Scientia Horticulturae 217 (2017) 197–208

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Research paper

Anatomically assisted cherry rootstock selection

Mirjana Ljubojević[a,*], Lana Zorić[b], Ivana Maksimović[a], Jovana Dulić[a], Maja Miodragović[a], Goran Barać[a], Vladislav Ognjanov[a]

[a] University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[b] University of Novi Sad, Faculty of Sciences, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

ARTICLE INFO

Article history:
Received 10 October 2016
Received in revised form 25 January 2017
Accepted 30 January 2017
Available online 7 February 2017

Keywords:
Breeding
Hydraulic conductance
Prunus cerasus
Prunus fruticosa
Rootstock anatomy
Stems anatomy

ABSTRACT

Main goal of this study was the establishment of unequivocal selection protocol in breeding size-controlling rootstocks with possible drought adaptability. Plant material, consisted of ‘oblačinska’ sour cherry (*Prunus cerasus* L. – OV) and ground cherry genotypes (*Prunus fruticosa* Pall. – SV), was propagated by softwood cuttings under controlled mist system. Analysis included detail anatomical investigation of fine and skeletal roots and rootstock stems. Each constituent contributed to the overall rootstock size-control capacity and potential drought adaptability. Fine roots contribution was estimated trough the active root surface area that significantly varied within and between investigated species, indicating different absorption capacities. Ratio between active—fine-roots surface area responsible for radial solution sinking and total root surface area, comprised of non-sinking skeletal roots and fine roots served as coefficient for reduced water uptake in theoretical hydraulic conductance calculations. Simultaneous examination of root system water capacity, axial conductance capacity corrected by radial sink capacity, and rootstock stem capacity, defined by anatomical characteristics, wood formation and thickening dynamics was established as reliable selection protocol for size–controlling capacity and hypothesized adaptability. Based on stated protocol greatest size-controlling effect is expect to be achieved within ground cherry genotypes – SV1, SV2, SV4, SV5 and SVKK, followed by ‘oblačinska’ cherry genotypes OV13, OV14, OV22, OV23, and OV24. Following the same approach, genotypes SV2–7 and OV14 are presumed to be the best adapted to drought and embolism caused by freezing/thawing and hydration/dehydration cycles.

1. Introduction

Raising a fruit plantation is a long-term and expensive investment, where the most important goal is to reduce costs and intensify production. Profitability of production depends on adequate choice of both variety and rootstock. Size–controlling cherry rootstocks were introduced in order to improve numerous cherry tree characteristics – vigor, adaptability, precocity, productivity, fruit size and quality, with significant reduction of production inputs (Sansavini and Lugli, 1998; Lang, 2000; Hrotkó, 2008). Rootstock supplies scion with water and mineral solution, plant hormones, presents a mechanical support to the scion, while the scion provides photo—assimilates, for both rootstock growth and storage. The grafted plants survival, to a large extent depends on compatibility and further rootstock and scion ability to ‘communicate’ effectively (Prassinos et al., 2009). Rootstock breeding programs, as long—term projects, should pay considerable attention on rootstock selections with specific pedo—climatic adaptability. In terms of changing climate factors and scarcity of potable water, rootstock breeding is facing serious issues related to soil water limitations; hence, examination must be focused on size—controlling rootstocks able to cope with soil drought without compromising acceptable growth and yields, rather than dwarfing effect solely. In a relation to drought tolerance, rootstocks should be able to ensure unimpaired scion growth and functioning even when water supply is limited (Serra et al., 2014).

Root system of perennial species is comprised of fine roots and strong skeletal, woody roots, which equally contribute to the fulfillment of above—mentioned rootstock functions. Roots just like a scion shoots, represent the dynamic structures of branching, that actually form a ‘system’ in the sense that their components are

---

* Corresponding author.
E-mail addresses: mirjana.ljubojevic@polj.uns.ac.rs, mirush84@gmail.com (M. Ljubojević), lana.zoric@dbe.uns.ac.rs (L. Zorić), ivanam@polj.uns.ac.rs (I. Maksimović), jovana.dulic@polj.uns.ac.rs (J. Dulić), maja.miodragovic@polj.uns.ac.rs (M. Miodragović), goranb@polj.uns.ac.rs (G. Barać), vognjanov@polj.uns.ac.rs (V. Ognjanov).

http://dx.doi.org/10.1016/j.scienta.2017.01.049
0304-4238/

connected in the organized networks (Pagès, 1999). Their functioning seriously depends on the entire root system architecture (RSA), underground three—dimensional structure of each plant that includes topological organization and geometrical characteristics. Topology covers a physical connection between the components, while geometry involves shape, size, orientation and spatial organization of the components (Godin et al., 1999; Godin, 2000; Gregory, 2006; Reubens et al., 2007). Those properties define root system anchorage, ability to reach water and mineral resources and their conductance. Root system structure and spatial distribution, as parts of RSA are of a great importance because different roots of one root system can be exposed to very wide range of physical, chemical and mechanical conditions in soil volume. Although environmental factors are often associated with aboveground plant organs, roots are strongly affected by environment – rhyzosphere, as well as root system/rhyzosphere interaction. Rhyzosphere properties include moisture, aeration, temperature, soil mechanical composition, microbial activity, etc., that altogether contribute to water and mineral availability and their absorption. It was recently proved that RSA in most cases is regulated by a set of quantitative loci that significantly interact with the environment and lead to RSA having a low heritability. Thus, novel approaches define RSA as a synthesis of developmental processes (root growth, formation, tropisms) that are potentially influenced by root age, plant phenology, and the environment. In these approaches, the emphasis is shifted from the static description of RSA towards the dynamic changes (Orman-Ligeza et al., 2014.). Efficiency of water uptake and transport is highly dependent on individual root dynamic elements – development and structure. Water uptake is directly proportional to root surface area – particularly active root surface area (ARA) that proved to accurately reflect size-controlling capacity of standard cheery rootstocks (Ljubojević et al., 2013). Water transport, on the other side, is proportional to diameter and maturity of the xylem vessels. Each root is capable of performing only one of the two functions at the most efficient way (Waisel and Eshel, 2002). Soil solution taken up by fine roots – radial path, continues to scion trough skeletal roots – axial path, making it a functional unit (Danjon and Reubens, 2007).

In fruit species, anatomically assisted rootstock selection is old approach (Beakbane and Thompson, 1947; Floor, 1957) that is taking considerable employment in contemporary breeding programs. This early approach based on xylem and phloem portions on total cross-section in contemporary analyses is shifted towards the xylem conduit size, number, production dynamics and tightly connected hydraulic capacities. Growth regulation based on root and rootstock stem xylem characteristics (especially the size and vessel density), and hydraulic conductance capacity are widely accepted selection parameters (Tombesi et al., 2010; Tombesi et al., 2011; Zorić et al., 2012; Hajagos and Végvári, 2013; Ljubojević et al., 2013; Martínez-Alcántara et al., 2013; Bruckner and DeJong, 2014; Chen et al., 2015). High hydraulic conductance capacity arisen from large xylem vessels provides persistent soil solution flow to the scion, but at the same time, large vessels tend to be more vulnerable to draught– and freezing—induced embolism. Opposite, limited hydraulic conductance connected to small xylem conduits carry a risk of growth and development reduction and cessation during the dry summer months. When those axial hydraulic properties are accompanied with poorly developed root system and low ARA, problems are impassable without significant irrigation inputs. Ljubojević et al. (2013) noted the importance of ARA estimation and incorporation in vigor prediction models, where authors proposed integrated approach that takes into consideration both radial and axial conductance capacities, as well as their interaction.

Based on stated climate-change problems and findings on a wide range of fruit species, detail cherry fine and skeletal roots and rootstock stems anatomical investigation was conducted, aiming to the selection of rootstocks with growth control capacity and potential drought cycles adaptability.

2. Materials and methods

2.1. Plant material

During the four years total of 56 accessions were selected *in situ* and 27 phenotypically most interesting accessions of 'oblačinska' sour cherry (*Prunus cerasus* L.-OV) and European ground cherry (*Prunus fruticosa* Pall.-SV) were brought and collected *ex situ*. Origin, detail qualitative and quantitative description of genotypes was previously published by Ognjanov et al. (2012). Based on stated investigation 16 genotypes were selected for subsequent rooting by softwood cuttings under controlled conditions, in order to minimize impact of ecological factors on dissimilarities in plant growth and development. Additionally two standard cherry rootstocks of different growth control capacities – low vigorous 'Gisela 5′ and semi-vigorous 'Colt', as well as one regional amateur rootstock 'Cigancica' were propagated in the same conditions. From mid-June rooting of softwood cuttings was carried out in the green house of the Department for Fruit science, Viticulture, Horticulture and Landscape architecture in Novi Sad (Serbia), under a fogging system with 95–98% relative humidity, where fogging intervals lasted 90 s with 720 s pause. The 12 cm long terminal cuttings were treated with exogenously applied auxin mixture consisted of 0.8% α-naphthylacetic acid (NAA) and 0.5% indolebutyric acid (IBA). Cuttings were planted and remained in the 'Steckmedium' ('Klassman') substrate until the rooting termination at the end of growing season, when they were transplanted into larger containers with substrate 'Potground H' ('Klassman') to which slow-released fertilizer 'Osmocot Exact' was added. Plants were grown for one more season in order to obtain the two-year old root systems and rootstock stems. During the transplantation, plants were washed and immersed in 0.2 mM methylene blue, as described by Mu et al. (2006). Determination of the concentrations was carried out spectrophotometrically, by measuring the light absorption (the wavelength $\lambda = 662$ nm). Red values of the light absorption were converted to concentrations using a standard curve that was constructed based on known concentrations of methylene blue and the corresponding light absorption levels. Finally, active root surface area (ARA) was determined by applying the expression—ARA (m^2) = C × 1.05 m^2, where C represents the amount of methylene blue absorbed by the root system in last tub marked as C.

2.2. Anatomical characteristics and conductance capacities measurements

Root system separation on woody (skeletal) and non-woody (fine) roots followed the root typology described by Vercambre et al. (2002). Fine roots of the each skeletal root were sampled from the area of the greatest abundance—approximately 1st cm from the branching point down to the 6th cm and included all roots identified therein. Three two-year-old skeletal woody roots (4.00–6.00 mm in diameter) from five replicate plants per genotype, were taken from different sides of the root system. Samples from two-year-old rootstock stems were taken from three replicate plants per genotype, at approximately 10 mm from the basal part of the stem—the typical grafting point. Plant material was fixed and preserved in 60% ethanol, with addition of 10% of glycerin. Cross-sections were obtained using hand microtome and cryostat Leica CM 1850 (cutting temperature −20 °C; section thickness 60 μm). Cross-sections were examined and measured by light microscope and Image Analyzing System Motic Images Plus. Measurements included both cross-section and xylem characteristics on four radial