

## EXPOLITING PHENOTIPIC VARIABILITY IN A G10 MAGIC POPULATION OF SOLANUM LYCOPERSICUM

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## ABSTRACT

Climate change, soil fertility deterioration, increasing soil salinity and water pollution are just some of the many challenges that the current and future agriculture are going to face. The combination of good agricultural practices and innovative plant breeding techniques are needed to overcome these problems. In this framework, a new crossing plan was used to increase the germplasm allelic diversity, track down specific sequence in the genome and develop new molecular markers associated to agricultural traits of high value for future cultivation environments. One of this tools is the multi-parent advanced generation inter-cross (MAGIC) populations. A MAGIC population is obtained by intercrossing multiple parental lines and self-crossing the progeny for several generation, to obtain recombinant inbred lines (RIL), each containing fragments of genome deriving from all the parents. In this research, a MAGIC population of tomato (Solanum Lycopersicum L.) was produced starting from 8 parental lines, choosed based on specific phenotipical traits and breeding relevance. 7 lines were derived from breeding programs of ISI Sementi and 1 from a wild accession of Solanum cheesmaniae (LA1407) obtained from TGRC (http://tgrc.ucdavis.edu). This last, was selected for its resiliency and tolerance to biotic and abiotic stress. All the 8 lines were genotyped with a genotyping by sequencing (GBS) approach. At this point, in order to analyze the stability of the lines and the segregation of the main parental traits, a phenotyping work, using classical tomato breeding tools, was performed.

The 8 tomato lines selected as parental were chosen with the aim of maximizing fruits and plants phenotypic variability (fig. 4 and Fig. 5) within the
progeny. Some of their scored traits are described in
the Tab.1. The four initial crosses were performed
using the cross plan proposed by Pasqual et al, 2015.
Each G1 (Generation 1) line was then crossed in pair
to obtain two different sets of double cross hybrids
(G2). The two offspring obtained from each set were
sowed and intercrossed producing 100 plants, each
containing parts of the 8 founder genomes. Some
through single cood descent (SSD) to monitor
phenotypic variation and to increase seed
availability for the next SSD steps (Fig 1) The
presence of alleles for tomato spotted wilt virus
resistance, Beta (B) locus for beta-carotene and self-
pruning trait (sp locus) were tested with molecular
markers on the segregant population genotypes to
demonstrate the successful crossing (Campanelli et
al, 2019). During 2021, lines of G9 populations were

PARENTAL	HYP	OBV	JNT	G/UG
ISI 1	AA	NT	п	UG
151.2	ANT	NT	3	UG
ISI 3	ANT	NT	J	UG
ISI 4	ANT	T	JL.	UG
LA1407	AA	NT	1	G
151 6	ANT	T	JL.	UG
ISI 7	ANT	NT	JL	UG
ISI 8	ANT	NT	JL.	G

Tab.1 Summary of the main traits of the eight founders



classical agronomical techniques for processing tomato, with a distance between plants of 25 cm and a distance between rows of 1,4 m. The phenotyping work was done using classical tomato breeding tools, including visual analysis and the use of the atLeaf+ chlorophyll meter to measure the chlorophyll content of the leaves. The traits scored were: -HYP. Hypocotil colour (anthocyanins presence/anthocyanins absence) -PUN Plants uniformity -OBV. Obscura venosa (transparent/nontrasparent) -LBF, Leaves before flowering -FLO. Date of flowering -JNT, Peduncle (jointed/jointless) -G/UG, Green shoulder (presence/absence) -CHL, Chlorophyll content (using the AtLeaf+) At the end of the ripening, 5 fruits were harvested from a single randomly choosen plant for each line, in order to extract seeds needed for the next

generation

The graphics below show chlorophyll content distribution of 487 SSD lines grouped in different classes (Fig. 2) and days before flowering (Fig.3). Both of them follow normal distributions (Fisher test). This suggests that these traits are likely quantitative and controlled by different genes and by their interaction with environmental effect.

Both histograms shows that there are lines that overcome the highest chlorophyll content and number of leaves before flowering measured among the founders. This suggest that different genes peculiar of each founder line are involved in chlorophyll production, keeping and turnover.





AA

55

16

Tab. 2 allelic distribution

NT

т

ANT

325

75

Fig. 3 Leaves before flowering distribution. Highlited, the range Fig. 2 Chlorophyll content distribution. In red, the minimum and between average and standard deviation. maximum levels scored in founders

This allowed to score a higher chlorophyll content in some SSD lines. A similar behaviour was observed for LBF trait, suggesting a similar genetic control.

The following table describes the frequences of different combinations of two couple of alleles: presence/absence of anthocyanins in the hypocotil (ANT/AA) and the *obscurg venosg* trait (T/NT). Data analysis highlighted the presence of lines showing a combination of AA/T alleles (Tab. 2), absent in the 8 parental founder genotypes.

An Anova test using R software was performed to find any correlation between each phenotyped trait. The only significant result was the correlation between the date of flowering (FLO) and the leaves before the first floral branch (LBF). The test showed a correlation of the 99% (p-value: 2^-16) suggesting a strong genetic influence on transition from vegetative to reproductive plant phase.



Fig. 4 Variability in shape and colour of fruits in different lines

Fig. 5 Example of variability in habitus, flowering and lea colour of different lines.

A phenotyping activity was performed on all 487 RIL lines scoring traits of agronomical interests. The presence of allelic combination absent in parental lines confirm that crosses between founders made at the beginning of the experiment occurred successfully. This phenotipical result confirm the genetic analysis of Campanelli et al, 2019. Among the generated lines, chlorophyll content distribution observed in specific RILs represent an important genetic source for improvement of green leaves colour on tomato plants. In detail, chlorophyll content is correlated with fruit "Brix, which is an important added value for processing tomato supply chain. Furthermore, stay-green tomato plant results to be healthier in field stress conditions. Investigation of chlorophyll content, heritability and its genetic control must be studied throughtout a specific cross programme. Cross pollinations among lines which showed the minimum and the maximum chlorophyll content will be planned to generate an F1 and F2 segregant populations. Flowering is considered one of the most important transition in plant physiology because represent the switch from vegetative to reproductive phase. Earliness of cultivations in terms of ripening and transplanting date is a crucial factor for farming, but it's difficult phenotype these traits during breeding activities. The correlation between date of flowering (FLO) and leaves before flowering (LBF) allows to measure in a simple way the vegetative length of lines with several days in advance. Literature on flowering date (FLO) and number of leaves before flowering (LBF) confirm that both quantitative traits are influenced by environmental effect (Gao et al, 2015). The correlation between traits is high, suggesting that (i) traits are controlled by the same genetic pool or (ii) more measurements are required because a correlation value of 99% is biologically unlikely. GxE effect must be studied in different environments (location and years) in order to study heritability and genetic penetrance. If confirmed, this result can be applied as a very useful tool for breeding selection, capable of informing about flowering and plant earliness. Furthermore, all MAGIC SSD lines are going to be genotyped with a GBS approach, creating a powerful base for -omics studies.

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