2</sub> plants was inherited as monogenic and dominant, while the remaining ones were recessive. A final selection of four EMS lines was performed. Twenty-four accessions from the CC and BS were also preliminary selected on the basis of their tolerant phenotype. The re-evaluation of these materials under greenhouse conditions revealed that they displayed similar values of plant height, and less incidence of leaf senescence of chlorosis with respect to control plants. Interestingly, three EMS mutant lines, three CC accessions, and one BS accession showed higher fruit set, with values ranging 7 to 33% respect to control plants. These results show that mutation breeding in tomato, as well as exploration of germplasm collections, can result in the identification of materials with enhanced tolerance to abiotic stresses (Chaudhary et al., 2019).

# High-throughput genotyping of CC and BS and GWAS analysis

When using the new tomato reference genome (SL4.0, released in 2019), a total of 246,936 SNPs, of which 32,779 were retained after filtering for quality criteria, were detected in the set of materials genotyped. Most materials had a low heterozygosity (around 2%). Six



different genetic clusters were observed in the materials, which together with MDS clustering revealed a certain genetic differentiation of the "da serbo"/"de penjar" type and of Spanish landraces and a higher genetic variability in the heirlooms and cultivars (Esposito et al., 2020). The "da serbo"/"de penjar" materials were enriched in several mutations in genes, such as *CTR1* and *JAR1*, related to stress tolerance and fruit maturation. In addition, a mini-core collection of 58 accessions representing most of the diversity of the materials evaluated was established.

A total of 59 significant associations between SNPs and traits were detected, which allowed the confirmation of already known genes as well as the identification of novel ones involved in traits of interest under organic cultivation. Among other stable associations, several flower and fruit traits colocalized with *SUN-LIKE PROTEIN 31* (*SISUN31*) on chromosome 11, and another cluster of SNPs on chromosome 5 were associated to style position, puffiness, diameter of the main root, and fruit weight. These associations allow the use of marker-assisted-selection for these traits.

## Screening of CC and BS for tolerance to diseases

As a result of the screenings performed, 21 accessions with a high percentage of tolerance to *P. infestans* with intermediate to low percentage of foliar necrotized areas have been identified in the CC and BS. These materials can be very useful for combining or pyramiding several genes that can provide a more durable resistance to *P. infestans* (Li et al., 2011). Regarding the screening against *Fol*, 23 accessions of the BS had no or very low symptoms. These 23 materials were re-tested and resistance was confirmed in four of them, while tolerance (symptoms  $\leq 1$  in the symptoms scale) was confirmed in 10 of them. None of these materials had been previously identified as carriers of resistance/tolerance to *Fol*, and therefore represent new sources of resistance to *Fusarium oxysporum* f. sp. *lycopersici* may represent new sources of resistance to this pathogen and confirm the interest of these largely unexplored materials for durable resistance/tolerance to *Fol* (Takken and Rep, 2010).

The results of the screening of the BS against TSWV shows that most of the accessions display severe or moderate symptoms. Interestingly, five accessions displayed no symptoms and gave a negative result for the presence of TSWV in the ELISA tests, indicating a potential resistance to TSWV. In addition, seven accessions, despite displaying symptoms had low (<0.4) levels for the ELISA test. Other 16 accessions displayed low levels of symptoms and/or low ELISA absorbance values. These 28 accessions were retested for confirmation of the tolerance/resistance to TSWV. Among these, six hybrids carrying the Sw5 gene had no symptoms and ELISA tests negative for TSWV presence. Six other accessions had very mild symptoms ( $\leq$ 1) and tested negative for TSWV presence. In addition, one breeding line gave negative values in the ELISA test, with values close to 0, indicating that it could display recovery after suffering the disease and therefore be of interest as source of tolerance. These newly discovered sources of tolerance could be used in combination with already established TSWV resistance genes, such as Sw-5, for durable resistance to TSWV (Qi et al., 2021). For ToMV, only one accession was negative for the presence of the virus in the ELISA test. Regarding symptoms, most accessions (94.6%) displayed values >1 in the symptoms scale and can be considered as having a non-acceptable level of susceptibility to ToMV. Apart from the only accession that was resistant, three displayed no symptoms of the disease, although tested positive in the ELISA assay, whereas seven had very mild symptoms. All these materials will be re-tested to identify new sources of tolerance to ToMV or other emerging tobamoviruses such as *Tomato brown rugose fruit virus* (ToBRFV) (Hak and Spiegelman, 2021) that can be useful for developing durable resistance against ToMV or other related viruses.

# Selected set (SS) establishment and characterization

The SS consisted of 2 breeding lines, 9 improved cultivars, 6 highly resilient "da serbo"/"de penjar" genotypes, 19 landraces for fresh consumption and 6 heirlooms selected for their interest for direct utilization in organic cultivation. The evaluation of the SS revealed a significant effect of the genotype (G), environment (E) and treatment (T) for most traits. A

high number of G×E interactions was also significant, but few significant G×T interactions were found. E×T interactions were significant for several traits. Few significant G×E×T interactions were significant. The SS was highly diversified in terms of fruit traits, representing a potential source of variability for the organic sector. The most remarkable differences found among the three treatments were a higher soluble solids content (12.4%) and decreased total yield (-15.2%) and fruit weight (-11.9%) under water-stress conditions. These results indicate that, as found by others (Cui et al., 2020), the reduction in yield under reduced irrigation is compensated with a higher content in soluble solids content and therefore fruits with more intense taste. The root radical angle and the density of fine roots was higher under water-stress conditions, highlighting how tomato roots tend to cope with water-stress more by increasing the angle and the number of fine roots. Under N deficit conditions the most remarkable effect was a reduction in total yield (-14.8%), suggesting that deprivation of N fertilization under organic conditions can result in reduced tomato yield (Wei et al., 2018). By comparing the yield of individual accessions in the two sites under the three conditions it was possible to identify materials with good performance and resilience. These materials are very interesting for their resilience and high yield potential under organic cultivation conditions.

## Breeding of new materials for organic conditions

The evaluation of 15 hybrids between six local cultivars (programme a) has resulted in the selection of 6 hybrids with enhanced yield and quality characteristics. Interestingly, the evaluation of the hybrids between three highly resilient "de penjar" accessions (programme b) resulted in materials which displayed heterobeltiosis for yield, with yields on average 55.6% higher than that of parents, which suggests that hybrids have a high potential to enhance the performance of this type of tomato under organic conditions (Figàs et al., 2018).

The  $Tm2^2$  gene for tolerance to ToMV (programme c) has been introgressed by backcrossing using marker assisted selection in a local landrace of the "Valenciana" type and in a highly resilient landrace of the "de penjar" type; in this programme, backcrossing was performed until the BC<sub>4</sub> generation, followed by two generations of selfing. This type of approach has been previously used with other tomato landraces (Carbonell et al., 2018). The two resulting introgressed lines were phenotypically indistinguishable from the original nonresistant accession, except for displaying resistance to this virus. Also, BC<sub>4</sub> generations have been obtained in the backcross programme to introduce  $Tm2^2$  and I2 genes in four recurrent local cultivars with good characteristics (programme d). Selection for both genes has been done using molecular markers. BC<sub>4</sub> lines were also selected for phenotypic characteristics similar to the recurrent parent. The resulting materials were selfed to obtain the  $BC_4S_1$ generation, where selection will be performed to obtain homozygous lines carrying the two resistance genes. These materials will allow the cultivation of the four local cultivars in conditions where ToMV and/or Fol incidence are expected. In the other backcross programme (programme e), the four recurrent parents were crossed with the two breeding lines carrying the resistance genes. So, a total of eight hybrids have been obtained. The eight hybrids have been backcrossed to their respective recurrent parents to obtain the first backcrosses (BC<sub>1</sub>s), and the BC<sub>1</sub> generations have been genotyped for the five genes of resistance. The final aim is to obtain 16 lines (4 per "recipient" parent) so that all lines carry the *Tm2*<sup>2</sup> allele of resistance plus one of the other four (Sw5, 12, Ve, or for Ty5). This strategy has been chosen as the accumulation of too many genes of resistance in local landraces may result in decreases of vield and quality (Rubio et al., 2016).

The evaluation of the parents and hybrids of complementary crosses under organic conditions (programme f) revealed that some of them displayed very good agronomic characteristics, with high and uniform fruit set. A wide range of fruit sizes existed among the hybrids, with representatives from different commercial categories. Many of the hybrids tested had a greater earliness and higher production than parents, thus reaching the highest yield, indicating that hybrids are also of interest under organic cultivation conditions (Avdikos et al., 2021). The evaluation of quality traits in the parents and hybrids also resulted in many hybrids having higher soluble solids content values than the parents, with several hybrids



displaying heterobeltiosis. Regarding acidity, many hybrids had also higher values than most parents, although a wide range of variation was observed. As a result, 11 hybrids were selected and further re-evaluated, confirming their potential under organic conditions. The hybrids involve only 7 out of the 15 parents used for their development, thus indicating a good combining ability. Similar results were obtained for the programme of hybrid development between recipient lines and donors of traits of interest (programme g), in which several hybrids were found to have very good yield and quality characteristics under organic conditions.

A total of 31 hybrids of potential interest as rootstocks have been obtained (programme h). When screened under heat and drought stress conditions, several hybrids exhibited leaves without significant damages three weeks after beginning the drought treatment. Remarkably, some hybrids were also among those with highest height values and showed significantly higher fruit set values when compared to control plants of 'Moneymaker'. These results indicated that some of the hybrids evaluated, particularly those having *S. habrochaites* as a parent, displayed tolerance to heat and drought stress conditions (Poudyal et al., 2017). The evaluation of the hybrids as rootstocks both in Almería and Sicily revealed that some of the hybrid rootstocks outperformed the control rootstock commercial cultivars for fruit yield. Results indicate that some of the rootstocks tested are of high potential interest for their use under organic conditions.

#### CONCLUSIONS

The results obtained have revealed that the evaluation and exploitation of diversity present in unexplored tomato germplasm can allow significant improvements in the selection and development of plant materials for organic cultivation. Evaluation of phenotypically and genotypically highly diverse materials under organic conditions has allowed selection of materials well adapted to these conditions, including materials resilient to abiotic stresses, and the identification of SNPs and candidate genes for traits of interest in organic cultivation conditions. Further, new sources of tolerance to four diseases with high prevalence under organic conditions, have been identified, which is of great interest for the development of durable resistance. Development of different breeding programmes for obtaining hybrids and for developing selected materials which introgress genes of resistance has allowed obtaining new improved materials specifically suited to organic cultivation. Overall, the BRESOV project results a dramatic improvement in the use of tomato diversity for tomato selection and breeding for organic conditions.

#### ACKNOWLEDGEMENTS

This research has been funded by the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 774244 (BRESOV project).

#### Literature cited

Avdikos, I.D., Tagiakas, R., Mylonas, I., Xynias, I.N., and Mavromatis, A.G. (2021). Assessment of tomato recombinant lines in conventional and organic farming systems for productivity and fruit quality traits. Agronomy (Basel) *11* (*1*), 129 https://doi.org/10.3390/agronomy11010129.

Carbonell, P., Alonso, A., Grau, A., Salinas, J.F., García-Martínez, S., and Ruiz, J.J. (2018). Twenty years of tomato breeding at EPSO-UMH: transfer resistance from wild types to local landraces – from the first molecular markers to genotyping by sequencing (GBS). Diversity (Basel) *10* (1), 12 https://doi.org/10.3390/d10010012.

Chable, V., Nuijten, E., Costanzo, A., Goldringer, I., Bocci, R., Oehen, B., Rey, F., Fasoula, D., Feher, J., Keskitalo, M., et al. (2020). Embedding cultivated diversity in society for agro–ecological transition. Sustainability *12* (*3*), 784 https://doi.org/10.3390/su12030784.

Chaudhary, J., Alisha, A., Bhatt, V., Chandanshive, S., Kumar, N., Mir, Z., Kumar, A., Yadav, S.K., Shivaraj, S.M., Sonah, H., and Deshmukh, R. (2019). Mutation breeding in tomato: advances, applicability and challenges. Plants (Basel) *8* (5), 128 https://doi.org/10.3390/plants8050128. PubMed

Conesa, M.À., Fullana-Pericàs, M., Granell, A., and Galmés, J. (2020). Mediterranean long shelf-life landraces: an untapped genetic resource for tomato improvement. Front. Plant Sci. *10*, 1651 https://doi.org/10.3389/fpls.2019.01651. PubMed

Cui, J., Shao, G., Lu, J., Keabetswe, L., and Hoogenboom, G. (2020). Yield, quality and drought sensitivity of tomato to water deficit during different growth stages. Sci. Agric. 77 (2), e20180390 https://doi.org/10.1590/1678-992x-2018-0390.

De Beukelaer, H., Smýkal, P., Davenport, G.F., and Fack, V. (2012). Core Hunter II: fast core subset selection based on multiple genetic diversity measures using Mixed Replica search. BMC Bioinformatics *13* (*1*), 312 https://doi.org/10.1186/1471-2105-13-312. PubMed

Esposito, S., Cardi, T., Campanelli, G., Sestili, S., Díez, M.J., Soler, S., Prohens, J., and Tripodi, P. (2020). ddRAD sequencing-based genotyping for population structure analysis in cultivated tomato provides new insights into the genomic diversity of Mediterranean 'da serbo' type long shelf-life germplasm. Hortic Res 7 (1), 134 https://doi.org/ 10.1038/s41438-020-00353-6. PubMed

Figàs, M.R., Prohens, J., Raigón, M.D., Pereira-Dias, L., Casanova, C., García-Martínez, M.D., Rosa, E., Soler, E., Plazas, M., and Soler, S. (2018). Insights into the adaptation to greenhouse cultivation of the traditional Mediterranean long shelf-life tomato carrying the alc mutation: a multi-trait comparison of landraces, selections, and hybrids in open field and greenhouse. Front. Plant Sci. *9*, 1774 https://doi.org/10.3389/fpls.2018.01774. PubMed

Hak, H., and Spiegelman, Z. (2021). The tomato brown rugose fruit virus movement protein overcomes Tm-2<sup>2</sup> resistance in tomato while attenuating viral transport. Mol Plant Microbe Interact *34* (9), 1024–1032 https://doi.org/10.1094/MPMI-01-21-0023-R. PubMed

Lammerts van Bueren, E.T., Jones, S.S., Tamm, L., Murphy, K.V., Myers, J.R., Leifert, C., and Messmer, M.M. (2011). The need to breed crop varieties suitable for organic farming, using wheat, tomato and broccoli as examples: a review. NJAS Wagening. J. Life Sci. *58* (*3-4*), 193–205 https://doi.org/10.1016/j.njas.2010.04.001.

Li, J., Liu, L., Bai, Y., Finkers, R., Wang, F., Du, Y., Yang, Y., Xie, B., Visser, R.G.F., and van Heusden, A.W. (2011). Identification and mapping of quantitative resistance to late blight (*Phytophthora infestans*) in *Solanum habrochaites* LA1777. Euphytica *179* (*3*), 427–438 https://doi.org/10.1007/s10681-010-0340-7.

Poudyal, D., Akash, M., Khatri, L., Shrestha, D.S., and Uptmoor, R. (2017). *Solanum habrochaites* introgression line grafted as rootstock in cultivated tomato maintains growth and improves yield under cold and drought stresses. J. Crop Improv. *31* (4), 589–607 https://doi.org/10.1080/15427528.2017.1319887.

Qi, S., Zhang, S., Islam, M.M., El-Sappah, A.H., Zhang, F., and Liang, Y. (2021). Natural resources resistance to *Tomato spotted wilt virus* (TSWV) in tomato (*Solanum lycopersicum*). Int J Mol Sci 22 (20), 10978 https://doi.org/10.3390/ ijms222010978. PubMed

Rubio, F., Alonso, A., García-Martínez, S., and Ruiz, J.J. (2016). Introgression of virus-resistance genes into traditional Spanish tomato cultivars (*Solanum lycopersium* L.): effects on yield and quality. Sci. Hortic. (Amsterdam) *198*, 183–190 https://doi.org/10.1016/j.scienta.2015.11.025.

Takken, F., and Rep, M. (2010). The arms race between tomato and *Fusarium oxysporum*. Mol Plant Pathol *11* (2), 309–314 https://doi.org/10.1111/j.1364-3703.2009.00605.x. PubMed

Tripodi, P., Soler, S., Campanelli, G., Díez, M.J., Esposito, S., Sestili, S., Figàs, M.R., Leteo, F., Casanova, C., Platani, C., et al. (2021). Genome wide association mapping for agronomic, fruit quality, and root architectural traits in tomato under organic farming conditions. BMC Plant Biol *21* (*1*), 481 https://doi.org/10.1186/s12870-021-03271-4. PubMed

Wei, Z., Du, T., Li, X., Fang, L., and Liu, F. (2018). Interactive effects of elevated CO<sub>2</sub> and N fertilization on yield and quality of tomato grown under reduced irrigation regimes. Front. Plant Sci. *9*, 328 https://doi.org/10.3389/fpls.2018.00328. PubMed

