Huther, Cristina Moll; Martinazzo, Emanuela Garbin; Schock, Anderson Augusto; Rombaldi, Cesar Valmor; Bacarin, Marcos Antonio
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Universidade Federal do Ceará

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Production components in transformed and untransformed ‘Micro-Tom’ tomato plants

Componentes de produção em plantas transformadas e não transformadas de tomate ‘Micro-Tom’

Cristina Moll Huther, Emanuela Garbin Martinazzo, Anderson Augusto Schock, Cesar Valmor Rombaldi and Marcos Antonio Bacarin

ABSTRACT - Changes to the amounts of certain proteins have resulted in several studies, among them the so-called heat shock proteins (HSP), which take many forms, most of them constitutive. However, other forms may be inducible by a particular stress factor. The ‘Micro-Tom’ tomato is considered a model for experimental studies due to having suitable characteristics, such as reduced size, short generation time, and ease of transformation. Growth and production components were therefore evaluated in ‘Micro-Tom’ tomato plants transformed for different levels of mitochondrial HSP (MT-sHSP23.6). Plants from genotypes of the ‘Micro-Tom’ tomato (untransformed, and transformed with overexpression and with expression silencing) were grown under controlled conditions of temperature, photoperiod and photon flux density. To obtain the data, successive collections were carried out at regular intervals (21 days) throughout the development cycle of the plants, starting from the 21st day after transplanting (DAT). Total dry matter, leaf area, dry-weight partitioning between the plant organs, and production components were determined in the three genotypes. From interpretation of the results, it was found that plants transformed with overexpression of MT-sHSP23.6 displayed greater production capacity, considering the fresh weight of the fruit; but in general, the data showed that genetic transformation did not bring about major changes in growth, since the three genotypes displayed similar behaviour.

Key words: Solanum lycopersicum Mill. Dry matter distribution. Fruit production.

RESUMO - Modificações nas quantidades de certas proteínas têm proporcionado vários estudos, dentre elas as denominadas heat shock proteins (HSP), que possuem muitas formas, sendo a maioria constitutiva. Entretanto, outras formas são induzíveis por algum determinado fator estressante. Dessa forma, tomateiro Micro-Tom vem sendo considerado como um modelo para estudos experimentais, pois possui características que o tornam adequado, tais como porte reduzido, tempo de geração curto, e facilidade de transformação. Assim, avaliaram-se o crescimento e os componentes de produção em plantas de tomate ‘Micro-Tom’ transformadas para diferentes níveis de HSPs mitocondrial (MT-sHSP23.6). Plantas de três genótipos de tomate ‘Micro-Tom’ (não transformados, transformados com superexpressão e com silenciamento da expressão) foram cultivadas em condições controladas de temperatura, fotoperíodo e densidade de fluxo de fótons. Para a obtenção dos dados, foram efetuadas coletas sucessivas a intervalos regulares de tempo (21 dias) ao longo do ciclo de desenvolvimento das plantas, iniciando as coletas a partir do 21º dia após o transplante (DAT). A matéria seca total, área foliar, partição de massa seca entre órgãos da planta e os componentes da produção dos três genótipos foram determinados. Com a interpretação dos resultados verificou-se que as plantas transformadas com superexpressão da MT-sHSP23.6 apresentaram maior capacidade produtiva, considerando a massa fresca dos frutos, mas em geral os dados indicaram que a transformação genética não acarretou grandes mudanças no crescimento, pois os três genótipos tiveram comportamento similar.


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*Autor para correspondência
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1Parte da Tese financiada pela Coordenação de Aperfeiçoamento Pessoal de Nível Superior - CAPES e pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq
2Departamento de Botânica, Instituto de Biologia, Universidade Federal de Pelotas, Pelotas-RS, Brasil, 96.010-900, cristinahuther@yahoo.com.br, emartinazzo@gmail.com, verdepracaramba@yahoo.com.br, cesarvrf@ufpel.edu.br, bacarin@ufpel.edu.br
INTRODUCTION

The tomato plant displays several development stages in its growth cycle, being widely used as a model in various areas of plant research (FAYAD et al., 2001). It is characterised by having a relatively compact genome, together with rich collections of germplasm and highly efficient transformation protocols. In addition, it includes other characteristics, such as being dicotyledonous, with compound leaves, symподial flowering and the formation of fleshy climacteric fruit (ALMEIDA, 2012; ALVARENGA, 2004).

The fruit can be classified and evaluated according to colouration (PRAIT; WORKMAN, 1962). Those authors stipulated a colouring scale divided into five categories, however only two categories were used in this work, green fruit (immature green and mature green) and red fruit (breaker, red and senescent).

When using the tomato in experiments, another advantage is the miniature variety known as ‘Micro-Tom’, which has become important as a model tomato plant, presenting natural mutations that cause its dwarf size; it is of determinate growth, but with viable fruit and seeds (MEISSNER et al., 1997), and is therefore suitable for the production of transgenic plants (PINO et al., 2010).

Various studies, such as Iqbal et al. (2010), Vásquez-Robinet et al. (2010) and Chen et al. (2010), demonstrate that the loss of function (suppression) or overexpression of a gene can bring numerous advantages, especially in cultivated species (KHUONG et al., 2014).

Modifications in the amounts of certain proteins, in order to change their expression to a lesser degree than is usual or to increase that expression, have resulted in several studies (QUEITSCH; HONG; LINDQUIST, 2000). One type of protein that has been the focus of study are the so-called heat shock proteins (HSP) (PEGORARO et al., 2011). HSP are a family of proteins that have many forms, most being constitutive, with a cytoprotective role, however other forms are inducible by a particular factor or stress situation (JACOBY et al., 2012); they also play a prime role in the involvement of thermotolerance (RAMPINO et al., 2009).

The possible, and often proven, interaction of these proteins in certain situations has influenced the use of genotypes that exhibit distinct behaviour in relation to the induction and accumulation of HSP gene transcripts (CHO; HONG, 2006; HUTHER et al., 2013).

Among these genes, the sHSP23.6 gene, which encodes a mitochondria-located low molecular weight protein (sHSP), has contributed to a better characterisation of molecular and physiological mechanisms (GURLEY, 2000).

Studies report (BASHA; WATERS; VIERLING, 1999; PEGORARO et al., 2012) that the sHSP23.6 gene under normal conditions may be involved during the developmental process of different organs, as well as being present in different stressful situations, and is noteworthy for its constitutive expression; however, depending on the stage of development, its levels of expression vary (WATERS, 2013).

In this way, the evaluation of transformed plants under normal conditions of growth, can clarify how they behave in relation to carbon performance and fixation, as well as in the partitioning of photoassimilates, and in the possible impacts that transformation can cause on balanced growth.

The aim of the present study was to compare growth and production components in tomato plants of the wild Micro-Tom variety, and the transgenic lines that confer different levels of expression on MT-sHSP23.6 (mitochondrial small heat shock proteins).

MATERIAL AND METHODS

Seeds from the Micro-Tom variety of tomato were used, both untransformed and transformed genetically for different levels of mitochondrial MT-sHSP23.6 expression, as described in Huther et al. (2013).

Seeds from the three genotypes were placed in Gerbox® boxes. After seven days, they were transplanted to plastic pots (500 mm³ capacity) containing a sand substrate. The seedlings were placed in growth chambers under controlled conditions: a photosynthetically active photon flux density of around 200 μmol m⁻² s⁻¹ at the central height of the plant canopy, a photoperiod of 10 hours, and a temperature of 21 ± 3 °C. Irrigation was carried out daily using distilled water, except for three times a week when 20 mL Hoagland and Arnon nutrient solution (1950) was used.

In order to obtain the primary data for leaf area and dry matter weight, successive collections were carried out at regular intervals of 21 days, with collections starting on the twenty-first day after transplanting (DAT), giving a total of six collections, with three replications per genotype and collection. For each sample, the plants were cut close to the substrate, separated into organs (leaves, stems, flowers and fruit), and packed in paper bags. The roots were washed over a fine-mesh sieve using running water.

With each collection, the leaf area, the dry weight of the parts of the plant (leaf, petiole, stem, roots, and reproductive parts when present), and the number of green and red fruit were determined.
The leaf area was determined for the green leaves \((A_f)\) using a Li-Cor Model Li-3100 area meter, (Li-Cor Inc., NE, USA), and expressed in square centimetres per plant \((\text{cm}^2/\text{plant}^{-1})\). The dry matter from each part of the plant was obtained after drying in a forced ventilation oven at a temperature of 65 ± 2 °C for at least three days, the total dry matter being considered the sum of the dry weight of the parts the plant. To analyse assimilate partitioning during plant development, the dry weight of each part of the plant was expressed as a percentage in relation to the total dry weight. The total number of fruit \((N_{fr})\) was determined by directly counting the fruit per plant, and fresh fruit weight \((W_{fr})\) was measured on a precision balance immediately after collection.

The primary data for leaf area \((A_f)\) were adjusted using orthogonal polynomials (RICHARDS, 1969); while the primary data for total accumulated dry matter \((W_t)\) were adjusted by the simple logistic equation (equation 1):

\[
W_t = \frac{W_m}{(1 + Ae^{-Bt})}
\]

where \(W_m\) is the asymptotic estimate for maximum growth, \(A\) and \(B\) are adjustment constants, \(e\) the natural base of the neperian logarithm, and \(t\) the time in days after transplanting (RICHARDS, 1969).

RESULTS AND DISCUSSION

The transformed plants displayed a larger leaf area than the untransformed (wild) plants (Figure 1). Plants of the three genotypes showed similar values for \(A_f\) in the initial growth phase, up to approximately 42 DAT. Plants of the transformed lines then displayed a higher growth rate for leaf area, resulting in a greater maximum leaf area. After the maximum values were reached, \(A_f\) was reduced, characterising leaf senescence. The maximum leaf areas were 369, 459, and 446 \(\text{cm}^2/\text{plant}^{-1}\) for wild plants, plants with overexpression of MT-sHSP23.6, and plants with silencing of MT-sHSP23.6 respectively, reached at 106, 113, and 107 DAT.

Fayad _et al_. (2001) consider that stabilisation and the subsequent fall in leaf area is caused by leaf senescence and abscission. The same authors mention that the leaf area of the EF-50 hybrid tomato in a protected environment reached its maximum growth at 93 DAT, and that in the ‘Santa Clara’ cultivar a reduction occurred from 58 DAT. According to Lopes (2010), there is a reduction from 74 DAT in the SM-16 hybrid tomato, which can be explained by abscission and natural senescence of the leaves, both common occurrences towards the end of the crop cycle, as well as more photoassimilates being directed to the reproductive structures, which become the preferred sink of the plant. For Bezerra Neto and Nogueira (1999), this
In 'Micro-Tom' tomato plants, the partitioning of assimilates, expressed as a percentage of dry weight per organ, followed the same pattern for all genotypes, and presented very similar photoassimilate distribution in the different plant organs for the various collection dates (Figure 3).

For the first collection at 21 DAT, the predominant total dry weight of the plant was related to leaf content, reaching 70% of the total in the wild genotype (Figure 3A), around 80% in the genotype with overexpression of MT-sHSP23.6 (Figure 3B), and approximately 65% in the silenced genotype (Figure 3C). The rest of the dry weight was divided between the roots and the stems. In the ‘SM-16’ tomato, from shortly after transplanting until 14 DAT, the leaves behaved as both source and sink, as they are responsible for the production of photoassimilates and are also the organ of greatest storage (LOPES, 2010). At 42 DAT, about 70% of the total dry matter in plants of the genotype with overexpression of MT-sHSP23.6 was allocated to the leaf tissue, the rest being distributed between the stems and roots; in the wild and silenced genotypes, around 60% consisted of leaf dry weight.

From 63 DAT, inflorescences and green fruit were seen, i.e. the reproductive phase in the three genotypes continued until the end of the analysis. According to Lopes (2010), stabilisation of the weight accumulated in the leaves possibly occurs due to the senescence phase overlapping the emission of new leaves, an event that occurs in tomato plants of determinate growth.

In Brazil, there are still few studies on the dynamics of biomass production and allocation in parts of the tomato (LOPES, 2010). Caliman (2008) emphasises that crop production is determined by plant growth through the allocation of biomass to the organs of commercial interest, and that dry weight does not refer to a single component substance of the plant, but to all the organic matter that is produced for the essential activities of photosynthesis and protein metabolism.

Heuvelink (1997) emphasises that the study of dry-weight distribution in plants is important to obtain a better understanding of the influence of the fruit on dry-matter partitioning between the fruit and the vegetable part of the tomato, in order to determine whether the generative force (source) is proportional to the number of fruit per stem.

Most of the photoassimilates produced are used for growth, being both partially and temporarily
stored in the form of starch and sugars, but some are exported to other plant organs (LOPES, 2010). Biomass production in the plant is proportional to the availability of photoassimilates, which in turn is related, among other factors, to the availability of light energy in the canopy and the concentration of CO₂ (CALIMAN, 2008).

In general, according to Alvarenga (2004), the tomato cycle can be divided into three stages: the first stage, which lasts from four to five weeks (from transplanting to the start of flowering); the second stage, with a duration of five to six weeks, starting with flowering and ending at the beginning of the fruit harvest; and the third stage, which lasts from the beginning to the end of the fruit harvest.

In studies with the Yubi Cherry tomato, which is of determinate habit, Albuquerque Neto and Peil (2012) state that development of the vegetative part takes place at a different time from that of the generative part (fruit), i.e. the plant grows, then flowers and fruits in sequence, presenting a very high number of lateral shoots that are retained and provide a large amount of dry weight for the leaves, which can equal the dry weight of other plant organs.

The most vigorous growth and the greatest rate for leaf emission are found in the stems that are located just below the inflorescences; definitely strategies of the tomato plant for maximising the translocation of photoassimilates for fruit growth (PIVETTA et al., 2007).

The leaves in the canopy of the tomato plant comprise around 36% of the dry matter corresponding to starch (EDWARDS; JOLLIFFE; EHRET, 2010). Those authors also considered that for nearby layers and/or layers below the canopy, the leaves display a reduction in starch content compared to the leaves of the canopy, and that these reduced levels of starch in the lower leaves indicate that they are possibly used in large measure for fruit growth. After flowering, the fruit begins to develop and grow, with the accumulation of dry matter in the shoots now taking place in the fruit (PIVETTA et al., 2007). Lopes (2010) describes that branches, inflorescences and fruit behave like a sink; the fruits however are the preferred sink for the plant, and so the leaf assimilates are vigorously directed to the fruit as a result of the predominance of the reproductive phase over the vegetative phase.

Further, in relation to the distribution of dry weight per parts of the plant, from 105 DAT, the tomato plants of all genotypes presented red fruit equivalent to around 30% to 35% in relation to dry weight, not exceeding 50% of the percentage value in each plant for the dry weight of the fruit. This differed from the values seen by Fayad et al. in plants of the EF-50 and ‘Santa Clara’ tomatoes, who found at the end of the cycle that of the total dry weight produced by the plant, 68% and 51% respectively was present in the fruit.

In the case of the variety known as ‘Micro-Tom’, where there is a smaller proportion of fruit compared to the other varieties present on the market, this variety is seen more as a genetic model for the family Solanaceae than for fruit production, even though the variety has viable fruit (FAYAD et al., 2001, 2002; MEISSNER et al., 1997).

According to Albuquerque Neto and Peil (2012), genotypes belonging to the group of mini tomatoes have greater proportional dry-matter partitioning for leaf formation, to the detriment of fruit formation, when compared to genotypes with larger fruit. It was also noted that in the final analyses of this experiment there was almost constant dry-matter distribution, which can be described by a saturation of the function of the number of fruit retained by each stem.

In the present experiment, plants of all the lines presented green fruit from the third analysis only, at 63 DAT (Figure 4A), with a peak in production at 84 DAT, after which production was comparable to the start of the analysis, finally displaying a small reduction in the number of green fruit per genotype. The red fruit were present from 105 DAT, maintaining an average for ripening in all genotypes until the end of the experiment (Figure 4C).

From 105 DAT, the fresh weight of the red tomato fruit was checked, where the genotype with overexpression of MT-sHSP23.6 stood out among the three genotypes; this was also seen at 126 DAT, where the untransformed plants were the same as the plants with overexpression of MT-sHSP23.6 (Figure 4D). The highest value for green-fruit fresh weight was reached together with the highest number of green fruit produced, at 84 DAT. The genotype with overexpression of MT-sHSP23.6 displayed the greatest weight, as was seen with the fresh weight of the red tomatoes of this genotype. The decrease in green-fruit fresh weight coincided with a reduction in the number of green fruit during the fruit-production cycle throughout the analyses (Figure 4B).

The growth of tomato fruit implies an irreversible flow process (GAO; SAGI; LIPS, 1998), being a powerful carbohydrate drain (ALMEIDA, 2012), and as high fruit yields are desirable, then the high distribution of biomass to the fruit is important, as well as a reduction in the number of fruit per plant (HEUVELINK, 1997), the purpose being to achieve fruit uniformity.

It is also worth noting that in all genotypes the fruit usually developed a reddish colour, as well as other characteristics such as the development of aromas and softening. The reddish colour in ripe tomatoes is due to the high amount of lycopene, which varies according to the type and degree of ripening of the tomatoes. Lycopene can also be found in tomato products, and due to being a carotenoid is found in larger quantities in the skins.
Figure 4 - Number of green fruit (A), fresh weight of green fruit expressed in mg plant⁻¹ (B), number of red fruit (C), and fresh weight of red fruit expressed in mg plant⁻¹, in ‘Micro-Tom’ tomato plants, transformed with overexpression of MT-sHSP23.6 (High HSP) and with silencing of MT-sHSP23.6 (Low HSP), and untransformed (WT), for days after transplanting.

CONCLUSIONS

1. Leaf area in the transformed genotypes was greater than in the untransformed plants; for production components, the most productive genotype, considering the fresh weight of the fruit, was the genotype with overexpression of MT-sHSP23.6;
2. The genetically transformed ‘Micro-Tom’ tomato plants behaved similarly in the analysis of total dry weight and in relation to assimilate partitioning.
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