Selection and Breeding of Robust Rootstocks as a Tool to Improve Nutrient-Use Efficiency and Abiotic Stress Tolerance in Tomato

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Keywords: grafting, *Solanum lycopersicum*, suboptimal temperature, salinity

Abstract

In April 2010, the research program 'Green Breeding' started in the Netherlands, which is subsidized by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV) and several private breeding companies. With this program, the Dutch government stimulates breeding research, which contributes to a more sustainable agriculture and horticulture. As part of this research program, this tomato rootstock project was granted for the period April 2010 to April 2013. It is aimed at identifying the key physiological traits that are characteristic of a vigorous rootstock, thus providing breeding companies with an effective selection method to breed robust rootstocks for both the conventional and organic production of tomatoes. Breeding for vigorous rootstocks is still a matter of trial and error. Practical selection tools like genetic markers are lacking because the knowledge about the physiology behind a successful rootstock (root-shoot interaction) is limited. In addition, the desired root traits studied in this project, nutrient-uptake efficiency, salinity and suboptimal-temperature tolerance, are complex and the identified Quantitative trait loci (QTLs) have hardly any value for practical breeding purposes. This project will therefore not focus on the identification of single allele-specific markers for traits that are expressed in a particular hybrid combination. Instead, it aims to identify biomarkers (particular physiological traits) as generic tools to develop a reliable screening method which supports the selection of vigorous rootstocks. If successful, the knowledge gained from this project will enable the selection and breeding of rootstock cultivars which improve the sustainability and profitability of both conventional and organic cultivation of tomatoes.

INTRODUCTION

In the Dutch glasshouse industry, growers rely on large inputs of mineral nitrogen (N), phosphorus (P) and potassium (K) fertilisers to realize high physical production levels and product quality (Voogt, 2009) to produce a profitable crop. By comparison, the annual application of fertilisers is on average about ten times higher for glasshouse than for open field crops (Voogt, 2004). Glasshouse horticulture offers possibilities for full process control; nevertheless it is still faced by low fertiliser efficiency. Therefore, in areas with a high density of glasshouses, the discharges of N and P contribute significantly to groundwater and surface water pollution (Voogt, 2009).

In 1997, the Dutch government introduced legislation aimed at reducing N and P emissions by 95% each by 2010, using 1985 as the year of reference (GlaMi, 1997). Since then, the glasshouse industry has implemented crop specific limitations in N and P use by switching from open to closed substrate culture systems and re-use of drainage water. In practice, substantial loss of water and minerals due to leakage and partial flushing to reduce accumulation of ions still occurs in closed growing systems (Voogt, 2009). The nutrient-use efficiency (NUE) of closed growing systems for both N and P is currently

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only 80%. Of the glasshouse-grown tomatoes, 10% are not grown hydroponically, but soil-grown. For soil-grown crops, systems with irrigation and fertilisation strategies based on crop demand and soil moisture content, like the fertigation model, have the best prospectives for sustainability. For all growing systems, further improvements are possible by reduction of N and P target values for the root environment (Voogt, 2004, 2009).

To stimulate realization of the above mentioned targets, the Dutch Ministry of Agriculture, Nature and Food Quality (LNV) has launched a new research program "Green Breeding" (2010–2020) with a total of €10 M to support plant breeding for sustainable, low-input agriculture and horticulture. The focus is on research for pest and disease resistance breeding, with emphasis on insect resistance and on improving adaptation to lower input of nutrients and changing climatic conditions. The results should be applicable in and serve both organic and conventional agriculture and horticulture. Both active and significant financial participation of the breeding companies is one of the preconditions. In February 2010, four projects were granted for the first phase (2010–2013). Besides the tomato project (rootstock selection to improve nutrient efficiency and abiotic stress resistance) described in this paper, these projects focus on potato (resistance to blight (*Phytphthora infestans*) and low-nutrient input), spinach (resistance to downey mildew (*Peronospora farinose*) and nutrient efficiency) and leek (resistance to thrips (*Thrips tabaci*)).

GRAFTING: A TECHNIQUE WHICH COMBINES THE BEST OF TWO WORLDS

Environmental stresses represent the most limiting conditions for horticultural productivity and plant exploitation worldwide. Important factors among those are water, temperature, nutrition, light, oxygen availability, metal ion concentration, and pathogens. One solution to these problems is to develop new cultivars that are more tolerant to such stresses. This is carried out with tremendous efforts, particularly from breeding companies. However, due to a lack of practical selection tools like genetic markers, it is a slow and inefficient process. A special technique of adapting plants to counteract environmental stresses is grafting elite, commercial cultivars onto selected vigorous rootstocks (Lee and Oda, 2003). With grafting, the best of two worlds can be combined: excellent above-ground traits (fruit quality and yield), conferred by an elite cultivar used as scion, and desired under-ground traits (resistance against soil-borne diseases and vigorous root growth), conferred by a suitable rootstock. Grafting is nowadays regarded as a rapid alternative tool to the relatively-slow breeding methodology aimed at increasing environmental-stress tolerance of fruit vegetables (Flores et al., 2010). In vegetable production, grafting has been used for more than 50 years in many parts of the world. With the introduction of the 'Japanese' grafting method in 1990, large scale grafting at lower costs became feasible. Grafting is not associated with the input of agrochemicals to the crops and is therefore considered to be an environmentally-friendly operation of substantial relevance to integrated and organic crop management systems (Rivard and Louws, 2008). For tomato, rootstocks are used to increase the resistance against soil-borne pathogens like *Verticilium* wilt and *Fusarium*, and above all, to improve scion vigour and endurance, which increase fruit production (Fig. 1). Rootstocks also allow growers to make a better use of their greenhouse facilities (CO_2 applications and heat storage). In recent years, the cultivated area of grafted *Solanaceae* and *Cucubitaceae* has increased tremendously because the objective of grafting has been greatly expanded from reduction of infections by soil-borne pathogens to enhance the tolerance against abiotic stresses. Among those are saline soils, soil-pH (alkalinity) stress, nutrient deficiency and toxicity of heavy metals. Other abiotic conditions for the application of rootstocks are thermal stress, drought and flooding, and persistent organic pollutants (Schwarz et al., 2010).

The demand for tomato rootstocks is still developing and holds at present an estimated market value of €30 M. The tomato rootstock market is currently dominated by two cultivars and any significant improvement of these is hampered by a lack of knowledge behind the key traits which determine rootstock performance and the interaction between rootstock and scion. Current rootstock breeding methods are relying on a trial-and-error approach to construct inter-specific hybrids of well-rooted wild tomato species and vigorous cultivated species. We state that no major improvement will be achieved in this market segment before we reach a deeper understanding of the physiology of tomato rootstocks and its interaction with the tomato scion.

This project explores the possibilities of using rootstocks as a tool to improve the nutrient-use efficiency and abiotic stress tolerance of elite tomato cultivars, and to deliver key-physiological parameters (biomarkers) which can be used for the selection and breeding of rootstocks which improve the sustainability and profitability of both the conventional and organic cultivation of tomatoes. This project may serve as a model study from which the developed selection method can also be used for the breeding of rootstocks of other grafted horticultural vegetable crops like bell pepper, cucumber and eggplant. In the next sections, we briefly describe the anticipated influence of the rootstock on plant performance with regard to nutrient-use efficiency and the resistance to suboptimal-temperature and salinity stress.

Rootstocks as a Tool to Improve Nutrient-Use Efficiency (NUE)

The major challenge for Dutch horticulture up to 2027, with a continually expanding global population, is to maintain or increase productivity while at the same time reducing nutrient use and losses to the environment (GlaMi, 2008). Improvements in NUE of greenhouse crops, defined as the yield per unit input of fertilizer, would help us to achieve these objectives. The NUE of a crop is determined by its genetic characteristics (genotype) and by the environment in which the crop (root) is grown. This project is aimed at finding selection criteria for the breeding of rootstocks which may enhance NUE of elite tomato cultivars under a wide range of growth (root) environment conditions. Several tomato grafting studies indicated that rootstocks affect leaf macronutrient content and may enhance nutrient uptake and NUE (Rivero et al., 2005; Leonardi and Giuffrida, 2006). Given the universal recognition that the worldwide phosphorous (P) rock reserves are finite, the main focus of this project will be on P-use efficiency (PUE). PUE is the product of the root P-uptake efficiency and the tissue P-utilization efficiency (White and Hammond, 2008). It appeared that selection for greater efficiency of root P uptake is the most effective strategy for developing crops with increased PUE (Lynch and Brown, 2008). Quite large variations were observed in phosphate (P_i)-acquisition efficiency among *Arabidopsis* ecotypes (Chevalier et al., 2003); however, no information is available about the variation in PUE within and among tomato species. One of the key traits for optimizing the efficiency of P acquisition is root elongation rate (Lynch, 1995). The main focus of this project will, therefore, be on elucidation of molecular and physiological mechanisms underlying variance in root elongation rate in tomato and the plasticity of this trait in response to changing growing conditions.

Rootstocks as a Tool to Improve Suboptimal-Temperature Tolerance

Temperature is one of the most important environmental factors inflicting relevant economic yield losses by reducing plant growth and development, causing wilt and necrosis, and retarding the rate of truss appearance and fruit ripening. Besides the air temperature, plant performance may also be limited by low soil temperatures. Commercial tomato production in Mediterranean areas may be limited by suboptimal root-zone temperatures during spring when air temperatures are already high. Also, tomatoes cultivated in closed greenhouses may suffer from suboptimal root-zone temperatures on hot days due to forced cooling which is located near the roots of the plants. Cultivation of tomato at suboptimal (root) temperatures (12–18°C) results in slower leaf expansion and initiation rate of new leaves, which delay the build-up of the crop and interception of irradiation. The reduction in leaf expansion and initiation rate at (root) temperatures below the optimum is not limited by the supply of carbohydrates, since leaves accumulate large amounts of starch, but more likely by root hormonal signals associated with the uptake of nutrients and water (Venema et al., 2008). Due to a lack of variation in response to low root temperatures among cultivars of the cultivated tomato species (Nieuwhof et al., 1999), breeders have to look for other sources of low-temperature tolerance, as found in related wild tomato species (Venema et al., 2005). One example is *S. habrochaites*, a wild tomato species which grows in relatively moist river valleys at elevations ranging from 500–3300 m above sea level in the Andes. Root related traits, such as elongation rate (Zamir and Gadish, 1987), transport rate of xylem sap and ions (Brunet et al., 1990), and root NH_4^+ absorption (Bloom et al., 1998) in high-altitude accessions of *S. habrochaites* were less affected by low-temperature stress in comparison with the cultivated tomato. In addition, shoots of the cultivated tomato wilt if their roots are exposed to chilling temperatures of around 5°C, whereas high-altitude accessions of *S. habrochaites* maintain turgor (Bloom et al., 2004). Grafting the cultivated tomato onto roots of a high-altitude accession of *S. habrochaites* improved shoot performance at suboptimal root-zone temperature (Venema et al., 2008) and in response to root chilling (Bloom et al., 2004). However, the underlying root-mediated physiological mechanism(s) remained unclear in both studies. Tomato grafted onto *S. habrochaites* revealed a higher capacity to adjust the root:shoot ratio to suboptimal-root temperature (Venema et al., 2008). The physiological mechanism(s) involved in this shift in assimilate partitioning from source leaves to roots is not clear and may be regarded as energetically wasteful from an agronomic point of view. However, this adaptive response recovers the functional equilibrium between root and shoot, allowing the root system to overcome restrictions in water and/or nutrient uptake. The difference in low-temperature induced root growth inhibition between tomato and *S. habrochaites* seemed to be related to the extent in which the root elongation rate was inhibited (Zamir and Gadish, 1987; Venema et al., 2008). The main focus in this project will, therefore, be on the elucidation of the physiological mechanisms underlying variance in root elongation rate at low temperatures.

Rootstocks as a Tool to Improve Salinity Tolerance

In addition to soils that are naturally saline, many prime agricultural soils are gradually being salinized by irrigation with poor-quality water and inadequate drainage. Tomato is sensitive to high levels of salts and as a result, their growth and economic yield are reduced substantially. Wild tomato species have often been considered a useful source of salt tolerance genes to transfer to the cultivated tomato (Albacete et al., 2009). Grafting tomato onto salt-tolerant wild species proved to be an effective tool to improve the adaptation of high-yielding tomato cultivars to salt stress (Fernández-García et al., 2002; Estañ et al., 2005; Cuartero et al., 2006; Albacete et al., 2009; Estañ et al., 2009). Salinity affects plant performance in two phases. In the initial ‘osmotic’ phase (seconds to minutes), the osmotic effect predominates and induces water stress due to high salt concentration in the root environment. During the second ‘ionic’ phase (weeks to months), growth is governed by toxic effects due to accumulation of Na^+ and Cl^- in leaf tissues, which provokes a wide variety of physiological and biochemical alterations that inhibit leaf expansion and appearance, and induce leaf senescence. To reduce the initial osmotic effects of salt stress, we are looking for rootstocks in which function and growth capacity are hardly affected by salinity. The mechanism(s) that down-regulates leaf growth and shoot development during the osmotic phase is not known, but a main role is hypothesized for root-derived hormones (Munns and Tester, 2008; Ghanem et al., 2008; Albacete et al., 2008, 2009). To reduce the accumulation of toxic levels of Na^+ and Cl^- during the second ‘ionic’ phase of salt stress, rootstocks have to be selected which have the ability to reduce the uptake and transport of saline ions to the shoot, termed “salt exclusion” (Estañ et al., 2005). In wheat, a root’s ability to retain K^+ correlated with the capacity to exclude Na^+ and thus salt tolerance (Cuin et al., 2008). Therefore, in this project we will use NaCl-induced K^+ efflux measurements on roots as a physiological marker to screen for salinity tolerance (Cuin et al., 2009). In contrast to cultivated tomato species, which roots generally exclude toxic ions, some salt tolerant accessions of related wild species like *S. pimpinellifolium* and *S. cheesmaniae* seem to have a "salt inclusion" mechanism. Plants with this mechanism accumulate high concentrations of Na^+ and/or Cl^- in the shoot and compartmentalize the ions in the vacuole (a halophytic response). For this mechanism it seems important to keep the K^+/Na^+ ratio in the vacuole intact (Munns and Tester, 2008). In recombinant inbred lines derived from a cross between a cultivated and a salt-tolerant wild tomato species, salinity tolerance (leaf growth and maintenance of the photosynthetic apparatus) was positively correlated with xylem K^+ and cytokinin (CK) concentrations, whereas the interaction between ethylene precursor ACC with CKs and abscisic acid (ABA) seems to be involved too (Albacete et al., 2009). These recent findings support our approach to specifically target the root system and exploiting root-to-shoot signalling to improve salt tolerance in tomato.

AIMS AND DELIVERABLES OF THIS PROJECT

So far, the breeding for vigorous rootstocks is a matter of trial and error since practical selection tools like genetic markers are lacking. This is because the knowledge about the physiology behind a successful rootstock (root-shoot interaction) is still very limited. In addition, desired root traits studied in this project, nutrient-uptake efficiency, salt and suboptimal-temperature tolerance, are complex and the identified QTL's have hardly any value for practical breeding purposes. This project will therefore not focus on the identification of single allele-specific markers for traits that are expressed in a particular hybrid combination. Instead, it aims to identify biomarkers (particular physiological traits) as generic tools to develop a screening method to select robust and vigorous rootstocks which improve the nutrient-use efficiency and adaptability of tomato to new growing, climatic and environmental conditions in open and protected cultivation. This screening method should be reliable and allow a high throughput rate. No techniques beyond the capabilities of a standard physiological/biochemical laboratory should be required for this method. Development of new cultivars which are environmentally-friendly and have a high adaptability is one of the main goals of modern plant breeding. If successful, this tomato rootstock project will contribute to the improvement of the sustainability and profitability of both conventional and organic cultivation of tomatoes.

ACKNOWLEDGEMENTS

J.H.V. was financially supported by the Dutch Ministry of Economic affairs, Agriculture and Innovation (EL&I) HP "Green Breeding" and three private breeding companies.

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Figures

Fig. 1. Schematic representation of the idealized beneficial effects of grafting a modern high-yielding (elite) tomato cultivar onto a vigorous rootstock as tool to improve nutrient-use efficiency and the adaptability to changing growing conditions. Left illustrates an ungrafted elite tomato cultivar and right the same cultivar grafted as a scion onto a vigorous rootstock. This figure is a modification of an original UA-CEAC image created by K. Tomlinson.