Analysis of genetic factors influencing transformation efficiency of *Rosa hybrida* cultivars

Von der Naturwissenschaftlichen Fakultät
Der Gottfried Wilhelm Leibniz Universität Hannover

zur Erlangung des Grades
Doktorin der Naturwissenschaften
(Dr. rer. nat.)

genehmigte Dissertation
von
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2019

Tag der Promotion: 20.09.2019

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Abstract

The production of ornamental roses makes substantial contributions to the global floriculture industry; furthermore, roses have been used for medicine, perfume and food purposes for centuries and are among the top five ornamentals worldwide. However, the traditional methods for breeding roses are time-consuming and may have unwittingly eliminated agronomically useful traits. One of the alternatives is genetic transformation, an efficient technology for improving useful agronomic rose traits without these limitations. To improve the efficiency of transformations in the rose, the propagation and regeneration capacity of 96 rose genotypes were investigated to find suitable varieties for regeneration and micropropagation, as well as for genetic modifications. By combining genetic analysis and association mapping, candidate genes associated with regenerating and propagating traits were identified.

For phenotypic analyses, the shoot regeneration and *in vitro* propagation traits of 96 rose genotypes were investigated. Shoot regeneration rates varied significantly between genotypes, with values from 0.88–88.33%, and shoot ratios (number of shoots per explant) varied from 0.008–1.2. Significant differences in callus size on CIM1 (callus inducing medium 1) were observed on a scale of 0–4 and 0.82–4 on CIM2. Significant variation in shoot multiplication rate was found with variation from 0.5–4.24 among genotypes. Significant variation in *in vitro* root number (ranging from 0.12–18.7), root length (0.26–25.76 cm) as well as *in vivo* root number, root length and root biomass were recorded among the genotypes. These analyses indicated significant genetic influence acting on these traits.

For genetic analysis, GWAS (Genome Wide Association Study) was performed to detect the molecular markers associated with the traits (root and shoot characteristics as well as callus formation). In this analysis, 12 SNP (Single Nucleotide Polymorphism) markers from ESTs (Expressed Sequence Tags) matching known candidate genes involved in shoot morphogenesis were detected. For callus formation, 26 SNPs that are significantly associated with callus formation on CIM1 and 13 SNPs significantly associated with callus formation on CIM2 were found. A total of 6 SNPs were found to be significantly associated with shoot multiplication rate. For rooting traits, 49 SNPs were significantly associated with *in vitro* root length, 98 SNPs were associated with *in vivo* root number, 218 SNPs were associated with *in vivo* root length and 4 SNPs were associated with *in vivo* root biomass. Additionally, by using the KASP (**k**ompetitive **a**llel**s**pezifische **P**CR) technology to verify significantly associated markers for shoot organogenesis in other populations of garden roses, the trihelix transcription factor GT2-like (Rh12GR_53908_964P) and a putative leucine-rich repeat receptor-like protein kinase (Rh12GR_21560_124Q) were determined to influence shoot organogenesis in roses. Other detected markers should be used in future experiments to validate the genes in other populations and examine their functionality in transgenic approaches.

Keywords: Rose, SNPs, GWAS, adventitious shoot formation, callus formation, axillary shoot, adventitious root formation

Zusammenfassung

Die Produktion von Rosen hat einen signifikanten Anteil an der globalen Produktion von Zierpflanzen. Außerdem werden Rosen für medizinische Zwecke, für die Herstellung von Duftstoffen und Nahrungsmitteln verwendet. Rosen sind eine der fünf wirtschaftlich wichtigsten Zierpflanzenkulturen weltweit. Konventionelle Methoden der Rosenzüchtung sind zeitaufwändig und haben wahrscheinlich zum ungewollten Verlust agronomisch wichtiger Merkmale geführt. Eine der Alternativen ist die gentechnische Veränderung von Rosen als eine effiziente Technologie, die es erlaubt wichtige Merkmale ohne diese Einschränkungen zu verbessern. Um die bestehenden Transformationsmethoden für Rosen zu verbessern, wurde die Vermehrungs- und Regenerationsfähigkeit von 96 Rosengenotypen untersucht, um geeignete Sorten für Regeneration und In vitro Vermehrung sowie für Transformationsexperimente zu identifizieren. Durch die Kombination genetischer Analysen und Assoziationskartierungen konnten Kandidatengene identifiziert werden, die mit Merkmalen der Regenerations- und Vermehrungseignung assoziiert sind.

Für die phänotypischen Analysen wurden Parameter für die Sprossregeneration und die In-vitro-Vermehrung in 96 Rosengenotypen analysiert. Die Sprossregenerationsraten variierten signifikant von 0,88-88,33% und „shoot ratios“ (Zahl der Sprosse pro Explantat) variierten von 0,008 bis 1,2. Signifikante genotypische Unterschiede wurden auch für die Kallusgröße auf zwei verschiedenen Medien, CIM1 und CIM2 ermittelt. Ebenfalls wurden signifikante Unterschiede zwischen Genotypen bei der Sprossvermehrungsrate in der In-vitro-Kultur (0,5-4,24) sowie in Bewurzelungsversuchen für die Wurzelanzahl in vitro (0,12-18,7), Wurzellänge in vitro (0,26-25,6 cm) sowie bei der Bewurzelung in vivo gefunden. Dies zeigte, dass ein erheblicher Einfluss genetischer Faktoren auf die Merkmale vorliegt.

Die genetische Analyse wurde mit Hilfe einer Genomweiten Assoziationsstudie (GWAS) vorgenommen, um Marker mit Assoziationen zu den Zielmerkmalen (Wurzel und Sprossmerkmale sowie Kallusbildung) zu identifizieren. In einer dieser Analysen wurden 12 SNPs („Single Nucleotide Polymorphism“) aus ESTs („Expressed Sequence Tags“) detektiert, die zu Genen mit potentieller Funktion in der Organogenese von Sprossen gehören. Für die Bildung von Kallus wurden 26 signifikant assoziierte SNPs für die Kallusbildung auf dem Medium CIM1 und 13 SNPs für die Kallusbildung auf CIM2 detektiert. Insgesamt wurden 6 assozierte SNPs für die Sprossvermehrungsrate gefunden. Für die Wurzellänge in der In-vitro-Bewurzelung wurden 49 SNPs identifiziert, während 98 SNPs mit der Wurzelzahl und 218 SNPs mit der Wurzellänge sowie 4 SNPs mit der Wurzelbiomasse in vivo assoziiert waren. Für das Merkmal Sprossorganogenese konnten einige der assoziierten Marker mit Hilfe von KASP (**k**ompetitive **a**llel**s**pezifische **P**CR) Assays in einer unabhängigen Population von Gartenrosen verifiziert werden und damit Marker aus Genen für einen trihelix Transkriptionsfaktor GT2 (Rh12GR_53908_964P) und eine putative „leucine-rich repeat

receptor-like“ Proteinkinase (Rh12GR_21560_124Q) bestätigt werden. Andere in dieser Arbeit gefundene Marker sollten in zukünftigen Experimenten in zusätzlichen Populationen und durch funktionelle Studien validiert werden.

Schlagwörter: Rose, SNPs, GWAS, adventitious shoot formation, callus formation, axillary shoot, adventitious root formation

Content

Abbreviations

°C	degree Celsius
°N	latitude North
°S	latitude South
AFLP	amplified fragment length polymorphism
bp	base pair
cm	centimeter
DNA	deoxyribonucleic acid
ESTs	expressed sequence tags
FeEDTA	ethylenediaminetetraacetic acid ferric
FeEDDTA	ethylenediamine di-2-hydroxylphenyl acetate ferric
GWAS	genome wide-association study
LD	linkage disequilibrium
LRR	leucine-rich repeat
QTL	quantitative trait locus
RFLP	restriction fragment length polymorphism
S NP	single nucleotide polymorphism
SSR	simple sequence repeat
ESTs	Expressed Sequence Tags

1. General introduction

1.1 Roses as important ornamental plants

1.1.1 Rose taxonomy, genetics and general botany

Roses are perennial shrubs or vine plants and belong to the genus *Rosa* (L) in the subfamily *Rosideae* within the family *Rosaceae*. Most rose species are innate to Asia, with smaller numbers native to North America, Europe and Northwest Africa (Erlanson 1938). *Rosa* species are found throughout the colder and temperate regions of the Northern hemisphere, from the Arctic to the subtropics, with over 180 species. Modern cultivars are mostly interspecific hybrids derived from only 10 of these species: *R. canina*, *R. chinensis*, *H. foetida*, *R. gallica*, *R. gigantea*, *R. moschata*, *R. multiflora*, *R. phoenicea, R. rugosa* and *R. wichurana* (Wissemann and Ritz 2005; Folta and Gardiner 2009). For example, *R. damascene,* more commonly known as the Damask rose, is a rose hybrid derived from *Rosa gallica* and *Rosa moschata,* known for its perfume and its pharmacological effects (Boskabady et al. 2011).

To date, approximately 30,000–35,000 cultivated rose varieties are known. Most modern cultivars do not belong to a single rose species but are instead complex hybrids derived from various species (Gudin 1999). They are generally referred to as *Rosa hybrida*. According to their horticultural classification, cultivated roses are frequently grouped as either hybrid tea (one flower), floribunda (cluster large-flowered), polyantha (cluster small-flowered) or miniature roses (Leus et al. 2018).

Roses comprise species with ploidy levels from 2x–8x. Wild species are often diploid (2n = 2x = 14) but almost all cultivated roses are tetraploids (2n = 4x = 28). Generally, roses are propagated by vegetative methods, such as cuttings, layering, budding and grafting, or by seeding to produce new cultivars and rootstocks.

1.1.2 Economic importance of roses

The rose, admired since ancient times for its beauty and fragrance, has multiple uses: cut flowers, miniature pot and landscape plants, oils (attar of rose) for perfume as well as culinary uses for rosewater and hips (fruits) as a source of vitamin C (Folta and Gardiner 2009). Therefore, roses are one of the most important ornamental plants in the world. The area of cut rose production worldwide is expanding, with remarkable progress in developing countries, for example, production area in Africa has increased from 810 hectares in 1997 to an estimated 5,000 hectares in 2009 (Gitonga et al. 2014). Some established major rose producers include the Netherlands, Colombia, Kenya, Israel, Italy, the United States and Japan. In the cut flower industry, of which roses account for two-thirds of all selections, about 130 billion rose stems are sold annually, and sales exceed €39 billion each year

(http://www.mysunnylawn.com/a30942.php). With imports of roses growing from €272 million in 2011 to €309 million in 2015, Germany now represents the largest market for cut roses in Europe (https://www.cbi.eu/market-information/cut-flowers-foliage/roses/germany)roses/Germany).The largest rose breeding companies have traditionally been located in Europe (e.g. the Netherlands, Germany and France). A summary of worldwide cut rose breeders is presented in Table 1.

Table 1. Cut rose breeding companies worldwide (Leus et al. 2018)

Rose breeding company	**Country**
Brown Breeding	Ecuador
Esmeralda Breeding	Ecuador
Delbard	France
Meilland International	France
Rosen Tantau	Germany
W. Kordes' Söhne	Germany
NIRP International	Italy
Franko	New Zealand
De Ruiter	The Netherlands
Interplant Roses	The Netherlands
Jan Spek Roses	The Netherlands
Schreurs	The Netherlands
United Selections	The Netherlands/Kenya
David Austin Roses	United Kingdom

1.1.3 Rose breeding

There is always a demand and need for new rose varieties with novel traits, such as new attractive flower colours, prickle-free stems, plant architecture, fragrance, recurrent flowering, long stems, high oil content, winter hardiness, resistance to pests and diseases, resistance to heat, easy propagation and suitability for growing under subtropical conditions. Conventional breeding through hybridisation faces problems because roses are highly heterozygous, with varying ploidy levels amongst species, difficulties in sexual hybridisation, low seed set and poor seed germination (Ahmad et al. 2010; Datta 2018).

A number of plant breeding methods, such as crossbreeding, mutagenesis induction and molecular breeding, are major methods of developing new varieties of roses. Nowadays, exploitation of molecular markers, genomic approaches, genetic linkage maps and genetic engineering are available for the genetic improvement of roses.

1.2 Tissue culture of roses

1.2.1 General plant tissue culture

Plant tissue culture plays an important role in the fundamental research and commercial propagation of roses, such as clone propagation, production of essential metabolites and genetic engineering. The scheme of plant tissue cultures, stress factors affecting tissue explants in tissue culture and molecular regulation of developmental events *in vitro* is outlined in Figure 1.

Plant tissue culture involves excising plant tissues (explants) and growing them on sterile nutrient media to use for a range of purposes. In a hormone-dependent manner, plant cells achieve totipotency and developmental plasticity, thereby harnessing the ability to dedifferentiate, proliferate and subsequently regenerate into mature plants under the appropriate culture condition (Skoog and Miller 1957; Steward et al. 1964). Plant tissue explants have the ability to reset their genetic and epigenetic programme in order to undergo development into other cell fates. Plant growth regulators (PGRs) or phytohormones greatly influence the fitness and adaptation of *in vitro* culture explants. As a consequence of these dynamic processes at the molecular level, variants or off-types are often identified among these clonally propagated progenies. The factors influencing *in vitro* regeneration and adaptation of plants vary, however, ranging from genotype, origin of explants, hormonal effects and culture conditions.

1.2.2 *In vitro* plant regeneration systems

Plant regeneration is one of the major prerequisites for the successful genetic transformation and micropropagation of any plant species. *In vitro* plant regeneration occurs through two major pathways: somatic embryogenesis (SE) or *de novo* organogenesis. Both pathways depend on phytohormone perception, cell division and dedifferentiation to obtain organ genetic competence, organ initiation and further development into differentiated tissues. Somatic embryogenesis and organogenesis can be induced either directly from tissues or indirectly from a callus. However, in most cases, SE is induced via an embryogenic callus, which then differentiates into embryos or embryo-like structures germinated into the embryo. A scheme for plant regeneration is illustrated in Figure 2.

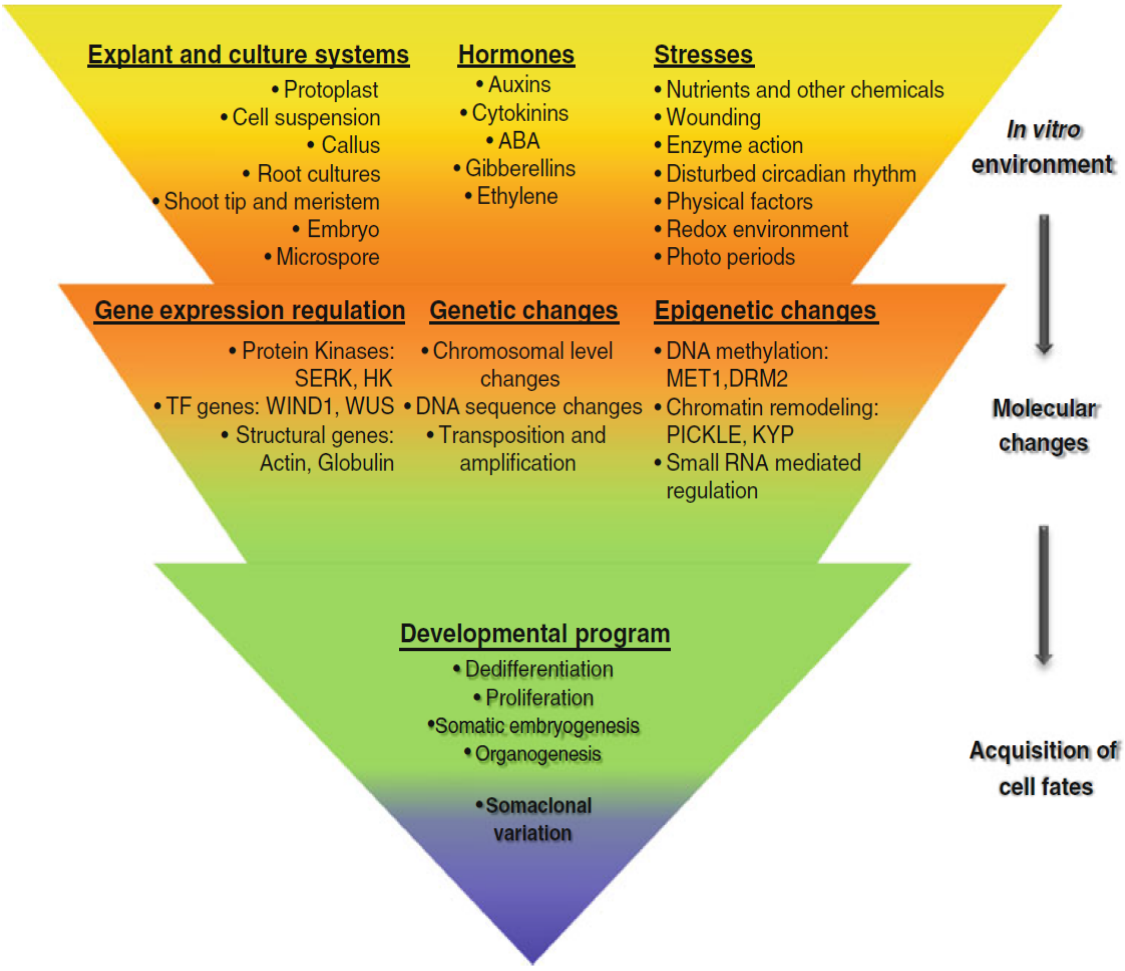

Fig 1. Plant *in vitro* culture and molecular changes caused in the process (Neelakandan and Wang 2012).

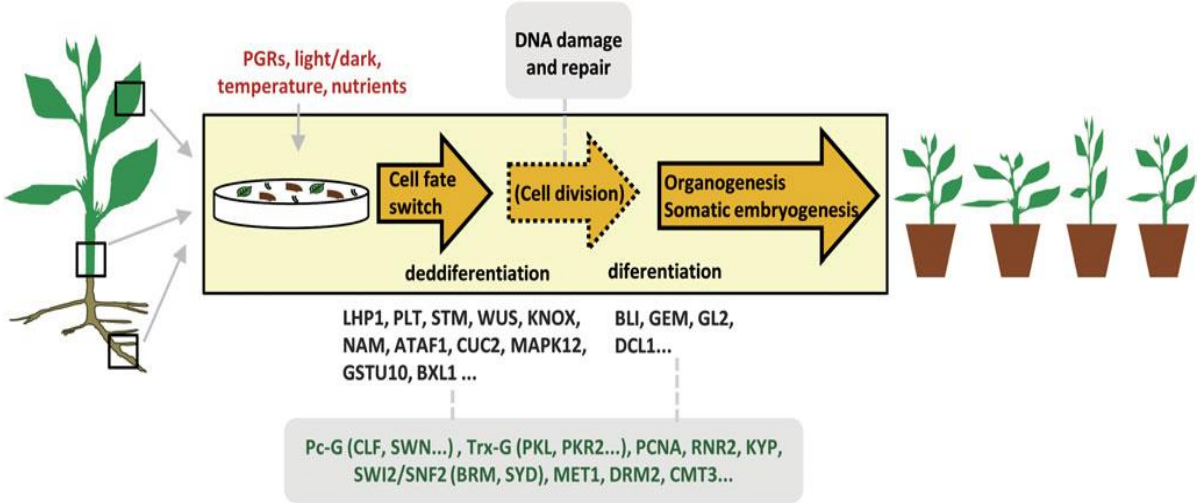

Fig 2. *In vitro* plant regeneration (Miguel and Marum 2011). Chromatin modifiers are in green and interacting genes or putative targets with a potential role during cell fate switch/cell division, and

differentiation of plant cells cultured in vitro are in black. Abbreviations— *Arabidopsis thaliana* activating factor 1: ATAF1, BRAHMA: BRM, BLISTER: BLI, BETAXYLOSIDASE1: BXL1, Chromomethyltransferase 3: CMT3, CURLY LEAF: CLF, CUP SHAPE COTYLEDON: CUC, DICER-LIKE 1: DCL1, DOMAINS REARRANGED METHYLTRANSFERASE 2: DRM2, PGRs: Plant growth regulators, GL2 EXPRESSION MODULATOR: GEM, GLABRA 2: GL2, GLUTATHIONE S-TRANSFERASE TAU 10: GSTU10, Knotted1-like homeobox: KNOX, Kryptonite: KYP, LIKE HETEROCHROMATIN PROTEIN 1: LHP1, MITOGEN-ACTIVATED PROTEIN KINASE 12: MAPK12, NO APICAL PROTEIN: NAM, PICKLE: PKL, PICKLE RELATED 2: PKR2, Polycomb-group: Pc-G, Proliferating cell nuclear antigen: PCNA, Ribonucleotide-diphosphate reductase 2: PLETHORA: PLT, Ribonucleotide-diphosphate reductase 2: RNR2, SWINGER: SWN: SPLAYED, SHOOT MERISTEMLESS: STM, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE: SPL, Sucrose nonfermenting 2:SNF2, SPLAYED: SYD, Trithorax group: Trx-G, WUS: WUSCHEL.

1.2.2.1 Callus formation

Callus induction is usually the initial step for *in vitro* plant regeneration. In nature, callus formation is important for sealing wounds, avoiding water loss and providing a cellular source for vasculature differentiation (Ikeuchi et al. 2016). Plant hormones, such as auxins and cytokinins (CK), are known to induce calluses in tissue culture. Incubation of various plant explants on a auxin-rich callus-inducing medium (CIM) could facilitate the callus formation (Pulianmackal et al. 2014).

Callus formation mechanisms have been previously studied, revealing how plant cells transduce wound signals to activate cell proliferation and callus induction (Ikeuchi et al. 2013). Callus formation requires *PASTICCINO* (*PAS*) genes for coordinating cell division and differentiating plant cells during development (Harrar 2003). Callus formation is usually achieved via reactivation of core cell cycle regulators, such as CYCLIN (CYC) and CYCLIN-DEPENDENT KINASES (CDK), and requires cell cycle re-entry of quiescent cells (Inzé and Veylder 2006). The AP2/ERF transcription factor wound-induced dedifferentiation (WIND1) is a key molecular factor involved in the control of cell differentiation in, for example, *Arabidopsis* (Iwase et al. 2011). The homologs of this gene, *WIND2*, *WIND3* and *WIND4,* are induced during wounding and promote callus formation (Iwase et al. 2011b). The LATERAL ORGAN BOUNDARIES DOMAIN (LBD)/ASYMMETRIC LEAVES2-LIKE (ASL) transcription factors are involved in controlling the callus formation programme in multiple organs of *Arabidopsis* (Fan et al. 2012). The genes *ETHYLENE RESPONSE FACTOR 115* and *PLETHORA3 (PLT3*), *PLT5* and *PLT7* are other recently identified factors involved in callus generation (Ikeuchi et al. 2017).

1.2.2.2 Organogenesis

Organogenesis is the formation of organs, either shoots or roots in a plant tissue culture. The formation of organs depends on the regenerative potential of the tissue as well as the balance of auxins and CK during culturing. There are two types of organogenesis *in vitro*: direct organogenesis

and indirect organogenesis (Bhatia and Bera 2015). The formation of shoots or roots without an intervening callus stage is called direct organogenesis, while indirect organogenesis is the formation of shoots or roots through a callus stage. Interactions of CK and auxins during plant organogenesis have been known for a long time. Cytokinins modulate auxin-induced organogenesis through the regulation of efflux-dependent intercellular auxin distribution (Pernisová et al. 2009). Auxins are transported by influx and efflux carriers within the polar system, and PINFORMED-dependent local auxin gradients are important for organ initiation (Bohn-Courseau 2010). Therefore, auxin is a major regulator of plant organogenesis for the shoot and root.

In recent years, research advances have provided molecular tools and resources to study molecular and genetic aspects of *in vitro* organogenesis in plants. For shoot organogenesis, quantitative trait loci (QTLs) analyses could identify a leucine-rich repeat receptor-like kinase, *RECEPTOR-LIKE PROTEIN KINASE1* (*RPK1*), which affects shoot organogenesis in *Arabidopsis* accessions (Motte et al. 2014). Dual expression of *PLT3*, *PLT5*, *PLT7* and *CUP-SHAPED COTYLEDON1* (*CUC1*) and *CUC2* take part in shoot meristem initiation during zygotic embryogenesis (Kareem et al. 2015). The *CLAVATA3* (*CLV3*) and *WUSCHEL* (*WUS*) proteins are involved in the signalling pathway as central regulators that coordinate cell proliferation and differentiation into shoot meristems (Chatfield et al. 2013; Somssich et al. 2016; Tian et al. 2018). Other regulators, such as *SHOOT MERISTEMLESS* (*STM*) and *PIN-FORMED1* (*PIN1*), further describe the radiating patterning of newly developing meristems and primordia initiation (Gordon et al. 2007). Other *AP2/ERF* transcription factors, such as *ENHANCER OF SHOOT REGENERATION1/DORNRÖSCHEN* (*ESR1/DRN*) and *ESR2/DRN-LIKE* (*DRNL*) are also induced on shoot inducing medium and enhance *CUC1* expression to stimulate shoot regeneration (Banno et al. 2001; Ikeda et al. 2006; Matsuo et al. 2009).

For root organogenesis, some plant species naturally generate roots from cuttings, and several plant hormones, such as auxins and CK, control this process (Bellini et al. 2014; da Costa et al. 2013). Accumulation of auxin at cut sites on the leaves of *Arabidopsis* induces the expression of two homeobox transcription factors, WUSCHEL RELATED HOMEOBOX11 (WOX11) and WOX12 (Liu et al. 2014b). The expression of *LATERAL ORGAN BOUNDARIES DOMAIN16* (*LBD16*), *LBD29* and *WOX5* are involved in lateral root development (Ditengou et al. 2008; Goh et al. 2012). In addition, some genes are members of the *AUXIN RESPONSE FACTOR* (*ARF*) family and directly activate *WOX11* expression in leaves and promote root formation (Liu et al. 2014a).

1.2.2.3 Somatic embryogenesis

Somatic embryogenesis is a developmental process unique to plants that includes a number of specific events: dedifferentiation of somatic cells, activation of cell division and reprogramming of their physiology, metabolism and gene expression patterns. In plant tissue culture systems, most of the SE induction processes depend on the type and concentration of plant growth regulators used. *In vitro* SE can be induced through two pathways: the direct and the indirect pathways. If the somatic embryo is

formed at the edge of an explant without an intermediary callus stage, then this can be considered as direct embryogenesis. In contrast, embryos induced from a callus are considered to be a case of indirect embryogenesis (Quiroz-Figueroa et al. 2002; Varis et al. 2018).

The mechanism for the induction of SE requires changing the of genetic programmes of cells that lead to the regulation of many genes (Riechmann et al. 2000). These changes involve the substantial participation of transcription factors (TFs). Some TFs and other factores were discovered during the induction of SE in different species, such as *ABA INSENSITIVE 3* (*ABI3*) (Shiota et al. 1998), *AGAMOUS LIKE* (*AGL*) (Thakare et al. 2008), *BABY BOOM* (Florez et al. 2015), *LEAFY COTYLEDON (LEC*) (Iwase et al. 2015), *RWP-RK DOMAIN-CONTAINING 4 GROUNDED ((RKD4/ GRD)* (Waki et al. 2011), *VIVIPAROUS1 (VP1)* (Footitt et al. 2003) and *WUSCHEL* (Arroyo-Herrera et al. 2008), and the genes *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1 (SERK1*) (Hecht et al. 2001; Pérez-Pascual et al. 2018). However, the expression of some TFs is specific to individual species so that an understanding of SE must involve species-specific analyses of the underlying factors.

SE signalling is a complex process that requires several molecular mechanisms including two major factors: 14-3-3 proteins and epigenetic processes. The 14-3-3 adaptor proteins are involved in the signal transduction pathway and participate in SE induction in *Carica papaya* (Vale et al. 2014). Epigenetic changes in tissue culture, such as chromatin remodelling, DNA methylation and small interference RNA (siRNA) regulation, also participate in the induction and development of somatic embryos. The changes in chromatin patterns are associated with the control of several genes involved in SE, such as *WUS, BABY BOOM 1 (BBM1*) *and LEC* (De-la-Peña et al. 2015; Yakovlev et al. 2016). DNA methylation is required in the SE induction of some plants, such as Siberian ginseng (Chakrabarty et al. 2003), pumpkin (Leljak-Levanić et al. 2004; Viejo et al. 2010) and European chestnut (Viejo et al. 2010). Small interfering RNAs (siRNAs) play important roles in regulating gene expression in plant development and respond to biotic and abiotic stresses (Kasai et al. 2013), and they are intensively regulated during the induction of SE in *Arabidopsis* (Szyrajew et al. 2017).

1.2.3 *In vitro* shoot proliferation

In vitro shoot proliferation via axillary shoots is one method for the rapid propagation of many plant species. Axillary bud outgrowth is controlled by apical dominance, as the main stem shoot apex influences axillary buds' growth. Mineral salts and carbohydrates are also essential elements required for healthy and vigorous growth of plants and shoot proliferation (George et al. 2007; Thorpe et al. 2008). PGRs play a significant role in affecting shoot multiplication in tissue culture (Gaspar et al. 1996). Three classes of plant hormones-auxins, endogenous PGR such as CKs and exogenous PGR such as strigolactones (or strigolactone derivatives)—regulate bud activation and thereby regulate shoot branching (Evers et al. 2011). CKs can promote shoot branching by

activating axillary buds (Müller and Leyser 2011). Auxin controls the level of a root-to-shoot moving signal that moves in axillary buds and regulates their outgrowth (Sachs and Thimann 1967). Strigolactones, a group of sesquiterpene lactones derived from carotenoids, promote shoot branching and only inhibit shoot branching in the presence of a competing auxin source (Crawford et al. 2010). Gibberellic acid 3 (GA3) is known for its effect on internode elongation and seed germination, but its role in shoot branching was found in *Arabidopsis* (Silverstone et al. 1997) and pea (Murfet and Reid 1993).

In recent years, physiological and molecular studies dealing with underlying genes controlling shoot proliferation were carried out. Genetic analysis was performed and discovered several of the factors involved in shoot proliferation in some plants. CK biosynthetic genes *ISOPENTENYLTRANSFERASE1* and *ISOPENTENYLTRANSFERASE2* (*PsIPT1* and *PsIPT2*) are expressed in the nodal regions of stems regulating shoot formation. The gene *SUPERSHOOT* controls axillary bud initiation, which is characterised by a massive over-proliferation of shoots in *Arabidopsis* (Tantikanjana et al. 2001). Other factors, such as TEOSINTE BRANCHED1, CYCLOIDEA, PCF transcription factor TB1/BRC1 and the polar auxin transport, move through the stem as potential integrators of those signals controlling branching (Domagalska and Leyser 2011; Rameau et al. 2014). Overexpressing gibberellic acid (GA) catabolism genes increases the branching of some phenotypes of several plant species (Agharkar et al. 2007). The *SHORT INTERNODES*-like gene (*SHI*) is one of a 10-member *SHIRELATED SEQUENCE* (*SRS*) gene family and regulates shoot growth and xylem proliferation in *Populus* (Zawaski et al. 2011). The *PHOTOPERIOD RESPONSE1 (PHOR1*)-like genes enhance shoot and root growth, as well as starch accumulation in *Populus* (Zawaski et al. 2012). Although some genetic factors were discovered, the molecular mechanisms and the integration of environmental and endogenous signals for shoot proliferation are quite complex and not fully understood.

1.2.4 Adventitious root formation

Root systems play a fundamental role in the growth and development of plants in uptake of and absorbing water and minerals, anchoring plants and synthesising hormones to regulate plant growth and development. Adventitious root (AR) formation is an essential step for the vegetative propagation of plants in horticulture, agriculture and forestry (Klerk et al. 1999). The formation of ARs is regulated by both environmental and endogenous factors, and among growth regulators, auxin plays an prominent role in regulating root development (Li et al. 2006; Pop et al. 2011). Other phytohormones, such as ethylene, can also promote or accelerate rooting (Santisree et al. 2012), whereas gibberellins inhibit AR induction but stimulate subsequent root elongation (Niu et al. 2013). Adventitious root development is a complex process affected by multiple factors, including phytohormones, light, nutritional status, genetic characteristics and associated stress responses, such as wounding (Geiss et al. 2018).

In recent decades, many factors influencing AR formation have been exploited. Molecular studies on root formation recently showed many transcription factors to be involved in the formation and development of ARs, such as AP2/ERF (Trupiano et al. 2013), INTEGUMENTA-like (AtAIL) (Rigal et al. 2012) and WUSCHEL-related homeobox (WOX) (Liu et al. 2014a). The genes *SHORT-ROOT* (*SHR*) control the radial patterning of *Arabidopsis* roots (Helariutta et al. 2000) while *SCARECROW* (*SRC*) modulates the root formation of *Arabidopsis* (Cui et al. 2012). *Crown-root less1* (*CRL*) genes are essential for root formation in rice, targeting an AUXIN RESPONSE FACTOR (ARF) in auxin signalling (Inukai et al. 2005). Auxin movement is intervened on by influx proteins, such as AUXIN RESISTANT1 (AUX1) and Like AUX (LAX) (Noh et al. 2001), which assist auxin movement into cells. The *ATP-binding cassette B19* (*ABC B19*) auxin transporter induction contributes to excision-induced AR formation in *Arabidopsis* hypocotyls (Christie et al. 2011; Sukumar et al. 2013). The target of rapamycin (TOR) signalling plays a key role in AR formation in *Arabidopsis* and potatoes (Deng et al. 2017). However, despite the increasing number of physiological and molecular studies on ARs, the molecular mechanisms and integration of environmental and endogenous factors are difficult to study and are, therefore, not yet fully understood and may be species-specific.

1.2.5 *In vitro* propagation of roses

In vitro rose propagation is an important tool for rapid multiplication of cultivars and the development of new varieties with desirable traits and maintaining disease-free genetic stocks. During the last few years, different methods have been used for the *in vitro* propagation of roses. No single method or explant type has been applied to all rose varieties (Pourhosseini et al. 2013). Many kinds of explants and cultivars of roses were used to establish effectively *in vitro* regeneration systems. The different regeneration and micropropagation pathways in roses were reviewed by Pati et al. (2006).

Direct regeneration of roses via shoot organogenesis of some cultivars has been described by some authors, including (Lloyd et al. 1988; Dubois and Vries 1996; Dubois et al. 2000 and Pati et al. 2004b). Shoot organogenesis forming through a callus phase was achieved by Ishioka and Tanimoto (1990) and Hsia and Korban (1996). Embryogenic callus formation in roses was induced on media with high concentrations of 2.4D (Hsia and Korban 1996) or NAA (Dohm et al. 2001a) or 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) (Estabrooks et al. 2007). Somatic embryogenesis and regeneration of some rose cultivars induced from callus were also described by (Wit et al. 1990, Khosh-Khui and Sink 1982a; Noriega and Söndahl 1991; Marchant et al. 1996; Kim et al. 2004 and Pour et al. 2015).

Shoot multiplication of roses has been applied in different cultivars using several kinds of media and plant growth regulators. The most common medium used for rose propagation is MS (Murashige and Skoog 1962). For *Rosa hybrida*, the replacement of FeEDTA by FeEDDHA in the medium led to better performance in shoot propagation (van der Salm et al. 1996). Cytokinins are a major PGR, whereas in

some cases, low concentrations of auxins or GA3 were also used for *in vitro* shoot proliferation and multiplication (Vijaya et al. 1991; Yan et al. 1996).

The *in vitro* rooting ability depends on the interaction of internal and external factors, such as cultivar, size and age of micro-shoots and media components. *In vitro* rooting response in roses was cultivar-dependent and influenced by the age and size of the micro-shoots (Khosh-Khui and Sink 1982b; Rout et al. 1991). Varying concentrations of inorganic salts and different auxins were used for *in vitro* root induction in previous reports. Half-strength MS medium, supplemented with NAA (0.54 µM), was suitable for inducing rooting in the cultivar of Bridal Veil (Khosh-Khui and Sink 1982b). Micro-shoots of roses also induced roots on media supplemented with low concentrations of auxins, such as IAA, IBA or NAA (Pierik 1997; Akhtar et al. 2015).

1.3 Genetic dissection of agronomic traits in plants

1.3.1 General genetic dissection of agronomic traits in plants

Most traits that are of interest in plant breeding are polygenic traits (qualitative traits) that do not follow patterns of Mendelian inheritance but rather display quantitative inheritance (Semagn et al. 2010). Quantitative traits are controlled by multiple genes, or quantitative trait loci (QTLs). The development of molecular markers is one of the most significant advances in the field of plant molecular biology and biotechnology via the detection and exploitation of DNA polymorphisms in plant systems. Two complementary approaches for QTL mapping, linkage mapping and association mapping (AM), are the most commonly used methods for the dissection of complex traits in many crop species. However, linkage mapping is limited by low degrees of polymorphism, small numbers of tested alleles or the availability of suitable crosses (Chen 2013).

Association mapping or linkage disequilibrium mapping (LD-mapping), has been widely used to dissect complex traits in plants based on the strength correlation between mapped genetic markers and traits (Abdurakhmonov and Abdukarimov 2008; Khan and Korban 2012). Association mapping can be used at four different genomic levels: the QTL level, candidate gene level, polymorphism level and whole genome level, and is illustrated in Figure 3. Association studies at the QTL level were used to confirm a previously identified QTL in a different germplasm or to search for a candidate gene within a QTL confidence interval (Zhao et al. 2007b). At the candidate gene level, AM was used to search for causal polymorphism within the validated candidate genes, but this technique requires prior knowledge about the candidate gene (Caporaso et al. 2009; Pasche and Yi 2010). Association studies at the candidate DNA polymorphism level are based on several potential causal polymorphisms within the candidate gene associated with the target trait used to test the transferability of the marker trait-association (Flores-Martínez et al. 2004). Whole genome AM, or genome-wide association studies (GWAS), is a forward or linear approach to identifying genetic factors across the whole genome contributing to the trait in question. GWAS uses many molecular markers, which cover the whole

genome, for a large number of individuals in order to identify functional common variants in LD for the target traits.

Many methodologies have been developed and widely used for AM, ranging from a simple students t-test to linear mixed models, which considers population structure as well as relatedness between individuals of an association panel (Chen 2013). Several approaches have been examined, such as Multiparent Advanced Generation Intercross (MAGIC) (Kover et al. 2009), Transmission Disequilibrium Test (TDT) (Mackay and Powell 2007) and other approaches that incorporate corrections for population structure, as in genomic control (GC) (Devlin et al. 2001; Wang et al. 2012) and structured association (SA) (Curtis et al. 2012; Zhao et al. 2007a). These were used to study marker trait associations in plants (Soto-Cerda and Cloutier 2012).

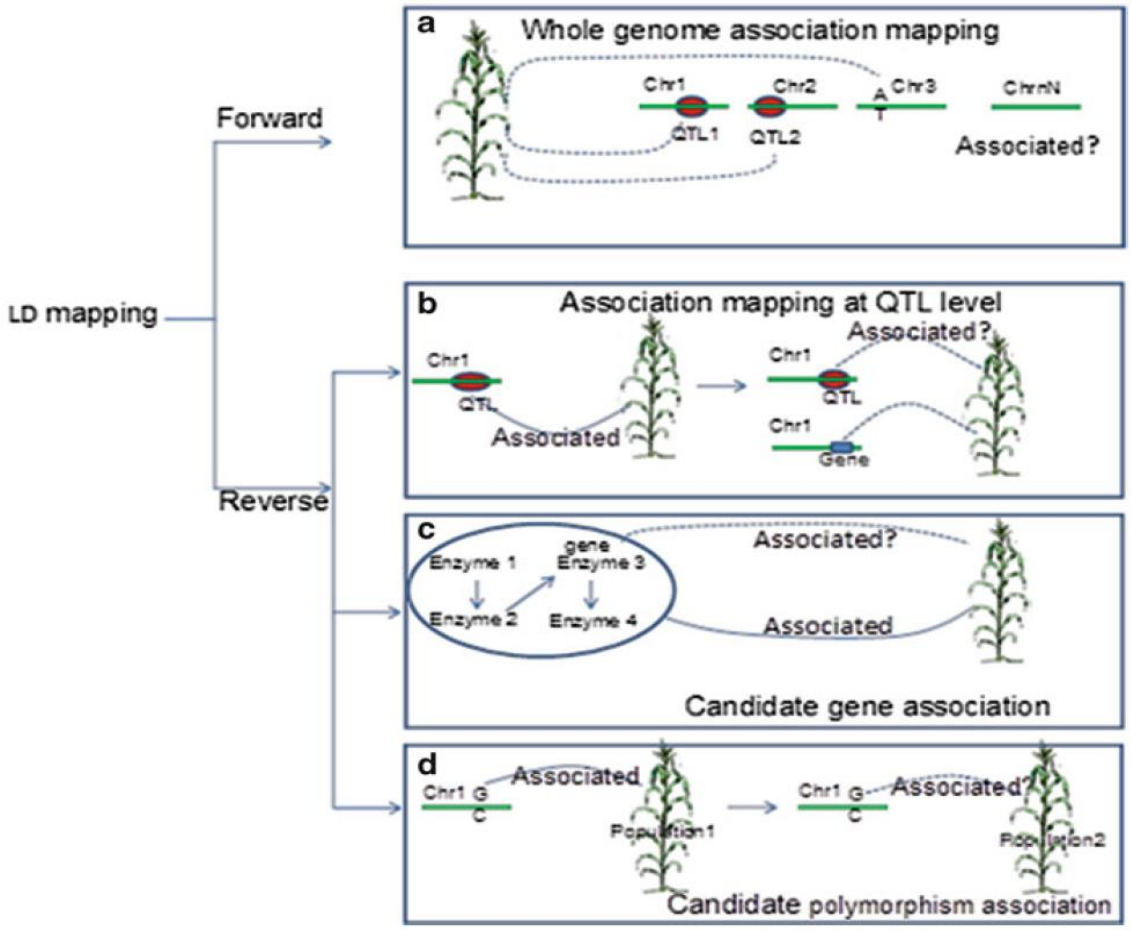

Fig. 3. Association mapping of a plant at four genomic levels (Chen 2013). (a) whole genome AM to identify genetic factors across the whole genome that contribute to the trait in question; (b) AM at QTL level, which can be employed to confirm a previously identified QTL in a different (larger) germplasm or to fine map a QTL; (c) candidate gene AM, which takes advantage of prior (inferred) functional information of candidate genes; (d) candidate polymorphism AM, which can be employed to develop

functional markers. The whole genome AM is a progressive genetic approach, while the other three are reverse genetic approaches.

One important aspect of AM is the phenotyping of the traits being studied. Some plant traits are recorded as categorical data, for example, disease phenotypes are often recorded by scales (e.g. scale 1–9) (Atwell et al. 2010). For genotyping, a set of markers that are unlinked, have a selectively neutral background and are scaled to accomplish genome-wide coverage will be used to broadly characterise the genetic composition of individuals. Due to lower mutation rate, higher genome density and better responsiveness to high-throughput detection systems (SNP chips or next-generation sequencing based methods), SNPs are becoming the marker of choice for complex trait dissection studies in plants.

Currently, there are many software packages available for the analysis of AM (Table 2) (Zhu et al. 2008). Trait Analysis by aSSociation, Evolution and Linkage (TASSEL) is the most common software used for AM in plants (Bradbury et al. 2007). TASSEL implements general linear models (GLM) and multiple regression models (mix linear models [MLM]) for controlling population and family structure. This programme requires a Q matrix from previous population structure analyses (Hubisz et al. 2009a) or a K matrix (Hardy and Vekemans 2002) and allows analyses of LD statistical and graphical display, population structure using Principle Component Analysis (PCA) and tree plots of genetic distances. The protocol for an AM analysis is illustrated in Figure 4.

Association mapping has been conducted in the model plant *Arabidopsis thaliana* (Filiault and Maloof 2012; Togninalli et al. 2018) and in many crops, such as rice (Huang et al. 2010), maiz (Xiao et al. 2017), wheat (Guo et al. 2017), soybean (Zatybekov et al. 2017), barley (Gawenda et al. 2015), sorghum (Morris et al. 2013), potato (Sharma et al. 2018), tomato (Mazzucato et al. 2008; Zhang et al. 2015), in forest trees, fruit crops (Cao et al. 2016; Khan and Korban 2012) and ornamental plants (Chong et al. 2016; Schulz et al. 2016).

Table 2. Common statistical software packages for association mapping (Zhu et al. 2008)

Software packet	Focus	Website	Comments
TASSEL	Association mapping	https://www.maizegenetics.net/tassel	Free, LD statistics, sequence analysis, association mapping (logistic regression, linear model and mixed model)
SAS	Generic	https://www.sas.com	Commercial, standard software widely used in data analysis and methodology work

R	Generic	http://www.r-project.org	Free, convenient for simulation work for research with good programming and statistics background
STRUCTURE	Population structure	http://pritch.bsd.uchicago.edu/structure.html	Free, widely used for population structure analysis
SPAGeDi	Relative kinship	http://www.ulb.ac.be/sciences/ecoevol/spagedi.html	Free, genetic relationship analysis
EINGENSTRAT	PCA, association	http://genepath.med.harvard.edu/~reich/Software.htm	Free, PCA was proposed as an alternative for population structure analysis
MTDFREML	Mixed model	http://aipl.arsusda.gov/curtvt/mtdfreml.html	Free, mixed model analysis for animal breeding data, also can be used for plant data
ASREML	Mixed model	http://www.vsni.co.uk/products/asreml	Commercial, mixed model analysis for animal breeding data, also can be used for plant data

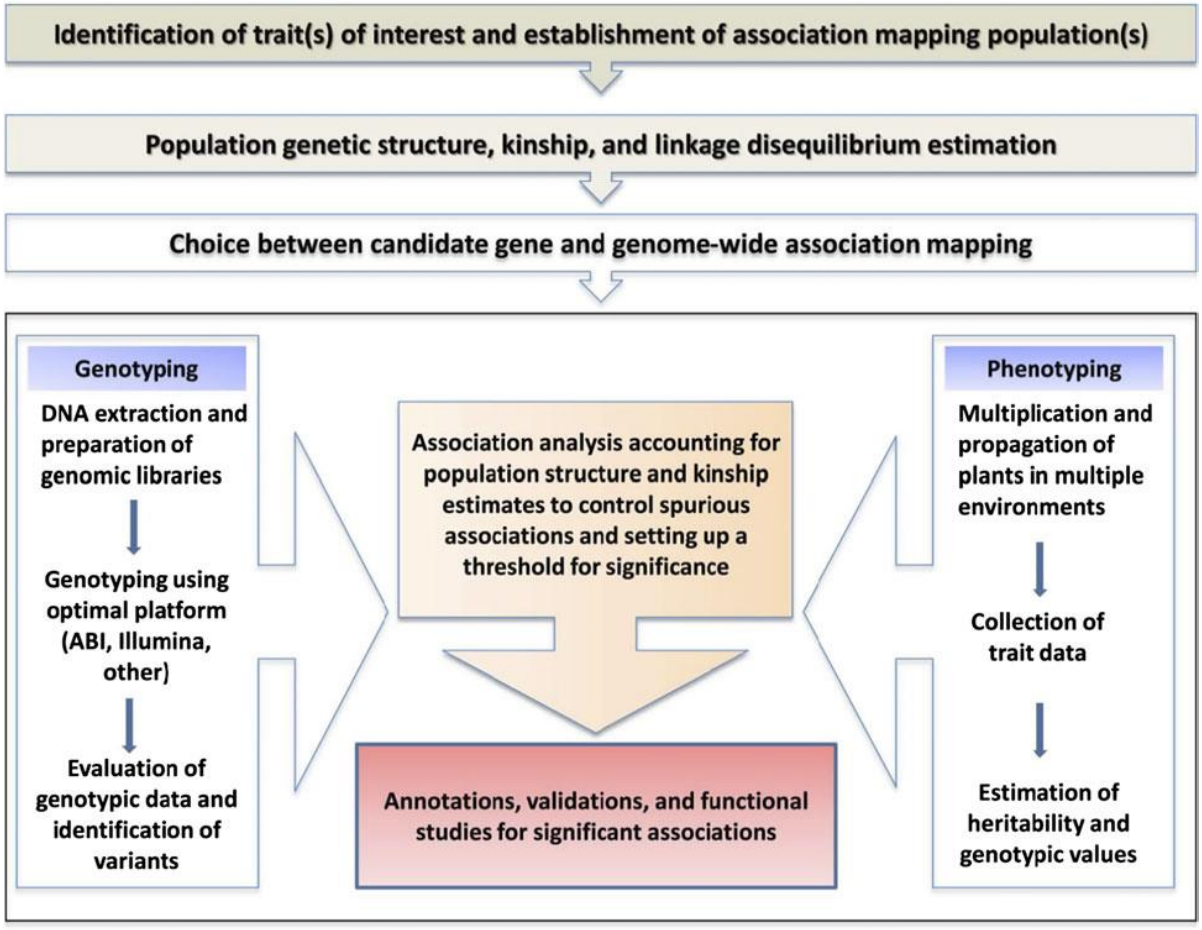

Fig. 4. A schematic representation of a protocol to conduct an AM study (Khan and Korban 2012).

1.3.2 Genetic dissection of key traits in roses

In recent years, several studies have performed genetic analysis and mapping in order to analyse segregating populations of roses. For genetic maps, an initial linkage map was constructed by RADPs and AFLP markers in a map for roses (Debener and Mattiesch 1999) . Construction of an integrated map of roses using AFLP, SSR, protein kinase (PK), resistance gene analogues (RGA), RFLP, sequence-characterised amplified region (SCAR) and morphological markers was done by Yan et al. (2005). Construction of a first integrated consensus map (ICM) based on the information of diploid populations was carried out by Spiller et al. (2011). The combination of the Tyramide-FISH technology and the HRM molecular marker system to anchor *Rosa* linkage groups to physical chromosomes may result in an effective integration of physical and genetic maps (Kirov et al. 2014). An ultra-high density linkage map of all homologous chromosomes of the tetraploid cut rose population was constructed based on the development of the 68 K WagRhSNP array (Vukosavljev et al. 2016). The first rose genome sequence from the wild, heterozygous *Rosa multiflora* was then released by Nakamura et al. (2018). A high-quality reference genome sequence of *Rosa chinensis*, or ‘Old Blush,’ was generated

to study the genome structure and genetic basis of major ornamental traits (Hibrand Saint-Oyant et al. 2018).

For decades, molecular genetic approaches have been developed to interpret ornamental traits and identify regions of important genes controlling these traits (Debener and Linde 2009b). Genetic factors for the flower traits of roses were found, such as flower colour (Gitonga et al. 2016; Henz et al. 2015), flowering date and number of petals (Roman et al. 2015), flowering traits (Hibrand-Saint Oyant et al. 2007), flowering time (Dong et al. 2017) and flower development (Dubois et al. 2011), as well as the amount of anthocyanin and carotenoid in petals (Schulz et al. 2016). Genetic analysis for vigour in roses was performed by (Yan et al. 2007), as was scent metabolic (Spiller et al. 2010). Genetic dissection was performed in plant architecture, flowering behaviour (Kawamura et al. 2015) and rose bush architecture (Li-Marchetti et al. 2017). The analysis of disease resistance genes against black spot (Tefere-Ayana et al. 2012; Terefe-Ayana et al. 2011; Whitaker et al. 2010; Zurn et al. 2018) and powdery mildew (Hosseini Moghaddam et al. 2007; Kaufmann et al. 2012; Linde et al. 2006; Linde and Debener 2003) revealed single loci as well as QTLs for these traits.

2. Thesis objectives

The main goal of this thesis is the analysis of genetic factors influencing the regeneration and propagation efficiency of *Rosa hybrida* cultivars. To perform this analysis, the thesis focuses on the following objectives:

- ❖ Genetic dissection of traits related to *in vitro* regeneration and propagation traits in roses by employing genome-wide AM in 96 rose genotypes. In particular, the following traits were analysed:
  - Direct shoot regeneration capacity from petioles
  - Callus induction
  - Shoot proliferation
  - Adventitious root formation
- ❖ Development of markers for regeneration traits
- ❖ Analyses of correlations between these traits and potential overlap in the genetic pathways with influence on these trait

3. Manuscripts and publications

3.1 Genetic dissection of adventitious shoot regeneration in roses by employing genome-wide association mapping

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Type of authorship: First author

Type of article: Research article

Contribution to the article: Planned and performed the experiments, completed the statistical analysis and wrote most of the manuscript.

Contribution of other authors: Dietmar Schulz conducted part of the data analysis.
Traud Winkelmann contributed to the experimental setup and wrote part of the manuscript.
Thomas Debener was involved in planning the experiments and wrote parts of the manuscript.

Journal: *Plant Cell Reports*

Date of publication: September 2017

Impact factor: 2.989

DOI: 10.1007/s00299-017-2170-8

Plant Cell Rep (2017) 36:1493–1505
DOI 10.1007/s00299-017-2170-8

ORIGINAL ARTICLE

Genetic dissection of adventitious shoot regeneration in roses by employing genome-wide association studies

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Received: 25 April 2017 / Accepted: 20 June 2017 / Published online: 24 June 2017

Abstract

Key Message **We analysed the capacity to regenerate adventitious shoots in 96 rose genotypes and found 88 SNP markers associated with QTLs, some of which are derived from candidate genes for shoot regeneration.**

Abstract In an association panel of 96 rose genotypes previously analysed for petal colour, we conducted a genome-wide association study on the capacity of leaf petioles for direct shoot regeneration. Shoot regeneration rate and shoot ratio (number of shoots/total number of explants) were used as phenotypic descriptors for regeneration capacity. Two independent experiments were carried out with six replicates of ten explants each. We found significant variation between the genotypes ranging from 0.88 to 88.33% for the regeneration rate and from 0.008 to 1.2 for the shoot ratio, which exceeded the rates reported so far. Furthermore, we found 88 SNP markers associated with either the shoot regeneration rate or the shoot ratio. In this association analysis, we found 12 SNP markers from ESTs (expressed sequence tags) matching known candidate genes that are involved in shoot morphogenesis. The best markers explained more than 51% of the variance in the shoot regeneration rate and more than 0.65 of the variance in the shoot regeneration ratio between the homozygote marker classes. The genes underlying some of the best markers such as a GT-transcription factor or an LRR receptor-like protein kinase are novel candidate genes putatively involved in the observed phenotypic differences. The associated markers were mapped to the closely related genome of *Fragaria vesca* and revealed many distinct clusters, which also comprised the known candidate genes that functioned in the organogenesis of plant shoots. However, the validation of candidate genes and their functional relationship to shoot regeneration require further analysis in independent rose populations and functional analyses.

Keywords Shoot regeneration · Genome-wide association study · SNP markers · Rose cultivars

Communicated by Dr. Xian Sheng Zhang.

Electronic supplementary material The online version of this article (doi:10.1007/s00299-017-2170-8) contains supplementary material, which is available to authorized users.

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Introduction

The regeneration of adventitious shoots is not only an essential step in the clonal propagation and plant genetic engineering, but it is also a useful tool in the research on the totipotency of plant cells. Typically, in vitro plant regeneration can be obtained through somatic embryogenesis or de novo shoot organogenesis under appropriate culture conditions in a hormone-dependent manner (Motte et al. 2014b). Although in vitro regeneration systems have been established for many plant species, the shoot regeneration ability of the economically important plants is highly variable, unpredictable and influenced by factors such as the origin of explant, culture conditions, hormonal

effects and a strong species and genotype specificity (Neelakandan and Wang 2012). Because plant regeneration is a prerequisite for most *Agrobacterium tumefaciens*-mediated transformation systems, the transformation protocols for many agriculturally important species are mainly hampered by the low rates of shoot regeneration.

Over the last years, a number of factors involved in shoot regeneration have been exploited by genetic, biochemical and molecular methods (Motte el al. 2014b). Shoot regeneration in plants is controlled by complex regulatory mechanisms of hormone signalling, transcription factors, protein kinases and epigenetic factors involved in different types of regeneration (Xu and Huang 2014). Examples of these genes are receptor protein kinases (RPK) and CLAVATA (CLV), which are transmembrane protein kinases involved in cellular signal transduction and are essential for the developmental processes of plant cells (Motte et al. 2014a; Ishida et al. 2014). Somatic embryogenesis receptor kinases (SERK) belong to a group of leucine-rich repeat receptor-like kinases (LRR-RLK), which play a major role in embryogenesis (Schmidt et al. 1997; Hecht et al. 2001; Talapatra et al. 2014). Transcription factors of the Apetala2/Ethylene Response Factor (*AP2/ERF*) family and WOUND INDUCED DEDIFFERENTIATION 1 (*WIND1*) are the central regulators of wound-induced cellular reprogramming in plants involved in cell dedifferentiation, callus formation and regulation of the expression of the ENHANCER OF SHOOT REGENERATION1 (*ESR1*) gene (Ikeda and Ohme-Takagi 2014; Iwase et al. 2015, 2016). The other members of this gene family are *BABYBOOM* (Florez et al. 2015), *EMBRYOMAKER*, LEAFY COTYLEDON (*LEC*), CUP SHAPE COTYLEDON (*CUC*) and *WUSCHEL*, which have been reported to enhance plant regeneration efficiency (Gliwicka et al. 2013; Florez et al. 2015; Rupps et al. 2016). Despite recent advances in understanding the molecular basis of shoot regeneration, many aspects of the process and the causes of regeneration recalcitrance are not well known and might be species specific.

The rose is one of the most important ornamental plants, and the production area of cut roses is expanding remarkably worldwide (Gitonga et al. 2014). Commercial rose cultivars are complex tetraploid hybrids with a genome that comprises genomic components of at least seven different species (Fougere-Danezan et al. 2015). Rose cultivars are generally propagated by vegetative methods such as cuttings, layering, budding and grafting. Nevertheless, such techniques are time-consuming, dependent on season and do not ensure healthy and disease-free plants. In vitro regeneration of rose shoots has been applied not only for the rapid multiplication of cultivars, but also in genetic engineering and the production of valuable metabolites (Debener and Oyant 2009). The suitability of rose cultivars for shoot multiplication was addressed by many studies (Farahani 2012; Xing et al. 2010; Pati et al. 2005; van der Salm et al. 1994; Ibrahim and Debergh 2001). Moreover, several plant regeneration protocols for various rose genotypes were studied for application in plant transformation and the production of metabolites in roses (Li et al. 2002; Vergne et al. 2010; Bao et al. 2012; Jang et al. 2016). Although several regeneration protocols were established for individual cultivars, no cultivar-independent method is available yet, and most rose cultivars have to be considered as recalcitrant regeneration plants. Shoot regeneration in rose cultivars is likely a complex trait, and the knowledge of the molecular basis influencing the shoot regeneration is still limited. For establishing efficient regeneration protocols for rose cultivars, it is important to understand the molecular basis of the variation between the different genotypes and identify the genes regulating shoot regeneration.

Quantitative trait locus (QTL) mapping has been increasingly used for the genetic dissection of complex horticultural traits in roses such as plant architecture, flowering behaviour (Kawamura et al. 2015), and flowering date and the number of petals (Roman et al. 2015). However, most of these studies were conducted in biparental populations exploring only a small portion of the available genetic variation in roses (Debener and Linde 2009; Henz et al. 2016; Hibrand-Saint Oyant et al. 2007; Kawamura et al. 2011, 2015). Currently, genome-wide association studies (GWASs) are promising methods for the genetic exploration of complex traits in plants based on populations of independent individuals (George and Cavanagh 2015). GWASs have been used in several crops, for example wheat (Liu et al. 2014), barley (Long et al. 2013), rice (Huang et al. 2010), pea (Kwon et al. 2012), peach (Cao et al. 2012), lettuce (Kwon et al. 2013), soybean (Haerizadeh et al. 2009; Kadam et al. 2016), and tomato (Shirasawa et al. 2013; Ruggieri et al. 2014). Association analysis has recently been used to identify loci associated with anthocyanin and carotenoid content in rose petals (Schulz et al. 2016). To locate genomic loci influencing shoot regeneration, we genotyped the same set of rose genotypes from the panel used by Schulz et al. 2016 and used the marker information from an Axiom SNP array for roses (Koning-Boucoiran et al. 2015).

The aim of the present study was to analyse the phenotypic variability for shoot regeneration in 96 rose cultivars and to identify the SNP (single- nucleotide polymorphism) markers and therefore the genomic regions that are significantly associated with the phenotypes, including SNPs from genes coding for orthologues of known factors of shoot regeneration. Moreover, another aim was to identify candidate genes that could facilitate future studies on the functional genomics of shoot regeneration in roses.

Materials and methods

Plant material

A total of 96 rose cultivars of different origins were used as described in Schulz et al. (2016) (Table S1). The panel included selected commercial cultivars based on the available information about pedigrees to minimize relatedness. Most of these rose cultivars are tetraploid, only one cultivar is diploid, and eight cultivars are triploid. Plants were cultivated in the greenhouse as potted plants (9 L containers in Einheitserde CL P, Einheitserdewerke Patzer, Sinntal-Altengronau, Germany) in three randomized blocks under semi-controlled conditions (heating set point 5 °C in the winter and 15 °C in summer with no additional light applied). The plants were fertilized every week with a liquid fertilizer (Peters Exel Growers N/P/K 14/6/14) during the growing season. The shoots used for experiments were collected from April to September in 2014 and 2015.

Adventitious shoot regeneration

Adventitious shoot regeneration experiments were carried out according to the protocol of Dubois et al. (2000). Shoot tips (5–7 cm long) with partly unfolded leaflets (Fig. 1) from the three clonal plants of each cultivar were collected in the greenhouse. The shoot tips were sterilized in 1% sodium hypochlorite for 5 min and then rinsed three times in sterilized deionized water (5 min each). The petioles together with the lower section of the leaflets were used as explants. Ten explants were placed onto an induction medium (IM, Table 1) in 9 cm Petri dishes. The explants were incubated in the dark for 8 days at 23 ± 2 °C and then transferred to the shooting medium (SM, Table 1). After transfer to the shooting medium (SM), the Petri dishes were placed under cool white fluorescent light at a photosynthetic photon flux density (PPFD) of 40 µmol m^{-2} s^{-1}, at 23 ± 2 °C and a 16 h photoperiod. For each genotype, the experiment was repeated twice with six replicates (Petri dishes) each. Phenotypic data were recorded as regeneration rate (i.e. the percentage of explants producing at least one shoot), and the number of shoots per regenerated explant were estimated as the number of regenerated shoots/the number of regenerated explants. However, to assess the regeneration capacity of rose genotypes, the regeneration rate (percentage of regenerating explants) and the shoot ratio (number of shoots per explant) were determined according to Saha et al. (2007) and Rostami et al. (2013). The data were recorded after 28 days of culture in the light.

Statistical analysis

All statistical analyses were conducted with the R software package version 3.2.5 (The R-Foundation for Statistical Computing 2016). The differences between cultivars and replications regarding the regeneration rate and adventitious shoot ratio were analysed with a generalized linear

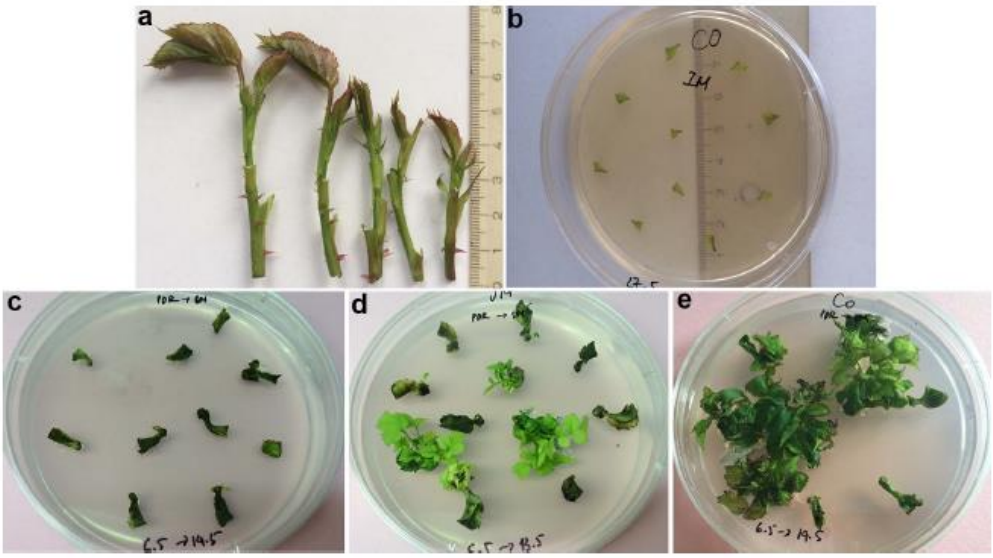

Fig. 1 Adventitious shoot regeneration in rose cultivars (*bar* 1 cm). **a** Shoots used for surface disinfection and explant preparation. **b** Petiole explants on IM medium. **c–e** Regeneration capacity of selected genotypes (**c** Sterntaler, **d** Jasmina, **e** Compassion) 28 days after transfer to the shooting medium SM

Table 1 Composition of IM and SM medium

Media	Salts and vitamins	Plant growth regulators	Carbon source	$AgNO_3$	Agar
IM	Haft-strength MS (Murashige and Skoog 1962) salts and full-strength MS vitamins	6.9 µM TDZ 0.49 µM IBA	30 g/l glucose	60 µM	8.0 g/l Plant Agar (Duchefa)
SM	Full-strength MS salts and vitamins	2.2 µM BAP 0.05 µM IBA 0.3 µM GA_3	30 g/l glucose	–	8.0 g/l Plant Agar (Duchefa)

The medium was adjusted to pH 5.8 and autoclaved at 121 °C for 20 min. The plant growth regulators TDZ, GA_3 and $AgNO_3$ were added after autoclaving

model. The normal distribution was tested with the "quasi binomial model". The correlation coefficient between the regeneration rate and the shoot ratio was calculated using Spearman's rank correlation coefficient.

SNP analysis

A total of 63,000 SNPs were analysed with the Axiom WagRhSNP array for cut and garden roses (Koning-Boucoiran et al. 2015). SNP genotypes were reduced to a diploid configuration for analysis with the TASSEL software package. This was done as described by Schulz et al. (2016), where all heterozygous loci were encoded as AB, and homozygous loci were encoded as either AA or BB.

Association mapping study

The analysis of associations between SNP loci and phenotypes was conducted with TASSEL version 3.0 (Bradbury et al. 2007) using the mixed linear model (MLM, Q+K model) as described in Schulz et al. (2016), with the minor allele frequency (MAF) at 0.05. The Q matrix was computed with STRUCTURE 2.3. (Pritchard et al. 2000) based on a subset of markers and the settings described in Schulz et al. (2016); the matrix output was used as covariates. The K matrix of pairwise kinship coefficients was calculated from the SNP data by the SPAGeDi software (Hardy and Vekemans 2002).

The significance of the association between traits and markers was defined using an adjusted P value (Bonferroni correction), and the threshold for the association was set to $-\log10\ P$ value > 6.1, accordingly. The estimated effects for each genotypic class were obtained directly from the mixed linear model. The effect of the genotypic class with the lowest frequency is set to zero; then, the effects of the other genotypes are given as deviations between their estimated values and the lowest frequency class.

Location of rose sequences in the *Fragaria vesca* genome

Strawberry orthologues of the sequences, which harboured significantly associated rose SNPs, were analysed with BLAST searches against the *F. vesca* genome v2.0a1 scaffold sequences and aligned using the sequence alignment editor Bioedit version 7.2.5 (Hall 1999). Only similar hits with e values lower than $1e^{-05}$ were considered. Gene and site annotations for the strongest match (lowest e value) for each sequence were recorded and plotted against the significance values from the TASSEL analysis.

Results

Direct shoot regeneration capacity

A panel of 96 rose genotypes was evaluated for variation in the direct shoot regeneration capacity. The shoots with folded leaflets were collected from the three clonal plants per genotype, and the time that explants of all 96 rose genotypes were sampled lasted 5 months due to the differences in the development of the genotypes.

Adventitious shoots were formed at the proximal end of the leaflet petioles within 28 days of culture on SM media under light. No additional adventitious shoots formed after this period. Adventitious shoot regeneration occurred to some extent in all genotypes, although with a high variation between the genotypes of the frequency of explants showing shoot regeneration and the number of shoots formed per explant (Fig. 1; Table S2). The capacity to regenerate adventitious shoots was expressed as the regeneration rate and the shoot ratio. The average regeneration rate per genotype ranged from 0.88 to 88.33% (Fig. 2; Table S2). In most genotypes, considerable variation among replicates expressed as standard deviation was recorded. The shoot ratio varied from 0.008 to 1.2 shoots per explant (Fig. 3). Statistical analysis of the regeneration rate and shoot ratio revealed a significant difference between genotypes at $P = 0.05$. The results from Tukey's test showed no significant differences (at $P = 0.05$) between the two repeat experiments for both parameters. The distributional assumptions of the two parameters, including the regeneration rate and shoot ratio, were tested by analysing the Q–Q plots under a binomial model. The

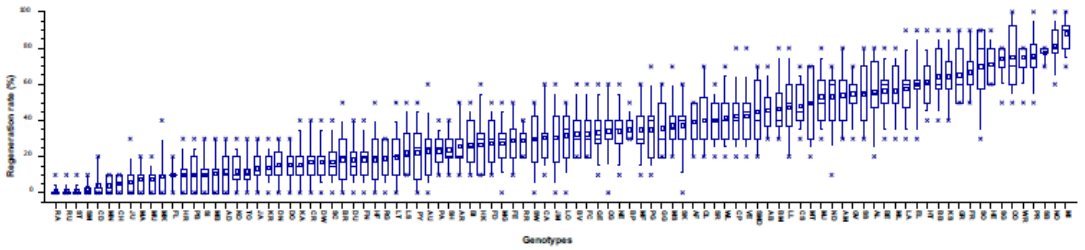

Fig. 2 *Box plots* of shoot regeneration rates for 96 rose genotypes based on two independent experiments with six biological replicates (Petri dishes with 10 explants) each. *Small square* mean; *continuous line* median; *asterisk* minimum, maximum; *box* first and third quartiles; and *whisker* standard deviation

distribution of the observed values was close to the expectation, with the exception that the distribution of the shoot ratio was slightly skewed to the left, indicating that our values were approximately normally distributed (Figs. S1, S2).

Regeneration rate and shoot ratio were highly positively correlated ($r = 0.9908$), and the correlation was statistically significant at a P value of 0.01(Fig. S3).

Marker–trait association analysis

To locate genetic factors influencing the measured regeneration parameters, we performed an association analysis in TASSEL 3.0 with the entire SNP data set, as described in Schulz et al. (2016), filtered for a minimum minor allele frequency (MAF < 0.05). A mixed linear model was used to reduce the false-positive associations, and the threshold P values were adjusted by Bonferroni correction for multiple testing at P values of $-[\log 10] = 6.100$. Overall, 47 SNPs were significantly associated with the regeneration rate and 61 markers were associated with the shoot ratio (Tables S3 and S4). A subset of 20 markers was associated with both traits as shown in Fig. 4 and Table S5. The lowest P value ($P = 1.15\text{E}{-62}$) was detected for the association of SNP marker RhK5_10015_277P (gene sn1-specific diacylglycerol lipase alpha) with the regeneration rate, and the lowest P value for the marker associated with shoot ratio was observed for RhK5_69_2438Q (a putative phosphoinositide phosphatase), with $P = 5.01\text{E}{-42}$.

Among the 88 significant SNPs associated with the regeneration traits, we found 12 SNPs from the ESTs matching known candidate genes involved in shoot morphogenesis. Of these, five SNPs were derived from receptor-like protein kinase genes, two SNPs from morphogenesis-related transcription factors, two SNPs from the ESTs of genes coding for epigenetic factors, and three SNPs from the ESTs of genes involved in plant hormone signalling. The SNPs from the ESTs related to receptor-like protein kinase genes were Rh12GR_21560_124Q, RhK5_8293_614Q, RhMCRND_6435_375P, Rh12GR_15592_504P, and RhMCRND_6327_1724Q. The two SNPs from ESTs related to transcription factors were Rh12GR_53908_964P (gene trihelix transcription factor GT-2-like) and RhMCRND_9379_1315Q (gene ethylene-responsive transcription factor RAP2-7-like). The ESTs from genes related to epigenetic factors were DNA methylation 3-like (Rh12GR_28168_792P and Rh12GR_28168_792Q) and mitogen-activated protein kinase kinase kinase ANP1-like (RhMCRND_12360_336P). The SNPs from the ESTs of genes involved in plant hormone signalling were RhK5_3149_367Q (gene DELLA protein GAI-like), RhK5_7232_851P (gene putative axial regulator YABBY 2) and especially Rh12GR_19922_162Q (gene for auxin transport protein BIG), which were found to be associated with a low P value (5.40E−56). There were some SNP markers associated with candidate genes for shoot morphogenesis such as RhMCRND_6327_1724Q, RhK5_3066_1552Q, RhK5_3066_1552Q, Rh12GR_15592_1555P, Rh12GR_54604_428Q (linked to receptor-like protein kinases), RhMCRND_23732_326Q (Homeobox_protein_knotted-1-like_3) and RhK5_7232_851P (Putative_axial_regulator_YABBY_2). These markers were associated with the phenotype, but their P values exceeded the threshold; thus, they were not considered further (Table S6).

The observed effects for the regeneration rate ranged from −38.8 to 52.3. For shoot ratio, the effects varied between −0.58 and 0.62. Tables 2 and 3 show significant SNPs that expressed the largest effects on the regeneration rate and shoot ratio, respectively. The best effects of the genotypic classes were found for markers Rh12GR_21560_124Q (a putative leucine-rich repeat receptor-like protein kinase) and Rh12GR_53908_964P (a putative trihelix transcription factor GT-2-like) for both the regeneration rate and the shoot ratio, respectively. Figures 5 and 6 illustrate the genotypic effects of these markers on the regeneration rate and the shoot ratio as calculated from the original data. Supplementary tables S8 and S9 contain the information on the sequences

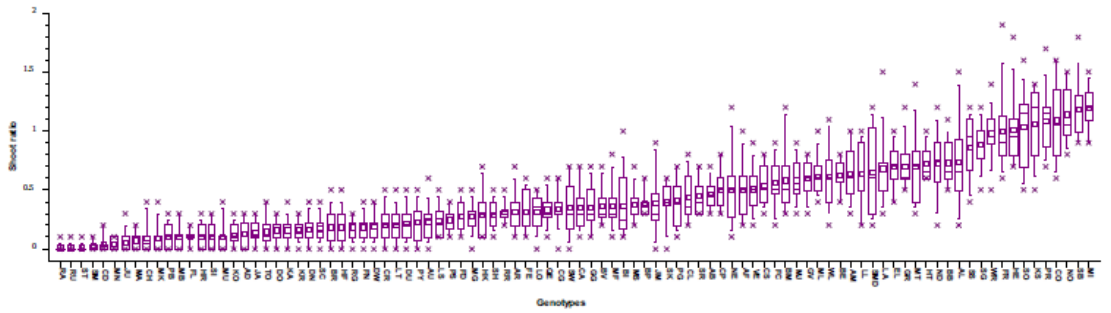

Fig. 3 *Box plots* of adventitious shoot ratios for 96 rose genotypes based on two to three independent experiments with six biological replicates each (Petri dishes with 10 explants). *Small square* mean; *continuous line* median; *asterisk* minimum, maximum; *box* first and third quartiles; and *whisker* standard deviation

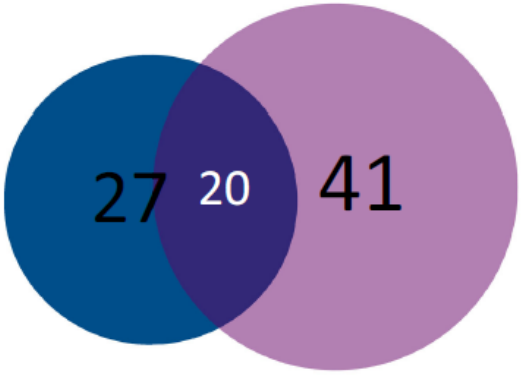

Fig. 4 Venn diagram showing the overlap between significant SNPs associated with shoot regeneration rate (*blue*) and shoot ratio (*purple*)

underlying these SNPs and the corresponding names of the SNPs on the Axiom Rose array.

Location of significant SNPs in the *F. vesca* genome and the identification of potential candidate genes

Until now, the only genetic map for rose that included 1929 SNP markers on 25 linkage groups (4 homologous sets of 7 chromosomes) was established by Vukosavljev et al. (2016), and no genome sequence for the rose is available yet. Therefore, significant SNPs associated with the regeneration rate and the shoot ratio were mapped via alignment to the reference genome sequence of *F. vesca* (Fig. 7). The results from the alignment analysis are summarized in Tables S3 and S4, respectively.

A new BLAST search was conducted for the sequences underlying all significantly associated SNPs (Tables S3 and S4) using the ESTs as queries and the *Fragaria* genome as a target. Moreover, a search for candidate genes related to the regeneration capacity was conducted among the collection of ESTs, which were used to develop the Affymetrix SNP chip by BLAST analyses. Moreover, these ESTs were also mapped to the *F. vesca* genome sequence to determine their position in relation to the associated rose SNPs. The results are shown in the genome plots (Table S7; Fig. 7).

The mapping results for SNPs for both the regeneration rate and the shoot ratio onto the strawberry genome showed that the majority of the markers fell into small clusters. For example, most of the markers mapping to strawberry chromosome 1 clustered in two groups—one at the end of the chromosome within a cluster of candidate genes comprising *SERK1*, *YABBY* and *WUSCHEL*, *CUC1* homologues and a second of four markers comprising the most significant associations. Other clusters with more than four markers were located on chromosomes three, four, five and six.

Discussion

In this study, we present data on genetic variation for the capacity of direct shoot regeneration from petiole explants among 96 rose genotypes. In addition to the phenotypic characterization, we identified genomic regions associated with shoot regeneration ability and located putative candidate genes with known functions in the plant developmental processes.

Direct shoot regeneration from leaf explants in roses

Due to the importance of roses as ornamentals, numerous studies have been conducted on rose tissue culture (reviewed in: Pati et al. 2006). The majority of the studies focused on aspects similar to in vitro propagation or somatic embryogenesis and their use in multiplication and biotechnology, whereas relatively little attention has been

Table 2 Significant SNP markers associated with the regeneration rate displaying the largest effects and sequence similarity to known candidate genes

Marker	*P* value	Effects			Function
		A:A	A:B	B:B	
Rh12GR_28168_792Q	**2.05E−08**	**0**	**34.132**	**20.986**	**Gene factor of DNA methylation 3-like (LOC101311119), transcript variant X2**
RhK5_3149_367Q	**4.37E−08**	**0**	**29.587**	**9.082**	**Gene DELLA protein GAI-like (LOC101314119), mRNA**
RhK5_8293_614Q	**4.78E−08**	**0**	**29.227**	**9.079**	**Gene probable receptor-like protein kinase At5g20050 (LOC101309575), mRNA**
RhMCRND_6435_375P	2.06E−07	8.109	28.088	0	Gene probable receptor-like protein kinase At5g20050 (LOC101309575)
Rh12GR_53908_964P	**3.16E−07**	**42.750**	**25.563**	**0**	**Gene trihelix transcription factor GT-2-like (LOC101315082)**
Rh12GR_21560_124Q	**6.70E−15**	**51.134**	**35.913**	**0**	**Gene probable leucine-rich repeat receptor-like protein kinase At5g49770 (LOC101315133)**
Rh12GR_11351_642P	4.13E−10	0	−16.745	–	Gene07909-v1.0-hybrid_30S_ribosomal_protein_S18_(probable)
Rh12GR_21282_4421P	**9.29E−10**	**52.271**	**35.014**	**0**	**Gene BTB/POZ domain-containing protein At1g04390 (LOC101302820), transcript variant X2**
RhMCRND_12360_336P	1.41E−09	−16.750	0	–	Gene mitogen-activated protein kinase kinase kinase ANP1-like (LOC101307975), mRNA
RhK5_11520_519P	1.49E−09	46.545	32.430	0	Gene serine/arginine repetitive matrix protein 2-like (LOC101309621), mRNA
RhMCRND_30734_1191Q	2.12E−07	44.140	29.530	0	Gene protein MOS2 (LOC101292784), transcript variant X6, mRNA
RhK5_9050_472Q	1.05E−07	–	17.671	0	Gene ATP-dependent RNA helicase DHX36 (LOC101299095), mRNA
RhK5_1098_361P	1.89E−07	–	41.726	22.746	Gene08916-v1.0-hybrid_ Dentin_sialoprotein,_Precursor_ (probable)
Rh12GR_19922_162Q	5.40E−56	9.835	2.991	0	Gene auxin transport protein BIG (LOC101292150), mRNA
RhK5_16002_503Q	1.76E−07	24.794	19.323	–	Gene putative protein FAR1-RELATED SEQUENCE 10 (LOC101307810), mRNA

A complete list of all SNPs associated with the regeneration rate is shown in Table S3. SNP markers associated with the shoot ratio are printed in bold

paid to direct organogenesis (Pati et al. 2004; Afshar et al. 2011; Pourhosseini et al. 2013). A few studies were conducted on the direct regeneration of shoots, most of which focused on the optimization of culture conditions. Only one study by (Dubois et al. 2000) compared the shoot regeneration rate of 24 rose genotypes and found significant variation between genotypes. Our data extend the study of (Dubois et al. 2000) in using a much larger and broader panel of genotypes as we did not restrict our collection to cut roses and rootstocks. However, our phenotypic variability was much larger, with some genotypes displaying very low regeneration rates in contrast with the results of (Dubois et al. 2000) who observed minimum rates of approximately 60%, which are closer to the observations from other *Rosaceae* members reporting recalcitrant genotypes and which do not regenerate, such as those of pear and plum (Lane et al. 1998; Yao et al. 2014). As shoot regeneration is an important aspect of plant biotechnology, our data may help improve the biotechnology protocols for the regeneration of roses in the future. The low variability between our experiments allows us to conclude that the contributor to variability is the genotype and that this information could be used for identifying the underlying genetic factors, although differences between single explants were obvious from high standard deviations. The high standard deviations are most likely caused by different degrees of injury in explant preparation and the differences in the physiological status of the explant material, resulting in particular micro-conditions within one Petri dish. Furthermore, as explants for the two experiments were collected over a period of more than 5 months with changing day lengths and light intensity, we concluded that shoot regeneration capacity was not influenced strongly by the changes in greenhouse culture conditions.

The regeneration of shoots occurred at the proximal end of the explant without a callus phase and within a relatively short time of 5 weeks. Since the axial bud was very carefully excised during explant preparation, the shoots were of adventitious origin. An advantage of this regeneration protocol is the use of greenhouse material avoiding laborious in vitro shoot multiplication, which bears the risk of accumulating somaclonal variants.

Table 3 Significant SNP markers associated with shoot ratio displaying the largest effects and sequence similarity to known candidate genes

Marker	*P* value	Effects			Function
		A:A	A:B	B:B	
Rh12GR_53908_964P	**2.87E−11**	**0.623**	**0.453**	**0**	**Gene trihelix transcription factor GT-2-like (LOC101315082)**
RhK5_3149_367Q	**1.72E−09**	**0**	**0.247**	**0.473**	**Gene DELLA protein GAI-like (LOC101314119)**
Rh12GR_28168_792Q	**2.17E−09**	**0**	**0.511**	**0.364**	**Gene DNA methylation 3-like (LOC101311119)**
RhK5_8293_614Q	**2.62E−08**	**0**	**0.451**	**0.230**	**Gene probable receptor-like protein kinase At5g20050 (LOC101309575)**
Rh12GR_28168_792P	8.62E−08	0	0.488		Gene DNA methylation 3-like (LOC101311119)
RhK5_2319_813P	9.44E−08	0.423	0		Gene17893-v1.0-hybrid_ Cell_division_protease_ftsH_homolog_(probable)
RhMCRND_6327_1724Q	4.05E−07	−0.216	0.199	0	Gene31125-v1.0-hybrid _Probable_receptor-like_protein_kinase_ At5g59700,_Precursor
Rh12GR_21282_4421P	**1.56E−11**	**0.736**	**0.533**	**0**	**Gene BTB/POZ domain-containing protein At1g04390 (LOC101302820), transcript variant X2, misc_RNA**
RhK5_5078_253P	1.06E−09	0.507	0.495	0	Gene grpE protein homolog, mitochondrial-like (LOC101297042), transcript variant X4
RhK5_7232_851P	5.10E−09	0.526	0.480	0	Gene putative axial regulator YABBY 2 (LOC101307367)
RhMCRND_9379_1315Q	3.50E−08	0.513	0.384	0	Gene ethylene-responsive transcription factor RAP2-7-like (LOC101295120)
Rh12GR_21560_124Q	**8.10E−08**	**0.650**	**0.473**	**0**	**Gene probable leucine-rich repeat receptor -like protein kinase At5g49770 (LOC101315133)**
Rh12GR_15592_504P	2.75E−07	0.452	0		Gene10374-v1.0-hybrid_ Probable_leucine-rich_repeat_receptor-like_protein_kinase _At2g33170, _Precursor_(putative)
RhK5_20938_917P	3.84E−07	0	0.410		Gene03256-v1.0-hybrid_ Golgin_subfamily_A_member_2 _(probable)
Rh12GR_1195_716Q	3.94E−07	0.622	0.467	0	Gene29960-v1.0-hybrid_ hypothetical_protein
RhK5_15232_250P	1.76E−07	0	0.336		Gene non-functional NADPH-dependent codeinone reductase 2-like (LOC101313111)
RhK5_9894_454Q	1.05E−09		0.452	0	Gene09394-v1.0-hybrid_Golgin_candidate_2_(AtGC2) _(probable)
Rh12GR_8077_1243Q	3.42E−07	0.389	0		Gene RING-H2 finger protein ATL54-like (LOC101301878)
Rh12GR_10115_1299P	5.54E−08		0	0.450	Gene phosphoglucan phosphatase LSF1, chloroplastic (LOC101294692)
Rh12GR_51628_738P	1.29E−07	0.525	0.501	0	Gene01203-v1.0-hybrid_NADH-quinone_oxidoreductase_subunit_C/D _(probable)
Rh12GR_51628_738Q	1.59E−07	0.522	0.483	0	Gene01203-v1.0-hybrid_NADH-quinone_oxidoreductase_subunit_C/D _(probable)

A complete list of all SNPs associated with shoot ratio is shown in Table S4. SNP markers associated with the regeneration rate are printed in bold

Marker–trait associations for shoot regeneration traits

Association genetics has become one of the most effective tools for the analysis of quantitative traits, providing higher resolution of markers and making use of a wider array of alleles in a given species compared to conventional QTL analysis in biparental populations (Nordborg and Weigel 2008). To date, several analyses were conducted to study organogenesis in soybean (Yang et al. 2011), tomato (Trujillo-Moya et al. 2011), *Brassica rapa* (Seo et al. 2013) and *Arabidopsis thaliana* ((Lall et al. 2004; Motte et al. 2014a), where several QTLs could be associated with traits related to shoot regeneration. An extensive study in *A. thaliana* identified a candidate gene (*RPK1*) for involvement in shoot regeneration, which is supported by functional genomic experiments (Motte et al. 2014a). Recently, we conducted a genome-wide association study on anthocyanin and carotenoid concentrations in rose petals where we used the same set of 96 genotypes as the current experiment and generated genotyping data by means of an Axiome SNP chip (Koning-Boucoiran et al. 2015; Schulz et al. 2016).

Our genome-wide association analysis revealed 88 markers associated with the two traits, and 20 markers were commonly associated with both traits (Fig. 4). Overlapping factors affecting the two traits were expected, as both traits were highly correlated ($r = 0.9908$) because the shoot ratio calculation included the regeneration rate due to the incorporation of all explants, and not just the explants that formed shoots. We also calculated the average number of

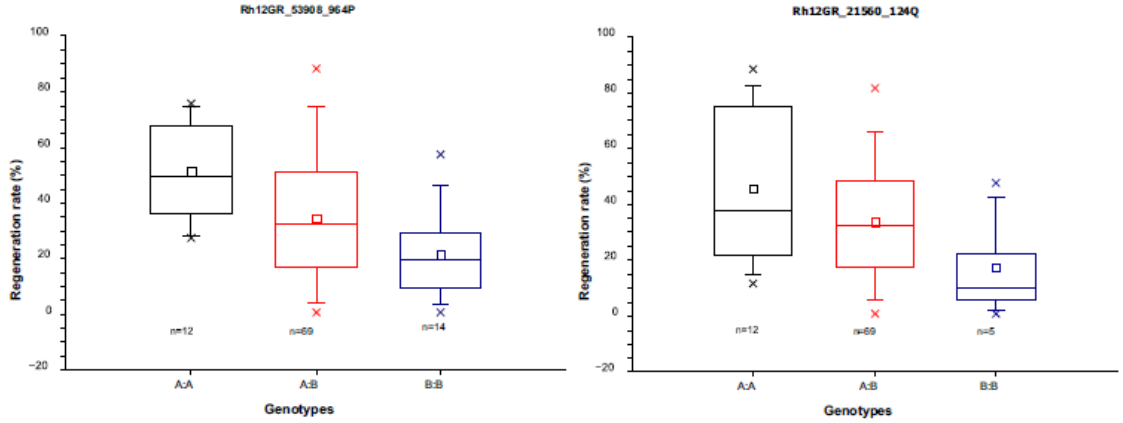

Fig. 5 Genotypic effects of SNP markers Rh12GR_53908_964P (trihelix transcription factor GT-2-like) and Rh12GR_21560_124Q (putative leucine-rich repeat receptor-like protein kinase) on the regeneration rate (*small square* mean; *continuous line* median; *asterisk* minimum, maximum; *box* first and third quartiles; and *whisker* standard deviation)

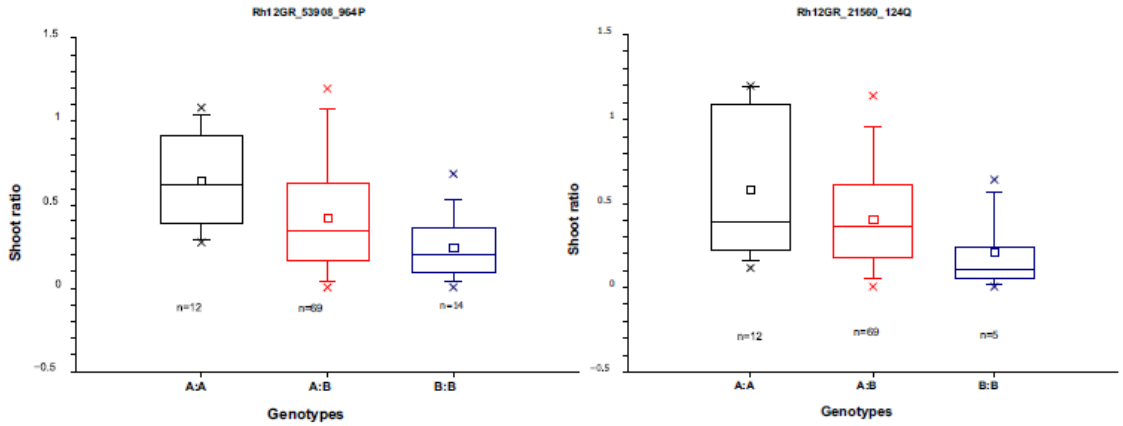

Fig. 6 Genotypic effects of SNP markers Rh12GR_53908_964P (trihelix transcription factor GT-2-like) and Rh12GR_21560_124Q (putative leucine-rich repeat receptor-like protein kinase) on the shoot ratio (*small square* mean; *continuous line* median; *asterisk* minimum, maximum; *box* first and third quartiles; and *whisker* standard deviation)

shoots per regenerating explant, but this parameter did not differ significantly between the genotypes (Table S4) and thus could not be used in the association analyses. However, since the shoot ratio apparently resulted in the identification of a different set of SNPs, its usefulness in the current study was shown. This was underlined by two of the markers (Rh12GR_53908_964P and Rh12GR_21560_124Q) with the highest effect on the phenotypes and that belonged to this overlapping group (Figs. 5, 6). These markers explained phenotypic differences from 0.83 to 87.9% for the regeneration rate and from 0.008 to 1.2 for the shoot ratio. The SNPs are located in the genes for a trihelix transcription factor GT2-like (Rh12GR_53908_964P) and a putative leucine-rich repeat receptor-like protein kinase (Rh12GR_21560_124Q), both of which might be interesting candidates for their role in shoot regeneration, which could be verified by overexpression or knockout approaches. GT2-like trihelix transcription factors were reported to have functions in the developmental processes such as embryogenesis and formation of perianth organs or trichomes (Kaplan-Levy et al. 2012; Barr et al. 2012).

Along with significant *P* values and effects, the clustering of markers to particular genomic regions is a hint of true association, as it has been shown in many cases that linkage disequilibrium leads to groups of markers that are linked to the causal genes displaying significant associations (Morton 2005). As the rose genome has not been

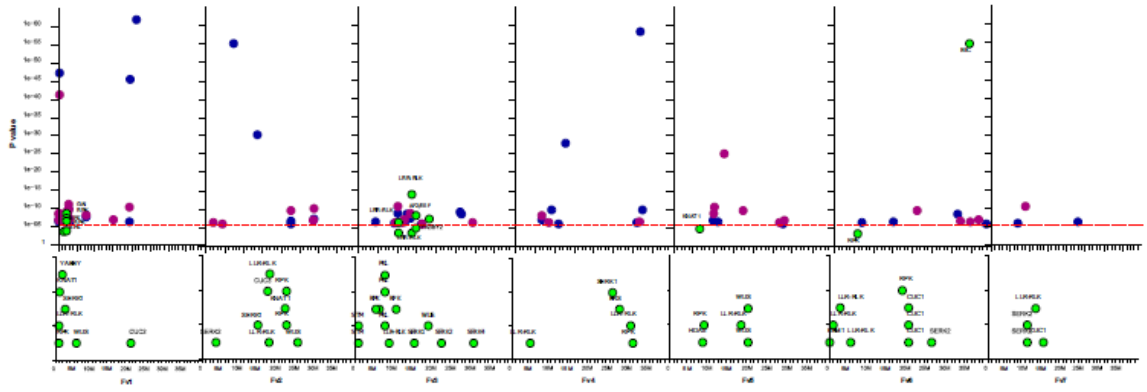

Fig. 7 Location of SNPs associated with the regeneration capacity in rose. The *upper* part shows SNPs that mapped to homologous sequences in the genome of *Fragaria vesca*. The position of known candidate genes for shoot regeneration in the genome of *F. vesca* is shown in the *bottom* part of the graph. The *blue circles* are significant SNPs associated with shoot regeneration rate, and the *purple circles* are SNPs related to the shoot ratio. The *green circles* are candidate genes. The *red dashed line* represents the Bonferroni threshold of the adjusted significant level $-[\log 10] = 6.100$

sequenced, it is not possible to analyse the "Manhattan plots" as is done for species with sequenced genomes. Therefore, we made use of the large degree of synteny between the *Fragaria* and the rose genome (Gar et al. 2011) to obtain positional information on the significantly associated markers. We identified two clusters on the *Fragaria* chromosome 1, one broad cluster on chromosome 3 and smaller clusters on chromosomes 4, 5 and 6. On the clusters at the start of chromosome 1 and the cluster on chromosome 3, a number of candidate genes known to influence the developmental processes could also be mapped. For example, several members of the receptor kinases gene family, such as *RPK1* and *LRR-RLK*, are known to play an important role in the development and differentiation of plant cells (Afzal et al. 2008; Motte et al. 2014a) mapped to these regions. Notably, the homologues to a receptor-like protein kinase gene that was identified as a major factor for shoot organogenesis in a GWAS in *Arabidopsis thaliana* (Motte et al. 2014a) were located in both clusters and a cluster on chromosome 4. Another highly associated SNP on chromosome 6 was located within a homologue of the *BIG* gene. This gene codes for an auxin transport protein and belongs to a polar auxin transport gene family (PIN). Furthermore, this gene has fundamental roles in the regulation of auxin action and is required for light-regulated responses during plant development (Gil et al. 2001; Adamowski and Friml 2015). Other genes involved in plant hormone signalling were found in linkage groups 1 and 3. One of these genes, the DELLA protein GAI, is considered to be a master regulator of gibberellin biosynthesis (Hedden and Thomas 2016). The gene *YABBY* modulates the gibberellin pathway (Yang et al. 2016), and the gene for the ethylene-responsive transcription factor RAP2 is involved in the control of primary and secondary metabolism, growth and developmental programmes (Licausi et al. 2013). In addition to the known candidate genes that were explored in the association analysis, we found some genes related to the epigenetic factors directing the developmental switches that occur during in vitro culture of plant cells such as a gene "factor of DNA methylation-3" (Shemer et al. 2015) and a gene for a mitogen-activated protein kinase kinase kinase (*MAPKKK*, Xu and Zhang 2015) on the linkage group Fv3. In contrast, other factors postulated to influence organogenesis in roses such as the *BABY BOOM* gene (Yang et al. 2014) and the *SERK* gene (Zakizadeh et al. 2010) could not be associated with any variation in shoot regeneration.

Some of the markers that we detected as significantly associated might be artefacts, as our data are based on only 96 genotypes, a number smaller than those used in most other studies conducted with cultivated plants (Weigel and Nordborg 2015). Therefore, the validation of markers should first be done by analysing an independent set of genotypes. However, as the phenotyping for shoot regeneration is extremely laborious, it might be useful for genotyping a large set of plants and only phenotype a subset of genotypes for the most contrasting marker dosages (e.g. homozygotes for each SNP class).

In our study, the *BIC* gene was associated with shoot regeneration with a very low *P* value of 5.4E−56. A future option for this candidate gene would be a functional genomics approach with overexpression of the favourable alleles in genotypes with low to medium regeneration rates or knockout experiments using genome editing tools. This would also unfold possibilities for future research on factors influencing organogenesis in roses. Moreover, novel

strategies for genetically engineering roses via the alternative regeneration pathways might be possible if the regeneration rates could be optimized, therefore serving as alternative approaches to current protocols using somatic embryogenesis as a regeneration pathway.

Conclusion

In the present study, we demonstrate a wide genetic variation for direct shoot regeneration capacity in a rose association panel. The preliminary results of a GWAS showed that associated markers could be confirmed in independent populations, which can be used for identifying the responsible genes and understanding the functional role of these genes in the regeneration process in future studies. The results provide insight into the genetic architecture of the regeneration capacity in roses, and this genetic information could be potentially useful in marker-assisted selection for regeneration capacity in rose. Whether the candidate genes associated with the observed differences in regeneration traits are causal factors or only linked to those genes can only be concluded after functional studies involving stable transformation of poorly regenerating genotypes with different alleles of these genes or by overexpressing these genes.

Author contribution statement NTHN conducted the experiments, completed the statistical analysis and wrote most of the manuscript. DS conducted part of the data analysis. TW contributed to the experimental setup and wrote a part of the manuscript. TD was involved in planning the experiments and wrote parts of the manuscript.

Acknowledgements This study was carried out in part with financial support from the Vietnamese Government and Department of Molecular Plant Breeding, Institute of Plant Genetics, Leibniz Universität, Hannover, Germany. We would like to thank Klaus Dreier and the gardeners from the Department of Molecular Plant Breeding for assistance and the Federal Plant Variety Office for providing greenhouse facilities in Hannover. We thank Dr. Frank Schaarschmidt from the Institute of Biostatistics, Leibniz Universität, Hannover, Germany, for help with the statistical analyses.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Supplements

Table S1: List of the association panel of rose genotypes was used in the study

DNAC ode	Cultivar	Code	Breeder	Country	Bred in (Y)	Type/habit	Flower	Polyploid
1	Parole	PR	W. Kordes&Söhne	GER	1991	Hybrid Tea	pink	Tetraploids
2	Queen Elizabeth	QE	Lammerts	USA	1954	Grandiflora, shrub	Pink	Tetraploids
3	Schneewittchen[1)]	SC	W. Kordes&Söhne	GER	1958	Floribunda, shrub	white	Triploid
4	Nemo	NE	Noack Rosen	GER	2001	Floribunda, ground cover	white	Tetraploids
5	Super Star[1)]	SS	Rosen Tantau	GER	1960	Hybrid Tea	salmon pink	Triploid
6	Small Maid. Blush	SM	Unknown	UK	1797	Alba, shrub	light pink	Tetraploids
10	Chippendale	CP	Rosen Tantau	GER	2005	Hybrid Tea	orange	Tetraploids
11	Climbing Allgold	CG	Douglas L. Gandy	UK	1961	Floribunda, climber	yellow	Tetraploids
12	Blue Parfum	BP	Rosen Tantau	GER	1978	Bedding	violet	Tetraploids
13	Feuerwerk	FE	Rosen Tantau	GER	1962	Shrub	orange, red	Tetraploids
14	Gebrüder Grimm	GG	W. Kordes&Söhne	GER	2007	Floribunda, bedding	orange	Tetraploids
15	George Vancouver	GV	Ag Can	CAN	1983	Hybrid Kordesii, shrub	Red	Tetraploids
16	König Stanislaus	KS	Rosen Tantau	GER	1998	Shrub	yellow	Tetraploids
17	Heidi Klum	HK	Rosen Tantau	GER	1999	Floribunda, bedding	violet	Tetraploids
18	Jasmina	JA	W. Kordes&Söhne	GER	1996	Climber	Pink	Tetraploids
20	Sonnenschirm	SO	Rosen Tantau	GER	1993	Floribunda, ground cover	yellow	Tetraploids
24	Heidetraum[1)]	HT	Noack Rosen	GER	1988	ground cover	carmine-pink	Triploid
26	Nostalgie	NO	Rosen Tantau	GER	1995	Hybrid Tea	white, pink	Tetraploids
27	Sommerwind[1)]	SW	W. Kordes&Söhne	GER	1985	Bedding	light pink	Triploid
28	New Dawn[1)]	ND	Somerset Rose Nurs.	USA	1930	Climber	light pink	triploid n
32	Mevrouw N. Nypels[2)]	MN	Mathias Leenders	NL	1919	Polyantha, shrub	Pink	Diploid
35	Mitsouko	MI	Delbard	F	1970	Hybrid Tea	yellow	Tetraploids
36	Black Baccara	BB	Meilland	F	2000	Hybrid Tea	Red	Tetraploids
37	Alinka	AL	Patrick Dickson	UK	1971	Hybrid Tea	Red	Tetraploids
38	Auslo (=Othello)	AU	David Austin Roses	UK	1986	Shrub	Red	Tetraploids
39	Ausmas (=Graham Thomas)	AM	David Austin Roses	UK	1983	Shrub	yellow	Tetraploids
40	Shalom	SH	PoulsenRoser A/S	DAN	1972	Floribunda, shrub	Red	Tetraploids
41	La Sevillana	LA	Meilland	F	1978	Floribunda, shrub	Red	Tetraploids
42	Mister Lincoln	ML	Swim & Weeks	USA	1964	Hybrid Tea	Red	Tetraploids
43	Rumba	RU	PoulsenRoser A/S	DAN	1958	Floribunda, bedding	orange	Tetraploids
44	Arthur Bell	AB	Sam McGredy Roses	NZ	1965	Floribunda, shrub	yellow	Tetraploids
46	Comtesse de Ségur	CS	Delbard	F	1992	Floribunda, shrub	Pink	Tetraploids
47	Mme Boll	MB	Daniel Boll	USA	1858	Portland, shrub	Red	Tetraploids
49	Compassion	CO	Harkness & Co Ltd.	UK	1972	Climber	salmon-pink	Tetraploids
50	Sutters Gold	SG	Herbert C. Swim	USA	1950	Hybrid Tea	yellow	Tetraploids
51	Scarlet Meidilland	SMD	Meilland	F	1987	shrub, ground cover	Red	Tetraploids
52	Rose de Resht	RR		Persia	1900	Damask, shrub	Red	Tetraploids
53	Celine Delbard	CD	Delbard	F	1986	Floribunda, shrub	salmon-pink	Tetraploids
54	Louise Odier	LO	Jules Margottin Père & Fils	F	1851	Bourbon, shrub	deep pink	Tetraploids

55	Ausfather (=Charles Austin)	AF(Ma et al. 2016)	David Austin Roses	UK	1973	Shrub	apricot	Tetraploids
56	Perpetually Yours	PY	Harkness & Co Ltd.	UK	1999	Climber	light yellow	Tetraploids
57	Mme Knorr	MK	Viktor Verdier	F	1855	Portland, shrub	Pink	Tetraploids
58	Papageno	PG	Sam McGredy Roses	NZL	1989	Hybrid Tea	red bled, stripes	Tetraploids
59	France Libre	FL	Delbard	F	1981	Hybrid Tea	orange	Tetraploids
61	Princess Alexandra	PA	PoulsenRoser A/S	DK	1988	Hybrid Tea	violet	Tetraploids
62	Mrs John Laing	MJ	Henry Bennet	UK	1885	Hybrid Perpetual, shrub	deep pink	Tetraploids
66	Black Magic	BM	Rosen Tantau	GER	1995	Hybrid Tea	dark red	Tetraploids
67	China Girl	CG	Mehring/ Tantau	GER	2005	Floribunda, bedding	yellow	Tetraploid
68	Perennial Blush	PB	Henry Bennet	UK	2007	climber/rambler	white, light pink	Tetraploid
69	Comtessa AL	CA	Rosen Tantau	GER	2006	Hybrid Tea	yellow, white	Tetraploid
70	Lipstick	LS	Rosen Tantau	GER	2001	ground cover	Pink	Tetraploid
71	Midsummer	MS	Rosen Tantau	GER	2007	Floribunda, bedding	orange-red	Tetraploid
72	Arabia	AR	Rosen Tantau	GER	2001	Shrub	orange blend	Tetraploid
73	Hansestd. Rostock	HR	Rosen Tantau	GER	2004	Floribunda, bedding	apricot	Tetraploid
74	Kastelrut. Spatzen	KA	Rosen Tantau	GER	2011	ground cover	white	Tetraploid
75	Elfe	EF	Rosen Tantau	GER	2000	Climber	yellow	Tetraploid
77	Jazz	JA	Rosen Tantau	GER	2003	ground cover	copper-orange	Tetraploid
78	MainzerFastnacht	MF	Rosen Tantau	GER	1964	Hybrid Tea	violet	Tetraploid
79	Dukat	DU	Rosen Tantau	GER	2010	Floribunda, climber	yellow	Tetraploid
80	My Girl	MG	Rosen Tantau	GER	2006	Hybrid Tea	white, yellow center	Tetraploid
81	Mariatheresia	MT	Rosen Tantau	GER	2003	Floribunda, bedding	light pink	Tetraploid
84	Knockout[1)]	KO	Radler	USA	1988	Shrub	Red	Triploid
85	Berolina	BE	W. Kordes&Söhne	GER	1984	Hybrid Tea	yellow	Tetraploid
89	Westerland	WL	W. Kordes&Söhne	GER	1969	Shrub	orange	Tetraploid
92	Frühlingsduft	FD	W. Kordes&Söhne	GER	1949	Shrub	white, pink shading	Tetraploid
93	Sebastian Kneipp	SK	W. Kordes&Söhne	GER	1997	Hybrid Tea	white, pink center	Tetraploids
94	Lavender Lassie[1)]	LL	W. Kordes&Söhne	GER	1960	Shrub	violet	Triploid
95	Dortmund	DO	W. Kordes&Söhne	GER	1955	Climber	Red	Tetraploids
96	Friesia	FR	W. Kordes&Söhne	GER	1973	Floribunda, bedding	yellow	Tetraploids
97	Sterntaler	ST	W. Kordes&Söhne	GER	1995	Shrub	yellow	Tetraploids
99	Raubritter[1)]	RA	W. Kordes&Söhne	GER	1936	Climber	light pink	Triploid
100	Herkules	HE	W. Kordes&Söhne	GER	2006	Shrub	pink, light lavender	Tetraploids
103	Fritz Nobis	FN	W. Kordes&Söhne	GER	1940	Shrub	rose-pink	Tetraploid
104	Beverly	BV	W. Kordes&Söhne	GER	1999	Hybrid Tea	Pink	Tetraploid
105	Juanita	JU	W. Kordes&Söhne	GER	1996	mini-shrub	light pink	Tetraploids
110	Windrose	WR	Noack Rosen	GER	1993	ground cover	Pink	Tetraploids
111	Donauprinzessin	DN	Noack Rosen	GER	1994	Floribunda, bedding	salmon-pink	Tetraploids
112	Münsterland	MU	Noack Rosen	GER	1986	Floribunda, shrub	light pink	Tetraploids
114	Venice	VE	Noack Rosen	GER	2003	Floribunda, ground cover	white	Tetraploids

115	Focus	FO	Noack Rosen	GER	1997	Hybrid Tea	light pink	Tetraploids
116	Simply	SI	Noack Rosen	GER	2003	ground cover	Pink	Tetraploid
118	Kronjuwel	KR	Noack Rosen	GER	1997	Floribunda, bedding	Red	Tetraploid
119	Tornella	TO	Noack Rosen	GER	2005	Shrub	Red	Tetraploid
120	Herzogin Friederike	HF	Noack Rosen	GER	2002	Shrub	Pink	Tetraploid
122	Blue River	BR	W. Kordes&Söhne	GER	1984	Hybrid Tea	magenta	Tetraploid
131	Cute Haze	CH	Rosen Tantau	GER	2010	ground cover, shrub	white	Tetraploid
132	Duftwolke	DW	Rosen Tantau	GER	1963	Bedding	Red	Tetraploid
133	Goethe Rose	GR	Rosen Tantau	GER	2004	Hybrid Tea	Red	Tetraploid
134	Albrecht Dürer Rose	AD	Rosen Tantau	GER	1996	Hybrid Tea	orange	Tetraploid
135	Stadt Rom	SR	Rosen Tantau	GER	2000	ground cover	carmine-pink	Tetraploid
136	Bienenweide	BI	Rosen Tantau	UK	2011	mini-shrub	Red	Tetraploid
137	Lolita	LT	W. Kordes&Söhne	GER	1972	Hybrid Tea	apricot	Tetraploid
138	Magenta	MA	W. Kordes&Söhne	GER	1954	Floribunda, shrub	violet	Tetraploid
139	Rose Gaujard	RG	Jean-Marie Gaujard	F	1957	Hybrid Tea	cherry-red	Tetraploid
140	Crimson Glory	CR	W. Kordes&Söhne	GER	1935	Hybrid Tea	purple, crimson	Tetraploid
141	Sunset Boulevard	SB	Harkness & Co Ltd.	UK	1997	Floribunda, shrub	salmon-pink	Tetraploid

Table S2: Comparison of regeneration rate between 2 repeats of 96 rose cultivars

Genotypes	Regeneration rate		Shoot ratio		Shoot number per explant	
	Mean	SD	Mean	SD	Mean	SD
Albrech Dürer Rose	11.67	11.15	0.13	0.12	1.06	0.18
Alinka	55.83	19.75	0.74	0.38	1.36	0.78
Arabia	25.83	13.11	0.32	0.17	1.32	0.40
Arthur Bell	45.83	13.11	0.46	0.13	1.03	0.26
Ausfather	39.17	11.65	0.50	0.24	1.23	0.31
Auslo (Othello)	23.33	15.57	0.23	0.16	1	0
Ausmas	54.17	17.30	0.64	0.24	1.18	0.23
Berolina	56.67	13.71	0.63	0.14	1.12	0.14
Beverly	32.5	12.88	0.37	0.16	1.13	0.25
Bienenweide	26.67	13.71	0.37	0.28	1.25	0.40
Black Baccara	64.17	15.05	0.73	0.20	1.15	0.20
Black Magic	46.67	17.23	0.58	0.29	1.22	0.27
Blue Parfum	35	6.74	0.38	0.11	1.03	0.08
Blue River	18.33	15.28	0.18	0.15	1	0
Celine Debard	4.17	7.93	0.04	0.08	1	0
China Girl	34.17	15.64	0.34	0.16	1	0
Chippendale	42.5	15.45	0.50	0.18	1.20	0.23
Climbing Algold	40	18.09	0.44	0.21	1.12	0.38
Compassion	75	18.34	1.09	0.35	1.46	0.37
Comtessa Al	30.83	15.64	0.36	0.19	1.15	0.23
Comtesse de Segus	48.33	11.93	0.54	0.17	1.11	0.14
Crimson Glory	17.5	12.88	0.20	0.15	1.13	0.21
Cute Haze	5	5.22	0.09	0.13	1.83	1.33
Donauprinzessin	15.83	10.84	0.17	0.12	1.07	0.15
Dormund	15.83	9.96	0.16	0.10	1	0
Duftwolke	17.5	10.55	0.19	0.12	1.09	0.30
Dukat	18.33	12.67	0.22	0.14	1.30	0.64
Elfe	60	15.95	0.70	0.20	1.17	0.17
Feuerwerk	29.17	14.43	0.32	0.17	1.07	0.20
Focus	32.5	12.88	0.56	0.21	1.76	0.46
France Libre	10	6.03	0.11	0.07	1.1	0.32
Friesia	66.67	13.71	1.00	0.35	1.49	0.33
Fritz Nobit	18.33	11.15	0.18	0.11	1	0
Frülingsduft	27.5	13.57	0.28	0.14	1	0
Gebrüder Grimm	35.83	15.64	0.36	0.20	1	0.21
George Vancouver	55	11.68	0.60	0.16	1.08	0.13
Goethe Rose	65.00	15.67	0.70	0.20	1.08	0.12
Hansestadt Rostock	10	9.53	0.11	0.10	1.13	0.35
Heidetraum	61.67	13.37	0.73	0.15	1.19	0.13

Heidi Klum	26.67	17.23	0.29	0.21	1.06	0.14
Herkule	70.83	9.96	1.01	0.31	1.41	0.30
Herzogin Fiederike	18.33	15.28	0.18	0.15	1	0
Jasmina	30.83	18.32	0.38	0.28	1.19	0.30
Jazz	13.33	9.85	0.14	0.11	1.05	0.16
Juanita	5.83	9.00	0.06	0.09	1	0
Kastelruther Spatzen	15.83	11.65	0.16	0.11	1.1	0.32
Knockout	11.67	9.37	0.12	0.09	1	0
König Stanislaus	64.17	15.64	1.06	0.32	1.64	0.25
Kronjuwel	14.17	7.93	0.16	0.09	1.14	0.32
La Sevillana	57.5	16.03	0.68	0.30	1.16	0.20
Lavender Lassie	47.5	18.65	0.64	0.27	1.34	0.28
Lipstick	22.5	14.22	0.24	0.14	1.13	0.31
Lolita	20	14.14	0.20	0.14	1	0
Louis Oldier	31.67	15.86	0.32	0.16	1	0
Magenta	7.5	7.54	0.08	0.08	1	0
Mainzer Fatnacht	35	15.67	0.37	0.19	1.03	0.10
Mariatheresia	50	20	0.70	0.32	1.42	0.33
Mevrouv Nathale Nypel	4.17	5.15	0.04	0.05	1	0
Midsummer	37.5	14.22	0.38	0.14	1	0
Mister Lincoln	56.67	14.35	0.62	0.16	1.10	0.20
Mitsouko	88.33	9.37	1.20	0.19	1.36	0.12
Mme Boll	10.83	10.84	0.11	0.11	1	0
Mme Knorr	9.17	11.65	0.09	0.12	1	0
Mrs John Liang	53.33	14.97	0.58	0.19	1.09	0.13
Münsterland	7.5	6.22	0.11	0.12	1.38	0.74
My Girl	27.5	12.88	0.28	0.13	1	0
Nemo	34.17	13.79	0.50	0.32	1.35	0.37
New Dawn	53.33	17.75	0.73	0.27	1.37	0.37
Nostagie	81.67	11.15	1.14	0.25	1.39	0.21
Papageno	35	17.32	0.41	0.20	1.18	0.31
Parole	75.83	13.79	1.08	0.28	1.42	0.18
Perenial Blush	10	10.44	0.10	0.10	1	0
Perpetually Your	22.5	17.12	0.23	0.17	1	0
Princess Alexandra	23.33	9.85	0.25	0.11	1.07	0.17
Queen Elizabeth	33.33	13.71	0.33	0.14	1	0
Raubitter	0.833	2.89	0.01	0.03	1	NA
Rose de Resht	29.17	7.93	0.31	0.09	1.06	0.16
Rose Gaujard	19.17	9.96	0.18	0.09	1	0
Rumba	0.833	2.89	0.01	0.03	1	NA
Scarlet Meidiland	45	18.83	0.65	0.37	1.38	0.34
Schneewittchen	17.5	11.38	0.18	0.11	1	0
Sebastian Kneipp	37.5	16.58	0.40	0.18	1.07	0.13
Shalom	24.17	9.96	0.29	0.12	1.26	0.41

Simply	10	11.28	0.11	0.12	1.14	0.38
Small Maiden	2.5	4.52	0.03	0.05	1	0
Sommerwind	30	17.06	0.35	0.24	1.20	0.44
Sonnenschein	70	21.32	1.03	0.34	1.47	0.37
Stadt Rom	40	12.06	0.45	0.15	1.13	0.18
Sterntaler	0.833	2.89	0.01	0.03	1	NA
Sunset Boulervar	77.5	4.52	1.18	0.26	1.53	0.32
Super Star	55	18.83	0.87	0.26	1.61	0.27
Sutter Gold	74.17	9.00	0.88	0.19	1.18	0.18
Tornella	11.67	10.30	0.15	0.14	1.30	0.42
Venice	42.5	16.03	0.51	0.20	1.19	0.16
Westerland	41.67	14.67	0.62	0.28	1.46	0.37
Windrose	75	9.05	0.98	0.21	1.30	0.19

Table S3: Significant SNPs associated to regeneration rate

Marker	p- value	Genotypic effects			Linkage group	Position	Function
		A:A	A:B	B:B			
RhK5_10015_277P	1.15E-62	3.950	-4.440	0	1	21193365	gene sn1-specific diacylglycerol lipase alpha (LOC101299525)
RhK5_69_2438Q	5.64E-48	-	-6.873	0	1	278318	gene probable phosphoinositide phosphatase SAC9 (LOC101296222), mRNA
RhK5_14289_440Q	2.63E-46	-	8.481	0	1	19604544	gene putative inactive cysteine synthase 2 (LOC101311409), transcript variant X5, misc_RNA
RhK5_8844_469P	1.17E-10	19.757	36.240	-	1	2607022	gene02112-v1.0-hybrid_IST1-like_protein_(probable)
Rh12GR_21174_1298Q	1.28E-08	0	33.551	22.931	1	7513167	gene acidic leucine-rich nuclear phosphoprotein 32-related protein (LOC101303231),
RhK5_8_6985Q	1.79E-08	0	29.632	8.684	1	2284025	gene dnaJ homolog subfamily C GRV2 (LOC101305987), mRNA
Rh12GR_28168_792Q	2.05E-08	0	34.132	20.986	1	543834	gene factor of DNA methylation 3-like (LOC101311119), transcript variant X2,
RhK5_8_7501Q	3.01E-08	0	29.754	9.504	1	2284025	gene DnaJ_homolog_subfamily_C_member_13_(RME-8)_(probable)
RhK5_3149_367Q	4.37E-08	0	29.587	9.082	1	2144285	gene DELLA protein GAI-like (LOC101314119), mRNA
RhK5_8293_614Q	4.78E-08	0	29.227	9.079	1	2035647	gene probable receptor-like protein kinase At5g20050 (LOC101309575), mRNA
Rh12GR_2555_1635P	7.93E-08	0	33.350	29.092	1	82309	gene uncharacterized LOC101305502 (LOC101305502), transcript variant X2,
Rh12GR_26729_1408Q	1.40E-07	0	34.088	21.638	1	2224055	gene transmembrane protein 19-like (LOC101291659), transcript variant X2,
RhMCRND_6435_375P	2.06E-07	8.109	28.088	0	1	2043659	gene probable receptor-like protein kinase At5g20050 (LOC101309575)
RhK5_13474_397Q	2.38E-07	0	32.510	29.638	1	15515	gene bifunctional protein FolD 1, mitochondrial-like (LOC101309186), transcript variant X2, mRNA
Rh12GR_53908_964P	3.16E-07	42.750	25.563	0	1	19327261	gene trihelix transcription factor GT-2-like (LOC101315082)
RhK5_6822_287P	3.30E-07	0	-15.804	15.876	1	2916809	gene NADPH:adrenodoxin oxidoreductase, mitochondrial-like (LOC101302840), mRNA
RhK5_11224_499Q	5.71E-56	0	-6.840	-	2	7302217	gene25272-v1.0-hybrid_Universal_stress_protein_A-like_protein_(probable)
RhK5_3180_1001P	3.48E-31	0	-38.768	-	2	13583691	gene aspartate--tRNA ligase, cytoplasmic (LOC101292913),

				39.935			mRNA
RhK5_4154_515Q	3.52E-08	0	32.068	15.374	2	28660478	gene probable CCR4-associated factor 1 homolog 7 (LOC101295595), mRNA
RhMCRND_13148_267Q	1.61E-07	0	32.247	16.810	2	22447628	gene uncharacterized RNA-binding protein C17H9.04c (LOC101291692), mRNA
RhMCRND_13148_267P	6.55E-07	0	31.191	16.165	2	22447628	gene uncharacterized RNA-binding protein C17H9.04c (LOC101291692), mRNA
Rh12GR_21560_124Q	6.70E-15	51.134	35.913	0	3	13620810	gene probable leucine-rich repeat receptor-like protein kinase At5g49770 (LOC101315133)
Rh12GR_11351_642P	4.13E-10	0	-16.745		3	26153032	gene07909-v1.0-hybrid_30S_ribosomal_protein_S18_(probable)
Rh12GR_21282_4421P	9.29E-10	52.271	35.014	0	3	10047667	gene BTB/POZ domain-containing protein At1g04390 (LOC101302820), transcript variant X2,
RhMCRND_12360_336P	1.41E-09	-16.75	0	-	3	26403030	gene mitogen-activated protein kinase kinase kinase ANP1-like (LOC101307975), mRNA
RhK5_11520_519P	1.49E-09	46.545	32.430	0	3	12432724	gene serine/arginine repetitive matrix protein 2-like (LOC101309621), mRNA
RhK5_5078_253P	2.66E-08	31.764	32.850	0	3	13277606	gene grpE protein homolog, mitochondrial-like (LOC101297042), transcript variant X4, mRNA
RhK5_6730_852Q	1.79E-07	-	0	7.766	3	4362951	gene29695-v1.0-hybrid_60S_ribosomal_protein_L11_(similar_to)
RhMCRND_30734_1191Q	2.12E-07	44.140	29.530	0	3	11900513	gene protein MOS2 (LOC101292784), transcript variant X6, mRNA
RhK5_41_5365P	5.83E-07	-	8.012	0	3	9222475	gene dedicator of cytokinesis protein 6 (LOC101307146), mRNA
Rh12GR_5896_1257P	3.62E-59	0	-6.859	-	4	31822284	gene pentatricopeptide repeat-containing protein At5g13770, chloroplastic (LOC101298417)
RhK5_6314_381Q	8.86E-29	0	-38.603		4	12740888	gene27395-v1.0-hybrid_Putative_lipase_ROG1_(probable)
RhK5_11458_475P	9.97E-11	29.508	35.140	0	4	9151218	pentatricopeptide repeat-containing protein At1g08070-like (LOC101304287)
RhK5_570_626P	1.20E-10	15.836	32.719	-	4	32379053	gene probable inactive serine/threonine-protein kinase scy1 (LOC101307983), transcript variant X2, mRNA
Rh12GR_22138_343Q	4.45E-08	0	7.650	-26.895	4	6633474	gene nucleolar GTP-binding protein 2 (LOC101314566)
RhMCRND_17848_232Q	3.63E-07	-	-7.646	0	4	30885384	gene ubiquitin-like-specific protease 1D (LOC101309441), transcript variant X2, mRNA
Rh88_10262_172P	6.72E-07	0	27.258	12.274	4	10812786	gene ammonium transporter 1 member 1 (LOC101312623),

RhK5_9050_472Q	1.05E-07	-	17.671	0	5	9776281	gene ATP-dependent RNA helicase DHX36 (LOC101299095), mRNA
RhK5_1098_361P	1.89E-07	-	41.726	22.746	5	11113259	gene08916-v1.0-hybrid_Dentin_sialoprotein,_Precursor_(probable)
RhK5_650_2680P	7.00E-07	0	-16.814	-9.117	5	27497816	gene28663-v1.0-hybrid_ Protein_phosphatase_1_regulatory_subunit_pprA_(probable)
Rh12GR_19922_162Q	5.40E-56	9.835	2.991	0	6	34387527	gene auxin transport protein BIG (LOC101292150), mRNA
RhK5_5772_666P	1.45E-09	-	0	17.362	6	31498149	gene protein PAT1 homolog 1 (LOC101303919), mRNA
RhK5_16002_503Q	1.76E-07	24.794	19.323	-	6	15016593	gene putative protein FAR1-RELATED SEQUENCE 10 (LOC101307810), mRNA
RhK5_11991_480Q	2.86E-07	-23.323	-15.509	0	6	7022344	gene 30S ribosomal protein S31, chloroplastic (LOC101295535), mRNA
RhK5_52_1245Q	7.67E-07	-	-15.227	0	6	38693445	gene mitochondrial phosphate carrier protein 3, mitochondrial (LOC101301602), mRNA
RhMCRND_21388_203P	2.91E-07	-	0	-15.036	7	22817326	Rosa multiflora breeding line 88/124-46 black spot resistance muRdr1 gene locus, complete sequence
RhMCRND_29428_215P	7.29E-07	-	-7.426	0	7	6833028	gene protein NRT1/ PTR FAMILY 8.2-like (LOC101294024), mRNA

Table S4: Significant SNPs associated to shoot regenerated ratio

Marker	**P value**	**Genotypic effects**			**Linkage group**	**Position**	**Function**
		A:A	**A:B**	**B:B**			
RhK5_69_2438Q	5.01E-42	-0.070	0		1	278318	gene probable phosphoinositide phosphatase SAC9 LOC101296222)
RhK5_8844_469P	4.61E-12	0.334	0.533	0	1	2607022	gene02112-v1.0-hybrid_IST1-like_protein_(probable)
Rh12GR_53908_964P	2.87E-11	0.623	0.453	0	1	19327261	gene trihelix transcription factor GT-2-like (LOC101315082)
RhMCRND_10865_425Q	1.15E-10	0	0.144	-0.375	1	2617571	gene02109-v1.0-hybrid_ Transcription_factor_IIIA_(Factor_A)_(probable)
Rh12GR_26729_1408Q	3.56E-10	0	0.518	0.369	1	2224055	Gene transmembrane protein 19-like (LOC101291659)
RhK5_15431_100Q	7.26E-10	0	0.446	0.228	1	2402769	PXMP2/4 family protein 2 (LOC101306080)
Rh12GR_2555_1635P	1.17E-09	0	0.505	0.458	1	82309	gene10158-v1.0-hybrid_ Aristaless-related_homeobox_protein_(ARX) _(similar_to)

RhK5_3149_367Q	1.72E-09	0	0.247	0.473	1	2144285	gene DELLA protein GAI-like (LOC101314119)
RhK5_6600_1018P	1.98E-09	0	0.511	0.364	1	11159	gene E3 ubiquitin ligase BIG BROTHER-related (LOC101309476)
Rh12GR_28168_792Q	2.17E-09	0	0.511	0.364	1	543834	gene DNA methylation 3-like (LOC101311119)
Rh12GR_21174_1298Q	3.06E-09	0	0.499	0.409	1	7513167	gene acidic leucine-rich nuclear phosphoprotein 32-related protein (LOC101303231)
RhMCRND_8993_916Q	5.38E-09	0.478	0.348	0	1	1719838	Gene equilibrative nucleotide transporter 8 (LOC101296713)
RhMCRND_6435_375P	6.77E-09	0.459	0.240	0	1	2043659	Gene protein arginine N-methyltransferase PRMT10 (LOC101309863)
RhK5_16132_1112P	1.05E-08	0	0.453	0.32932	1	427534	Gene1-acyl-sn-glycerol-3-phosphate acyltransferase 1, chloroplastic-like
RhK5_8_7501Q	2.51E-08	0	0.454	0.231	1	2284025	Gene dnaJ homolog subfamily C GRV2 (LOC101305987)
RhK5_8293_614Q	2.62E-08	0	0.451	0.230	1	2035647	Gene probable receptor-like protein kinase At5g20050 (LOC101309575)
RhK5_570_626P	3.04E-08	0.287	0.462	0	1	1949526	Gene nudix hydrolase 19, chloroplastic (LOC101304799)
RhMCRND_35035_91P	3.07E-08	0.382	0		1	630484	NA
RhK5_8_6985Q	3.60E-08	0	0.450	0.221	1	2284025	Gene dnaJ homolog subfamily C GRV2 (LOC101305987)
RhK5_2808_664Q	3.82E-08		0.267	-0.200	1	2900933	Gene transcription factor bHLH68 (LOC101302559),
RhK5_13474_397Q	8.28E-08		0.474	0.423	1	15515	Gene bifunctional protein FolD 1, mitochondrial-like (LOC101309186), transcript variant X2
Rh12GR_28168_792P	8.62E-08	0	0.488		1	543834	Gene DNA methylation 3-like (LOC101311119)
RhK5_2319_813P	9.44E-08	0.423	0		1	14963189	gene17893-v1.0-hybrid_ Cell_division_protease_ftsH_homolog_(probable)
Rh12GR_268_1450Q	1.22E-07		0.201	-0.205	1	1433291	Gene CCR4-NOT transcription complex subunit 10 (LOC101309764)
RhK5_8_6985P	1.65E-07	0	0.451	0.209	1	2284025	Gene dnaJ homolog subfamily C GRV2 (LOC101305987)
RhK5_2209_720P	3.23E-07	0	0.284	0.462	1	2236689	palmitoyl-acyl carrier protein thioesterase, chloroplastic (LOC101292829)
RhMCRND_6327_1724Q	4.05E-07	-0.216	0.199	0	1	1362699	gene31125-v1.0-hybrid_ Probable_receptor-like_protein_kinase_ At5g59700,_Precursor
RhK5_3288_1105Q	4.40E-07	-0.205	0.212	0	1	1668003	Gene glucan endo-1,3-beta-glucosidase 14 (LOC101315175)
RhK5_4154_515Q	5.24E-11	0	0.510	0.317	2	28660478	Gene probable CCR4-associated factor 1 homolog 7 (LOC101295595)
RhMCRND_13148_267Q	1.96E-10	0	0.505	0.318	2	22447628	Gene uncharacterized RNA-binding protein C17H9.04c

							(LOC101291692)
RhK5_10777_1068Q	9.14E-08	0.221	0.455	0	2	28460198	Gene allene oxide synthase (LOC101312801)
RhK5_12835_275P	3.43E-07	0	-0.080	-0.576	2	1940388	Gene QWRF motif-containing protein 2-like (LOC101313278)
RhK5_5241_289P	6.63E-07	-0.406	0.082	0	2	4351519	Gene uncharacterized LOC101312697
Rh12GR_21282_4421P	1.56E-11	0.736	0.533	0	3	10047667	Gene BTB/POZ domain-containing protein At1g04390 (LOC101302820), transcript variant X2, misc_RNA
RhK5_5078_253P	1.06E-09	0.507	0.495	0	3	13277606	Gene grpE protein homolog, mitochondrial-like (LOC101297042), transcript variant X4
RhK5_7232_851P	5.10E-09	0.526	0.480	0	3	14803678	Gene putative axial regulator YABBY 2 (LOC101307367)
RhMCRND_9379_1315Q	3.50E-08	0.513	0.384	0	3	18138098	Gene ethylene-responsive transcription factor RAP2-7-like (LOC101295120)
RhK5_10985_137P	6.29E-08		0.369	0	3	11740811	Gene early nodulin-like protein 3 (LOC101299560)
Rh12GR_21560_124Q	8.10E-08	0.650	0.473	0	3	13620810	Gene probable leucine-rich repeat receptor-like protein kinase At5g49770 (LOC101315133)
Rh12GR_15592_504P	2.75E-07	0.452	0		3	10280224	gene10374-v1.0-hybrid_Probable_leucine-rich_repeat_receptor-like_protein_kinase_At2g33170,_Precursor_(putative)
RhK5_2003_1038Q	3.51E-07	-0.468	0.152	0	3	29388443	Gene vacuolar cation/proton exchanger 5-like (LOC101296644), transcript variant X1
RhK5_20938_917P	3.84E-07	0	0.410		3	11227847	gene03256-v1.0-ybrid_Golgin_subfamily_A_member_2_(probable)
Rh12GR_1195_716Q	3.94E-07	0.622	0.467	0	3	9558174	uncharacterized LOC101290940
RhMCRND_26644_241Q	7.57E-07	0	0.462		3	16375733	Gene vicilin-like antimicrobial peptides 2-2 (LOC101293839)
Rh12GR_22138_343Q	4.13E-09	0	0.053	-0.461	4	6633474	NA
RhK5_15232_250P	1.76E-07	0	0.336		4	31617028	Gene non-functional NADPH-dependent codeinone reductase 2-like (LOC101313111)
RhMCRND_375_2859P	2.59E-07	0	0.166		4	8390220	Gene probable bifunctional methylthioribulose-1-phosphate dehydratase/enolase-phosphatase E1 1 (LOC101301667), transcript variant X2
RhK5_7039_1185Q	5.68E-26	0	0.065		5	12765184	gene10659-v1.0-hybrid_Phosphatidylinositol-4-phosphate_5-kinase_5_(AtPIP5K5)_(probable)
RhK5_1760_733P	1.64E-11	0.522	0		5	10233569	Gene NF-X1-type zinc finger protein NFXL1 (LOC101300822)

RhK5_9268_616Q	1.46E-10	0	0.527	0.486	5	17437885	gene29327-v1.0-hybrid_2',3'-cyclic-nucleotide_2'-phosphodiesterase,_Precursor_(probable)
RhK5_9894_454Q	1.05E-09		0.452	0	5	9982041	Gene golgin candidate 2 (LOC101294472)
RhK5_6094_1216P	9.83E-08	0.445	0.447	0	5	27768768	Gene VID27-like protein (LOC101314755)
Rh12GR_8077_1243Q	3.42E-07	0.389	0		5	26599027	Gene RING-H2 finger protein ATL54-like (LOC101301878)
RhK5_8158_242P	1.45E-10	0	0.528	0.396	6	21083448	Gene sec1 family domain-containing protein MIP3 (LOC101310332)
Rh12GR_10115_1299P	5.54E-08		0	0.450	6	36850178	Gene phosphoglucan phosphatase LSF1, chloroplastic (LOC101294692)
Rh12GR_51628_738P	1.29E-07	0.525	0.501	0	6	32105969	gene01203-v1.0-hybrid_NADH-quinone_oxidoreductase_subunit_C/D_(probable)
Rh12GR_51628_738Q	1.59E-07	0.522	0.483	0	6	32105969	gene01203-v1.0-hybrid_NADH-quinone_oxidoreductase_subunit_C/D_(probable)
RhK5_145_1950P	1.64E-07	0	0.471	0.630	6	34576352	Gene protein LONGIFOLIA 1 (LOC101295071)
Rh12GR_10683_924P	2.51E-11	0	0.524	0.328	7	9007811	gene04777-v1.0-hybrid_ Late_cornified_envelope_protein_1E_(probable)
RhK5_1507_416Q	2.55E-09	0	0.473	0.654	FvbUn	927961	Gene transmembrane 9 superfamily member 5 (LOC101304944)
Rh12GR_47076_193Q	8.22E-09	-0.211	0.270	0	NA		NA

Table S5: Overlapped SNP markers significantly associated with regenerartion rate and shoot ratio

Markers	Linkage groups	Site (bp)	Function
RhK5_13474_397Q	1	15515	gene bifunctional protein FolD 1, mitochondrial-like (LOC101309186), transcript variant X2, mRNA
Rh12GR_21174_1298Q	1	7513167	gene acidic leucine-rich nuclear phosphoprotein 32-related protein (LOC101303231),
RhK5_8_6985Q	1	2284025	gene dnaJ homolog subfamily C GRV2 (LOC101305987)
RhK5_3149_367Q	**1**	**2144285**	**gene DELLA protein GAI-like (LOC101314119), mRNA**
Rh12GR_28168_792Q	**1**	**543834**	**DNA methylation 3-like (LOC101311119)**
Rh12GR_53908_964P	**1**	**19327261**	**gene trihelix transcription factor GT-2-like (LOC101315082)**
Rh12GR_2555_1635P	1	82309	gene uncharacterized LOC101305502 (LOC101305502), transcript variant X2,
RhMCRND_6435_375P	1	2043659	Gene protein arginine N-methyltransferase PRMT10 (LOC101309863)
RhK5_69_2438Q	1	278318	gene probable phosphoinositide phosphatase SAC9 (LOC101296222), mRNA
Rh12GR_26729_1408Q	1	2224055	Gene transmembrane protein 19-like (LOC101291659)
RhK5_8293_614Q	**1**	**2035647**	**probable receptor-like protein kinase At5g20050 (LOC101309575)**
RhK5_8844_469P	1	2607022	gene02112-v1.0-hybrid_IST1-like_protein_(probable)
RhK5_8_7501Q	1	2284025	dnaJ homolog subfamily C GRV2 (LOC101305987)
RhMCRND_13148_267Q	2	22447628	gene uncharacterized RNA-binding protein C17H9.04c (LOC101291692)
RhK5_4154_515Q	2	28660478	Gene probable CCR4-associated factor 1 homolog 7 (LOC101295595)
Rh12GR_21560_124Q	**3**	**13620810**	**Gene probable leucine-rich repeat receptor-like protein kinase At5g49770 (LOC101315133)**
Rh12GR_21282_4421P	3	10047667	gene BTB/POZ domain-containing protein At1g04390 (LOC101302820), transcript variant X2, misc_RNA
RhK5_5078_253P	3	13277606	gene grpE protein homolog, mitochondrial-like (LOC101297042), transcript variant X4, mRNA
Rh12GR_22138_343Q	4	6633474	nucleolar GTP-binding protein 2 (LOC101314566)
RhK5_570_626P	4	32379053	gene probable inactive serine/threonine-protein kinase scy1 (LOC101307983), transcript variant X2, mRNA

Table S6: The SNP markers linked with the candidate genes of shoot morphogenesis with p value are exceeded the threshold in association mapping

Markers	Linkage groups	Site (bp)	p- value	Function
RhMCRND_6327_1724Q	1	1362699	1.21E-04	gene31125-v1.0-hybrid_ Probable_receptor-like_protein_kinase_At5g59700,_Precursor
RhK5_3066_1552Q	6	5912176	3.46E-04	gene13875-v1.0-hybrid_ Probable_receptor-like_protein_kinase_At3g55450
RhK5_3066_1552Q	1	2035647	8.18E-05	gene30977-v1.0-hybrid_ Probable_receptor-like_protein_kinase_At5g20050,_Precursor_(similar_to)
RhMCRND_23732_326Q	5	6603507	1.91E-05	gene30834-v1.0-hybrid_ Homeobox_protein_knotted-1-like_3_(similar_to)
RhK5_7232_851P	3	14803678	9.45E-06	gene22887-v1.0-hybrid_ Putative_axial_regulator_YABBY_2_ (similar_to)
Rh12GR_15592_1555P	3	10280224	5.32E-04	gene10374-v1.0-hybrid_ Probable_leucine-rich_repeat_receptor-like_protein_kinase_ At2g33170, _Precursor_(putative)
Rh12GR_54604_428Q	3	13654098	1.06E-04	gene03532-v1.0-hybrid_ Probable_leucine-rich_repeat_receptor-like_protein_kinase _At5g49770, _Precursor_(similar_to)

Table S7: The position of SNPs marker linked to known candidate genes of plant regeneration in *Fragaria vesca* genomes

Gene	SNPs	E value	Position	LG	Gene prediction
RPK	Rh12GR_14922_1495	0	794934	1	gene30859-v1.0-hybrid_ Probable_leucine-rich_repeat _receptor-like_protein_kinase _At5g61480,_Precursor
SERK1	Rh12GR_1608_1034	0	2440222	1	gene30893-v1.0-hybrid _ Somatic_embryogenesis _receptor_kinase_1_(AtSERK1), _Precursor_(similar_to)
SERK1	RhK5_11506_1455	0	2440864	1	gene30893-v1.0-hybrid_ Somatic_embryogenesis _receptor_kinase_1_(AtSERK1), _Precursor_(similar_to)
WUS	RhK5_5737_1045	0	5522291	1	gene13035-v1.0-hybrid _ WUSCHEL-related_homeobox _13_(putative)
YABBY	RhK5_6546_136	6.00E-60	1700128	1	gene31056-v1.0-hybrid_ Axial_regulator_YABBY_5 _(similar_to)
LLR-RLK	RhMCRND_1994_1513	0	794278	1	gene30859-v1.0-hybrid _Probable_leucine-rich_repeat _receptor-like_protein_kinase _At5g61480,_Precursor
KNAT1	RhMCRND_22932_282	1.00E-52	898263	1	gene30834-v1.0-hybrid _Homeobox_protein_knotted -1-like_3_(similar_to)

CUC2	RhMCRND_5472_322	0	20423033	1	gene20686-v1.0-hybrid_ Protein_CUP-SHAPED_ COTYLEDON_2_(ANAC098) _(similar_to)
WUS	RhMCRND_8675_603	0	5521743	1	gene13035-v1.0-hybrid _ WUSCHEL-related_ homeobox_13_(putative)
WUS	Rh12GR_12387_540	1.00E-143	25005749	2	gene09136-v1.0-hybrid _ WUSCHEL-related_ homeobox_4_(putative)
RPK	Rh12GR_12647_3549	0	22206058	2	gene11629-v1.0-hybrid_ Probable_receptor-like_ protein_kinase_At5g20050, _Precursor
SERK1	Rh12GR_14184_412	1.00E-105	14519925	2	gene17120-v1.0-hybrid_ Somatic_embryogenesis_ receptor_kinase_1_(AtSERK1) ,_ Precursor_(probable)
SERK2	Rh12GR_2200_1059	0	3428758	2	gene27511-v1.0-hybrid_ Somatic_embryogenesis_ receptor_kinase_2_(AtSERK2), _ Precursor_(putative)
RPK	Rh12GR_38687_506	0	22209645	2	gene11630-v1.0-hybrid_ Probable_receptor-like_ protein_kinase_At5g20050,_ Precursor
LLR-RLK	Rh12GR_48138_145	1.00E-93	17476469	2	gene08310-v1.0-hybrid _Probable_leucine-rich_repeat _receptor-like_protein_kinase _ At1g35710,_Precursor
LLR-RLK	RhK5_397_1112	0	17657796	2	gene08337-v1.0-hybrid _ Probable_leucine-rich_ repeat_receptor-like_protein_ kinase_ At5g61480,_Precursor
LLR-RLK	RhK5_610_1276	0	17528289	2	gene08315-v1.0-hybrid_ Probable_leucine-rich_repeat _receptor-like_protein_kinase _At1g35710,_Precursor
CUC3	RhK5_8502_186	e-123	17167416	2	gene08112-v1.0-hybrid _Protein_CUP-SHAPED_ COTYLEDON_3_(ANAC031) _(probable)
KNAT1	RhMCRND_20164_1189	0	21842854	2	gene30834-v1.0-hybrid_ Homeobox_protein_knotted-1 -like_3_(similar_to)
SERK1	Rh12GR_10927_1180	1.00E-131	14892994	3	gene23148-v1.0-hybrid_ Somatic_embryogenesis_ receptor_kinase_1_(AtSERK1) , _Precursor_(probable)
RPK	Rh12GR_15592_1122	0	10280224	3	gene10374-v1.0-hybrid_ Probable_leucine-rich_repeat _receptor-like_protein_kinase _At2g33170,_ Precursor_(putative)
LLR-RLK	Rh12GR_17873_899	1.00E-175	8503095	3	gene16840-v1.0-hybrid_ Probable_leucine-rich_repeat _receptor-like_protein_kinase_ At1g35710,_Precursor

WUS	Rh12GR_27771_1412	1.00E-127	18481791	3	gene27205-v1.0-hybrid_ WUSCHEL-related_ homeobox_8_(probable)
PKL	Rh12GR_31415_698	0	7497488	3	gene20001-v1.0-hybrid_ CHD3-type_chromatin-remodeling_factor_ PICKLE_(probable)
STM	Rh12GR_3167_1116	1.00E-112	701728	3	gene19507-v1.0-hybrid_ Homeobox_protein_ SHOOT_MERISTEMLESS_ (similar_to)
SERK2	RhK5_100_122	0	21873804	3	gene27511-v1.0-hybrid_ Somatic_embryogenesis_ receptor_kinase_2_(AtSERK2) , _Precursor_(putative)
SERK4	RhK5_19889_699	1.00E-48	30106441	3	gene00174-v1.0-hybrid_ Somatic_embryogenesis_ receptor_kinase_4_ (AtSERK4) ,_Precursor_(probable)
PKL	RhK5_38_1418	0	7501632	3	gene20001-v1.0-hybrid_ CHD3-type_chromatin-remodeling_factor_PICKLE_ (probable)
RPK	RhK5_447_1149	0	6089985	3	gene28878-v1.0-hybrid_ Probable_receptor-like_protein _kinase_At1g30570,_ Precursor_(similar_to)
PKL	RhK5_6196_213	1.00E-157	7497428	3	gene20001-v1.0-hybrid_ CHD3-type_chromatin-remodeling_factor_PICKLE_ (probable)
RPK	RhK5_7412_1133	0	5084615	3	gene29734-v1.0-hybrid_ Receptor-like_protein_kinase _At3g21340,_ Precursor_ (probable)
PKL	RhMCRND_30_1017	0	7498849	3	gene20001-v1.0-hybrid_ CHD3-type_chromatin-remodeling_factor_PICKLE _(probable)
STM	RhMCRND_3683_1367	1.00E-117	701728	3	gene19507-v1.0-hybrid_ Homeobox_protein_ SHOOT_MERISTEMLESS_ (similar_to)
PKL	RhMCRND_8996_924	0	7496980	3	gene20001-v1.0-hybrid_ CHD3-type_chromatin-remodeling_factor_PICKLE _(probable)
RPK	Rh12GR_17924_227	1.00E-145	30836650	4	gene23604-v1.0-hybrid_ Probable_receptor-like_ protein_kinase_At5g39030, _Precursor_(similar_to)
LLR-RLK	Rh12GR_2716_138	0	4577512	4	gene20751-v1.0-hybrid_ Probable_leucine-rich_repeat_ receptor-like_protein_kinase_ At2g33170,_Precursor_(putative)
ERS	RhK5_11738_331	2.00E-86	27445495	4	gene03871-v1.0-hybrid_ AP2/ERF_and_B3_domain-containing_transcription_repressor

					TEM1(putative)
SERK1	RhK5_5029_351	1.00E-127	25626641	4	gene07257-v1.0-hybrid_ Somatic_embryogenesis_receptor _kinase_1_ (AtSERK1),_ Precursor_(similar_to)
LLR-RLK	RhMCRND_3061_1175	0	30177395	4	gene04150-v1.0-hybrid_ Probable_leucine-rich_repeat_ receptor-like_protein_kinase_ At1g35710,_Precursor
HDA5	Rh12GR_10356_1095	1.00E-114	8037579	5	gene25808-v1.0-hybrid_ Histone_deacetylase_5_ (similar_to)
WUS	Rh12GR_31633_585	4.00E-80	19375585	5	gene20491-v1.0-hybrid_ WUSCHEL-related_homeobox_8_ (similar_to)
LLR-RLK	Rh12GR_52199_269	9.00E-54	17704152	5	gene29278-v1.0-hybrid_ Probable_leucine-rich_repeat_ receptor-like_protein_kinase_ At5g49770,_Precursor
RPK	RhK5_13032_161	0	8374569	5	gene26060-v1.0-hybrid_ Probable_receptor-like_protein_ kinase_At5g61350,_ Precursor_(similar_to)
WUS	RhMCRND_11067_872	1.00E-143	19375654	5	gene20491-v1.0-hybrid_ WUSCHEL-related_homeobox_8_ (similar_to)
RPK	Rh12GR_14608_3029	0	18436380	6	gene25207-v1.0-hybrid_ Probable_leucine-rich_repeat_receptor-like_protein_kinase_At2g33170, _Precursor
LLR-RLK	Rh12GR_16897_552	0	2683473	6	gene12902-v1.0-hybrid_ Leucine-rich_repeat_receptor-like_protein_kinase_ PEPR2_(PEP1_receptor_2), _Precursor_(probable)
SERK2	Rh12GR_17307_454	0	25872793	6	gene23091-v1.0-hybrid_ Somatic_embryogenesis_ receptor_kinase_2_(AtSERK2),_ Precursor_(probable)
BBM1	Rh12GR_2750_155	0	76528	6	gene21524-v1.0-hybrid_ AP2-like_ethylene-responsive_ transcription_factor _BBM1_(BnBBM1) _(similar_to)
CUC1	RhK5_11560_168	0	19983259	6	gene17720-v1.0-hybrid_ Protein_CUP-SHAPED_ COTYLEDON_1_(ANAC054) _(probable)
CUC1	RhK5_15454_453	1.00E-167	19981953	6	gene17720-v1.0-hybrid_ Protein_CUP-SHAPED_ COTYLEDON_1_ (ANAC054)_(probable)
LLR-RLK	RhK5_339_1717	0	5353489	6	gene13646-v1.0-hybrid_ Probable_leucine-rich_repeat_ receptor-like_protein_kinase_ At5g49770,_Precursor
LLR-RLK	RhK5_6494_1553	0	859821	6	gene16693-v1.0-hybrid_ Probable_leucine-rich_repeat_

					receptor-like_protein_kinase_ At5g61480,_Precursor
CUC1	RhMCRND_7775_1226	0	19982653	6	gene17720-v1.0-hybrid_ Protein_CUP-SHAPED_ COTYLEDON_1_(ANAC054) _(probable)
CUC1	RhMCRND_9571_1050	1.00E-169	19982402	6	gene17720-v1.0-hybrid_ Protein_CUP-SHAPED_ COTYLEDON_1_ (ANAC054)_(probable)
SERK2	Rh12GR_2826_1128	0	10380535	7	gene19419-v1.0-hybrid_ Somatic_embryogenesis_receptor_ kinase_2_ (AtSERK2),_ Precursor_(probable)
CUC1	RhK5_4129_645	1.00E-101	14657010	7	gene18589-v1.0-hybrid_ Protein_CUP-SHAPED_ COTYLEDON_1_ (ANAC054)_(probable)
SERK2	RhK5_5539_1258	0	10379646	7	gene19419-v1.0-hybrid_ Somatic_embryogenesis_receptor _kinase_2_ (AtSERK2),_ Precursor_(probable)
LLR-RLK	RhMCRND_25528_1622	1.00E-148	12586984	7	gene19262-v1.0-hybrid_ Probable_ leucine-rich_repeat_ receptor-like_protein_kinase_ At5g49770,_Precursor

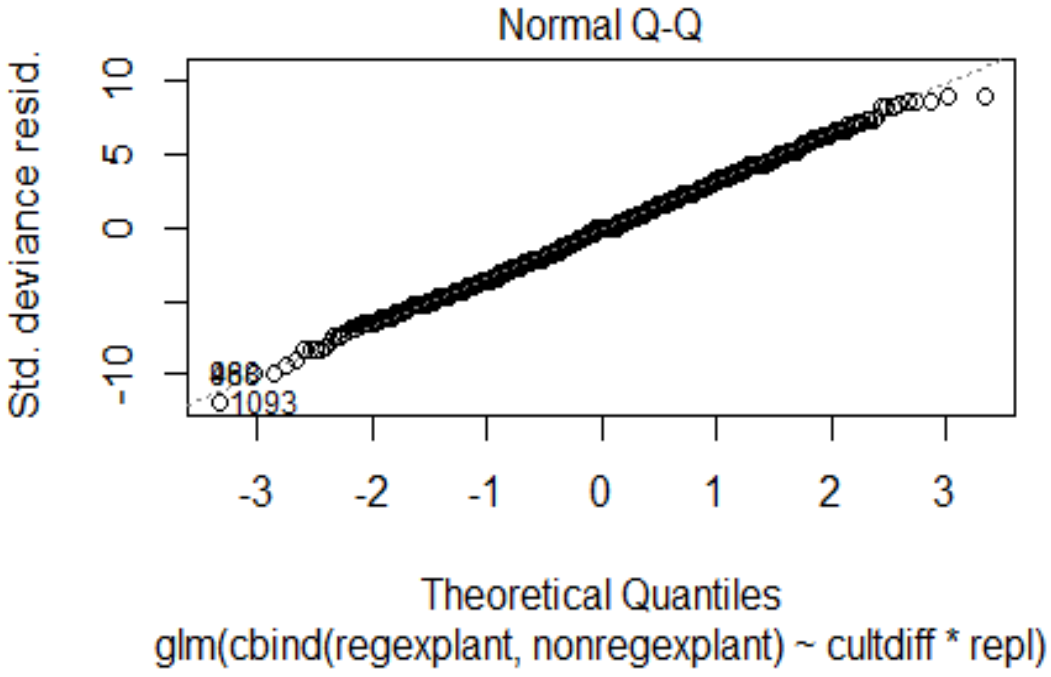

Fig S1: The distribution analysis of regeneration rate

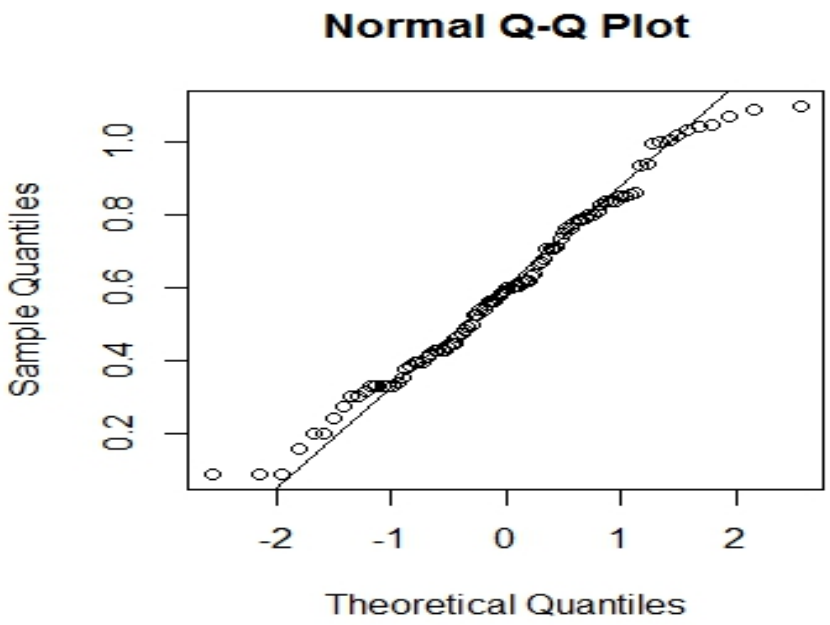

Fig S1: The distribution analysis of shoot ratio

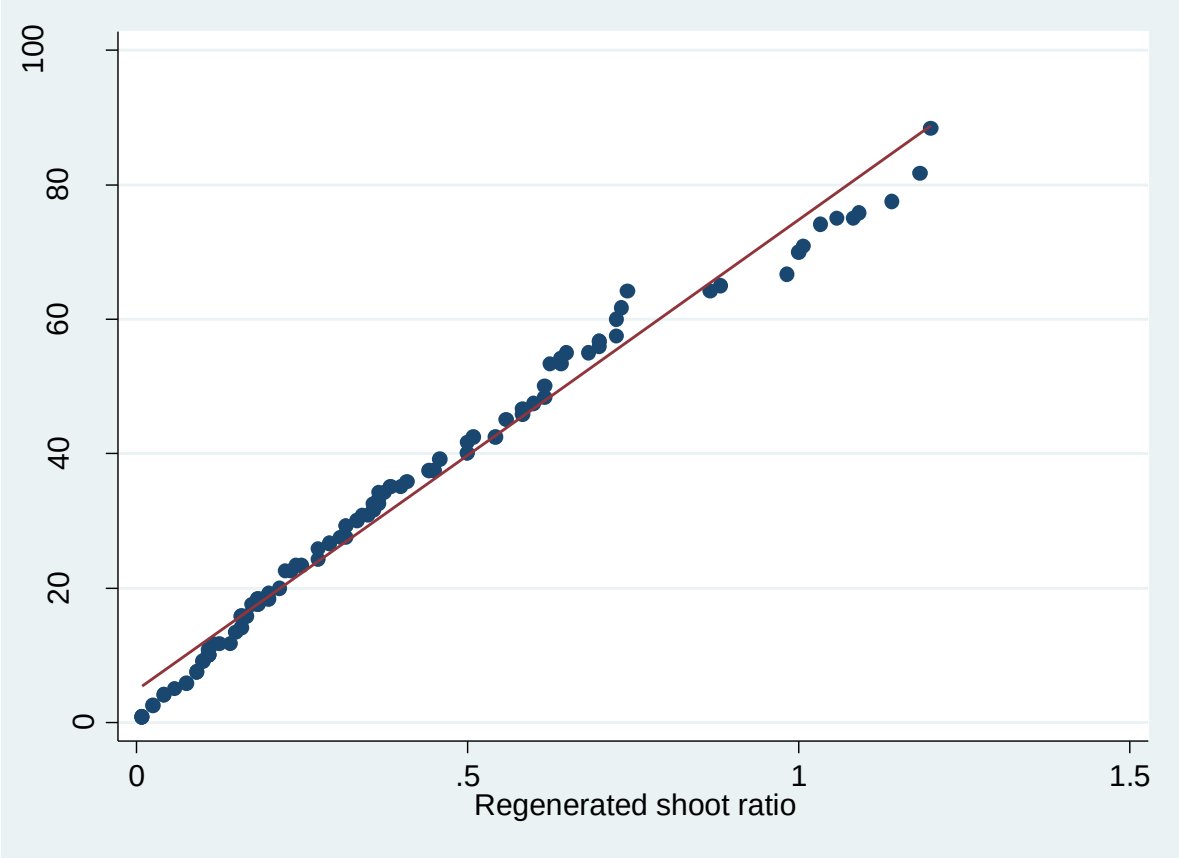

Fig. S3: Scatter plot of regeneration rate versus shoot ratio (Pearsons correlation coefficient r= 0.9908)

3.2 Development of markers for shoot organogenesis in roses

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Type of authorship: First author

Type of article: Research article

Contribution to the article: Planned and performed the experiments, completed the statistical analysis and wrote most of the manuscript.

Contribution of other authors: Traud Winkelmann contributed to the experimental setup and wrote part of the manuscript.
Thomas Debener was involved in planning the experiments and wrote parts of the manuscript.

Journal: *Acta Hortic*

Date of publication: February 2019

DOI: 10.17660/ActaHortic.2019.1232.2

Development of markers for shoot organogenesis in roses

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Abstract

Shoot organogenesis is an essential step for genetic engineering and the study of developmental processes in roses. Several adventitious shoot regeneration protocols have been established, but the number of responding cultivars is very limited. Furthermore, large differences in regeneration capacity between genotypes were reported rendering many cultivars as recalcitrant for shoot regeneration. Therefore, knowledge about the genetic complexity of the capacity for adventitious shoot regeneration and genes influencing this trait would be helpful for optimising regeneration protocols. Previously we analysed an association panel of 96 genotypes for in vitro shoot regeneration from petioles, and we detected a number of markers associated with the phenotypic traits. Here we present results of experiments to verify significantly associated markers in independent populations of garden roses using the KASP (Kompetitive Allele Specific PCR) technology.

Keywords: in vitro, KASP assay, *Rosa hybrida*, shoots regeneration, SNP markers

INTRODUCTION

Shoot regeneration via organogenesis or somatic embryogenesis is a critical step in plant propagation and genetic engineering as well as in the study of developmental processes in plants. Understanding the molecular factors involved in shoot regeneration will help to improve the regeneration capacity of the plants. Over the last years, a number of factors related to shoot regeneration such as protein kinases, hormone signaling, transcription factors, and epigenetic factors have been exploited (Neelakandan and Wang, 2012). For example, leucine-rich repeat receptor kinases (LRR-RKs) play a prominent role for developmental and defense-related processes such as cell proliferation, stem cell maintenance, hormone perception, defence responses, wounding responses, and symbiosis (Torii, 2004). Somatic embryogenesis receptor kinases (SERK) have a demonstrated function in plant embryogenesis (Talapatra et al., 2014; Li et al., 2015). Transcription factors of the Apetala2/Ethylene Response Factor (*AP2/ERF*) family are involved in the regulation of somatic embryogenesis and developmental processes (Piyatrakul et al., 2012; Licausi et al., 2013). The other members of this gene family such as *BABYBOOM*, *EMBRYOMAKER*, *LEAFY COTYLEDON* (*LEC*), *CUP SHAPE COTYLEDON* (*CUC*) and *WUSCHEL* play a role in regulation of embryogenesis, organ development and shoot regeneration (Daimon et al., 2003; Gaj et al., 2005; Tsuwamoto et al., 2010; Florez et al., 2015; Zhang et al., 2015). Another gene family, GT2-like trihelix transcription factors, are involved in developmental processes such as embryogenesis and the formation of perianth organs or trichomes (Barr et al., 2012; Kaplan-Levy et al., 2012). Hormone signalling PIN (Adamowski and Friml, 2015) and PICKLE affects chromatin remodelling (Zhang et al., 2014) and shoot regeneration. However, the regeneration of plant is controlled by complex regulatory mechanisms, and different types of regeneration are triggered under different circumstances (Xu and Huang, 2014).

Roses are among the commercially most important ornamental plants. Shoot organogenesis omitting the callus phase has been pursued in modern breeding of rose as well as a method for research purposes (Dubois et al., 2000; Ibrahim and Debergh, 2001; Pati et al., 2004; Haghighat et al., 2011; Pourhosseini et al., 2013). In these studies, the focus

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Table 1. Comparison of homozygous genotypes of the association panel consisting of 96 rose genotypes detected with the Axiom WagRhSNP assay and the KASP assay. Genotypes for which the KASP assay did not confirm the SNP results are indicated by italics.

Primer	Homozygous genotypes: A:A		Homozygous genotypes: B:B	
	Axiom WagRhSNP array	KASP assay	Axiom WagRhSNP array	KASP assay
RK124Q	Mitsouko	Mitsouko	Rumba	Rumba
	Auslo	Auslo	Lipstick	Lipstick
	Arthur Bell	Arthur Bell	Lavender Lassie	Lavender Lassie
	Compassion	Compassion	Juanita	Juanita
	Rose de Resht	Rose de Resht	Simply	Simply
	Berolina	Berolina	Comtessa Al	Comtessa Al
	Frühlingduft	Frühlingduft	Arabia	Arabia
	Fritz Nobit	Fritz Nobit	My girl	My girl
	Crimson Glory	Crimson Glory	Kronjuwel	Kronjuwel
	Sunset Boulevard	Sunset Boulevard	Kastelruther Spatzen	Kastelruther Spatzen
			Jazz	Jazz
TF964P	Parole	Parole	Jasmina	Jasmina
	Blue Parfum	Blue Parfum	Sommerwind	Sommerwind
	Mister Lincoln	Mister Lincoln	La Sevillana	La Sevillana
	Sutters Gold	Sutters Gold	Perennial Blush	Perennial Blush
	Rose de Resht	Rose de Resht	Lipstick	Lipstick
	Elfe	Elfe	Arabia	Arabia
	Westerland	Westerland	Dortmund	Dortmund
	Frühlingsduft	Frühlingsduft	Raubritter	Raubritter
	Friesia	Friesia	Juanita	Juanita
	Herkules	Herkules	Simply	Simply
	Climbing Allgold		Cute Haze	Cute Haze
			Stadt Rom	Stadt Rom
			Bienenweide	Bienenweide

Statistical analysis

The regeneration rate of the tested genotypes was statistically analysed for effect of marker genotypes by the non-parametric Kruskal-Wallis rank-sum test with the R software package version 3.2.5 (The R-Foundation for Statistical Computing, 2016).

RESULTS AND DISCUSSION

Validation of genotyping by SNP markers using the KASP assay in the association panel consisting of 96 rose genotypes

The sequences of SNP markers of TF964P and RK124Q that were found to be associated with shoot organogenesis in our previous study (Nguyen et al., 2017) were used to design KASP assays. Firstly both KASP markers were validated in the original association panel of 96 rose genotypes (Figure 1). For both markers cluster positions between individuals varied slightly probably due to technical reasons in the marker analysis. Whereas Marker TF964P had homozygotes clearly separated from the heterozygotes, Marker RK124Q had only one homozygote class clearly separated from the heterozygotes. The second homozygous group (marked in red) formed the upper end of a common cluster with the heterozygotes. However, genotypes homozygous for each of the markers in the Axiom WagRhSNP assay were also homozygous in the KASP assay. The position of the heterozygotes slightly differed between KASP assay and Axiom WAgRhSNP but in both assays they fell into the heterozygous clusters. The results therefore indicate that the KASP assay of these SNP markers can be used in other populations of rose to screen for

was on optimising the culture conditions and the genetic variation of shoot regeneration of a relatively small number of cultivars. In our previous study, shoot organogenesis was carried out in a population of 96 rose genotypes, and genetic factors influencing shoot organogenesis were analyzed by association mapping using a large set of SNP markers (Nguyen et al., 2017). In this study (Nguyen et al., 2017), 88 SNP markers that are significantly associated with shoot regeneration from the petiole were detected. The best SNP markers that were located in the genes for a trihelix transcription factor GT2-like (Rh12GR_53908_964P), and a putative leucine-rich repeat receptor-like protein kinase (Rh12GR_21560_124Q) might also be interesting candidates to be tested for their role in organogenesis of roses. In order to develop markers suitable for marker-assisted selection for shoot organogenesis in rose, these SNP markers need to be validated in independent rose genotypes. KASP (Kompetitive Allele Specific PCR) is SNP genotyping assay based on dual FRET (Fluorescent Resonant Energy Transfer) to analyse the dosage of alleles in biallelic SNPs. Due to its high throughput, robustness and cost effectiveness, KASP has been used in massive SNP genotyping in rice (McCouch et al., 2010), wheat (Neelam et al., 2013), soybean (Shi et al., 2015; Patil et al., 2017), peanut (Zhao et al., 2017). In this study, we used the KASP assays to validate two SNP-markers for shoot organogenesis in rose, trihelix transcription factor GT2-like (Rh12GR_53908_964P), and a putative leucine-rich repeat receptor-like protein kinase (Rh12GR_21560_124Q) in different genotypes of rose populations independent of the original association panel.

MATERIALS AND METHODS

Plant materials

The 96 rose genotypes, mostly tetraploid cultivars (87 tetraploid, 8 triploid, 1 diploid) of the association panel have been described in previous studies (Schulz et al., 2016; Nguyen et al., 2017) (Table 1). In addition, DNA of 345 tetraploid rose genotypes from various breeders and countries was extracted from plants of the rose collection of the "Bundessortenamt" (Federal Office for Variety Protection) in Hannover, Germany.

KASP assay development

The two selected SNPs, Rh12GR_53908_964P (TF964P) and Rh12GR_21560_124Q (RK124Q), which were previously shown to be associated with shoot regeneration in roses (Nguyen et al., 2017) were used for the validation assay. Sequence information of these SNPs was retrieved from the publication on the rose Axiom SNP chip (Smulders et al., 2015). Two allele-specific forward primers, along with tail sequences and one common reverse primer were synthesized for SNP genotyping assays by LGC Genomics (www.lgcgroup.com). The reaction mixture was prepared following the manufacturer's protocol with minor modifications in the number of PCR-cycles (www.lgcgroup.com/products/kasp-genotyping-chemistry/reagents). KASP assays were run with 10 µL final reaction volume containing 5 µL KASP master mix, 0.14 µL primer mix, 2 µL of 10-20 ng μL^{-1} genomic DNA and 2.86 µL H_2O. Amplification was performed on a StepOne™ Real-Time PCR System (Thermo Fisher Scientific). The following cycling conditions were used: 15 min at 95°C, followed by 15 touchdown cycles of 20 s at 94°C, 1 min at 65-57°C (dropping 0.8°C per cycle) and then further cycling of 20 s at 94°C, 30 s at 55°C was performed until genotypic clusters were sufficiently separated.

Assessment of shoot organogenesis

Shoot organogenesis experiments were carried out according to Nguyen et al. (2017). Phenotypic data were recorded as regeneration rate and calculated by the percentage of the explants regenerating at least one shoot.

Table 1. Comparison of homozygous genotypes of the association panel consisting of 96 rose genotypes detected with the Axiom WagRhSNP assay and the KASP assay. Genotypes for which the KASP assay did not confirm the SNP results are indicated by italics.

Primer	Homozygous genotypes: A:A		Homozygous genotypes: B:B	
	Axiom WagRhSNP array	KASP assay	Axiom WagRhSNP array	KASP assay
RK124Q	Mitsouko	Mitsouko	Rumba	Rumba
	Auslo	Auslo	Lipstick	Lipstick
	Arthur Bell	Arthur Bell	Lavender Lassie	Lavender Lassie
	Compassion	Compassion	Juanita	Juanita
	Rose de Resht	Rose de Resht	Simply	Simply
	Berolina	Berolina	Comtessa Al	Comtessa Al
	Frühlingduft	Frühlingduft	Arabia	Arabia
	Fritz Nobit	Fritz Nobit	My girl	My girl
	Crimson Glory	Crimson Glory	Kronjuwel	Kronjuwel
	Sunset Boulevard	Sunset Boulevard	Kastelruther Spatzen	Kastelruther Spatzen
			Jazz	Jazz
TF964P	Parole	Parole	Jasmina	Jasmina
	Blue Parfum	Blue Parfum	Sommerwind	Sommerwind
	Mister Lincoln	Mister Lincoln	La Sevillana	La Sevillana
	Sutters Gold	Sutters Gold	Perennial Blush	Perennial Blush
	Rose de Resht	Rose de Resht	Lipstick	Lipstick
	Elfe	Elfe	Arabia	Arabia
	Westerland	Westerland	Dortmund	Dortmund
	Frühlingsduft	Frühlingsduft	Raubritter	Raubritter
	Friesia	Friesia	Juanita	Juanita
	Herkules	Herkules	Simply	Simply
	Climbing Allgold		Cute Haze	Cute Haze
			Stadt Rom	Stadt Rom
			Bienenweide	Bienenweide

Statistical analysis

The regeneration rate of the tested genotypes was statistically analysed for effect of marker genotypes by the non-parametric Kruskal-Wallis rank-sum test with the R software package version 3.2.5 (The R-Foundation for Statistical Computing, 2016).

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genotype was 25%, only. The effect of marker TF964P was somewhat lower with genotypes homozygous for allele A displaying an average regeneration rate of 30%, whereas the genotypes homozygous for allele B displayed an average of 17.60% (Figures 2 and 4).

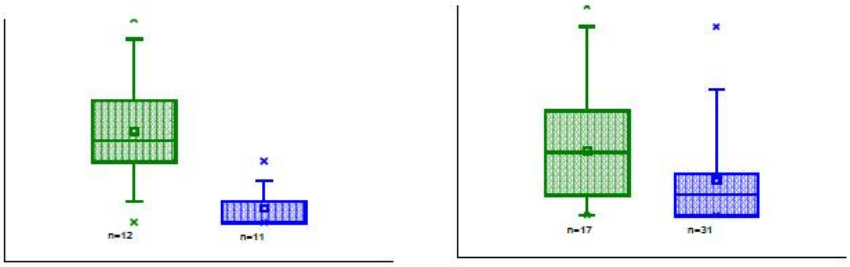

Figure 2. Box plots of shoot regeneration rates of homozygous genotypes for markers RK124Q (left) and TF964P (right) based on experiments with five biological replicates (petri dishes with 10 explants) each. Small square = mean; continuous line = median; asterisk = minimum, maximum; box = 1st and 3rd quartiles; and whisker = standard deviation.

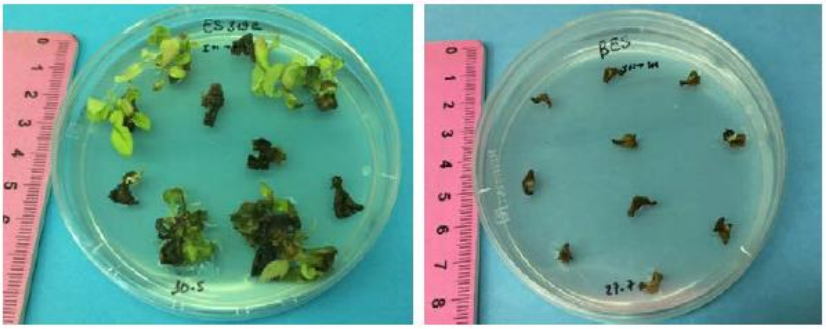

Figure 3. Examples of shoot organogenesis of genotypes homozygous for the A allele (left) and homozygous for the B allele for marker RK124Q (right).

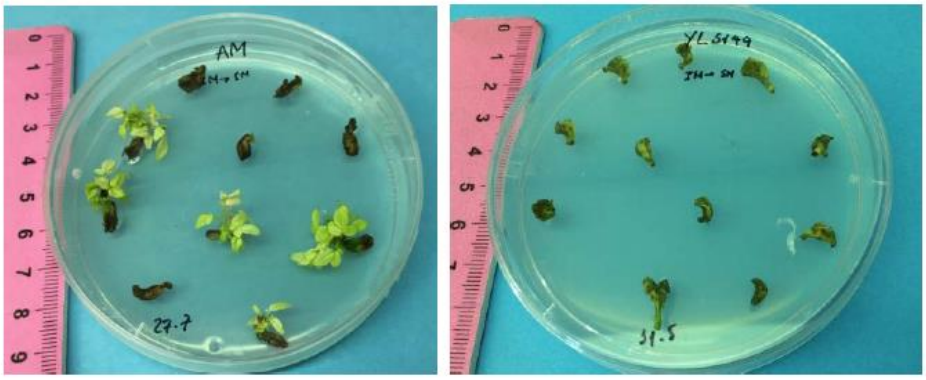

Figure 4. Examples of shoot organogenesis of genotypes homozygous for the A allele (left) and homozygous for the B allele (right) for marker TF964P.

Interestingly, 9 of the genotypes for which we performed regeneration experiments were either homozygous for the A allele or the B allele for both markers. The average regeneration rate of the nine plants homozygous for the A allele of both markers was 41.8%, whereas the nine plants homozygous for the B allele of both markers was 9.7% (Figures 5 and 6). Unexpectedly, compared to the individual results for marker RK124Q, no further increase was observed. This might be either due to the small number of available double homozygotes that we tested or to the fact that marker RK124Q is linked to a locus which is epistatic over the gene to which marker TF964P is linked. A distinction between these two possibilities can only be made in analyzing larger plant populations and segregating populations.

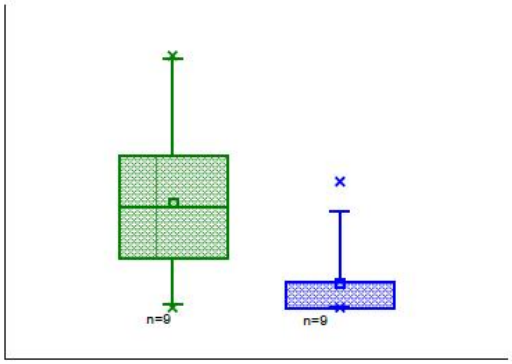

Figure 5. Box plots of shoot regeneration rates of genotypes which are homozygous for either allele A or allele B for both RK124Q and TF964P markers. The data are based on experiments with five biological replicates (petri dishes with 10 explants) each. Small square = mean; continuous line = median; asterisk = minimum, maximum; box = 1st and 3rd quartiles; and whisker = standard deviation.

Figure 6. Examples for shoot organogenesis of genotypes homozygous for the A allele (left) and homozygous for the B allele (right) for both markers, RK 124Q and TF964P.

CONCLUSIONS

The results of our KASP assay indicate that this method can be used for the selection of genotypes with significantly higher rates of shoot organogenesis in roses. In addition, the

markers may provide starting points for functional analyses of the genes causing the observed effects, because both markers are derived from expressed genes with a known function in developmental processes, the trihelix transcription factor GT2-like (Rh12GR_53908_964P), and a putative leucine-rich repeat receptor-like protein kinase (Nguyen et al., 2017). Future work may include functional studies for both genes in regeneration experiments where the expression of both genes might be altered (reduced or increased) or in which different alleles with differing effects can be expressed in low and highly regenerating genotypes.

ACKNOWLEDGEMENTS

This work was carried out with financial support of the Department of Molecular Plant breeding, Institute of Plant Genetics, Leibniz Universität Hannover, Germany. We would like to acknowledge the Federal Plant Variety Office in Hannover for providing plant materials and Dr. Helgard Kaufmann for providing plant DNA materials.

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3.3 Genetic analysis of adventitious root formation in vivo and in vitro in a diversity panel of roses

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Type of authorship: First author

Type of article: Research article

Contribution to the article: Planned and performed the *in vitro* experiments, completed the statistical analysis and wrote most of the manuscript.

Contribution of other authors: Sophia Tänzer conducted the *in vivo* rooting experiments. Jasmin Rudeck conducted the *in vitro* rooting experiments. Traud Winkelmann contributed to the *in vitro* experimental set up and wrote part of the manuscript. Thomas Debener was involved in planning the *in vivo* experiments and wrote parts of the manuscript.

Status of publication: Prepare to submit to *Frontier in Plant Science*

Genetic analysis of adventitious root formation *in vivo* and *in vitro* in a diversity panel of roses

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Abstract

In a diversity panel of 95 rose genotypes, we induced adventitious root formation under both *in vitro* and *in vivo* conditions and performed a genome-wide association study to analyse rooting performance using genotype information from the 68 K Axiom WagRhSNP chip. For each tested condition, three independent experiments were carried out. Significant variations in *in vitro* root number (ranging from 0.12 to 18.7) and total root length (0.26- 25.76 cm) as well as *in vivo* root number, root length and root biomass were recorded among the genotypes. For the *in vitro* parameters, we found 49 SNPs that were significantly associated with *in vitro* root length, whereas the other parameters did not exhibit any significant associations. For the *in vivo* parameters, we found 98 SNPs associated with root number, 218 SNPs associated with root length and 4 SNPs associated with root biomass. Some of these SNPs were located in genes with homology to rooting-related genes such as those encoding the WUSCHEL- related homeobox 8-like and ETHYLENE INSENSITIVE 3-like proteins, which were associated with *in vitro* root length, and Auxin_response_factor 19, Protein_AUXIN_RESPONSE_4, and Transcription_factor_MYC2 (AtMYC2), which were associated with *in vivo* root number and root length. We mapped the SNPs to the recently published high-quality genome sequence of the rose and detected several regions in the genome that harbour additional homologues of genes known to be related to rooting traits in other species, such as SCARECROW on chromosome 3 and WUSCHEL-related homeobox genes on chromosomes 1, 4 and 6. These markers will serve as the starting point for future experiments to validate the genes in other populations and examine their functionality in transgenic approaches.

Introduction

The rose is one of the most important plants in the floriculture industry because of its beauty and elegance. It is used not only for ornamental purposes but also in food, pharmaceutical products and perfumes (Debener and Linde 2009a). Roses belong to the genus *Rosa* L., comprising approximately 200 species and more than 20,000 cultivars (Pacurar et al. 2014a; Wissermann and Ritz 2005). Most commercial rose cultivars are tetraploid, exhibit a complex hybrid origin with wide phenotypic variability and are highly heterozygous (Kirov et al. 2014b). Due to intensive interspecific hybridizations, modern rose cultivars often present low fertility; thus, breeders face various levels of sterility in rose propagation (Koning-Boucoiran et al. 2012; Pipino et al. 2011). The conventional methods for propagating rose cultivars are cutting, budding and grafting. In addition, *in vitro* propagation is becoming increasingly popular for certain genotypes and is widely used for large-scale plant multiplication of rose in some parts of the world (Pati et al. 2006).

Vegetative propagation is often employed for the multiplication of highly heterozygous outcrossing crop species and is an important tool for the propagation of valuable plants, especially in horticulture

and forestry. This method is effective for maintaining desirable characteristics in superior rose cultivars, especially since commercial varieties are generally highly heterozygous and polyploid (Nasri et al. 2015). However, there are still some problems resulting from the strong genotypic differences in rooting ability among rose cultivars (Dubois and Vries 1991). Many studies have been conducted on physiological parameters influencing rooting in roses, but no information is available on the molecular mechanisms of adventitious root formation. Adventitious root formation is an essential step not only for the propagation of cuttings but also for grafting on unrooted rootstock stems (stenting). Adventitious roots (ARs) provide structural support and contribute to water and nutrient absorption, and they can be induced by stresses such as wounding, flooding, or etiolation (Davis and Haissig 1994; Steffens and Rasmussen 2016). The induction of ARs is a complex process regulated by multiple environmental and endogenous factors (Bellini et al. 2014; Díaz-Sala 2014; Druege et al. 2016).

In roses, auxins, especially IBA, are widely used for accelerating the formation of adventitious roots in certain cultivars under both *in vitro* and *in vivo* conditions (Ahmadi 2012; Dubois and Vries 1991; Khatik and Mishra 2017; Pierik 1997a; Z.A. Rather and Tsewang Tamchos 2017). Other auxins such as IAA and NAA are also used for rooting in some rose genotypes (Akhtar et al. 2015a; Monder and Pacholczak 2017). In addition to auxins, AR formation in rose is influenced by other chemicals, such as citric acid and malic acid applied foliarly, and by light quality (Ghazijahani et al. 2017; Pawłowska et al. 2017).

Recently, progress has been made in exploiting several factors involved in adventitious root formation, among which auxin is known to play a central role (Haissig and Davis 1994; Pacurar et al. 2014a; Pacurar et al. 2014b; Pop et al. 2011). Other phytohormones such as ethylene can also promote or accelerate rooting (Negi et al. 2010; Niu et al. 2013; Santisree et al. 2011; Santisree et al. 2012) whereas gibberellins inhibit adventitious root induction but stimulate subsequent root elongation (Niu et al., 2013). However, knowledge of the function and control of plant hormone homeostasis and the intricate signalling network of these hormones during AR formation is still fragmented. Molecular studies on root formation recently showed many transcription factors to be involved in the formation and development of ARs, such as the AP2/ERF, INTEGUMENTA-like (AtAIL), and WUSCHEL- related homeobox (WOX) transcription factors (Trupiano et al. 2013);(Hu and Xu 2016, 2016; Liu et al. 2014a; Liu et al. 2014b; Rigal et al. 2012) and the *SCARECROW* (*SRC*) and *SHORT-ROOT* (*SHR*) genes (Cui et al. 2012; Helariutta et al. 2000). Despite the increasing number of physiological and molecular studies on ARs, the molecular mechanisms and the integration of environmental and endogenous factors are difficult to study are, not yet understood and might be species specific. Understanding the genetic complexity and molecular basis of AR formation in rose will help to improve rooting performance in rose breeding programmes and rose production.

Over the last several years, some complex horticultural traits of roses have been analysed by using molecular markers; these trait include plant architecture, flowering behaviour and flowering dates as well as the number of petals, flower colour and disease resistance genes (Henz et al. 2015; Hibrand-Saint Oyant et al. 2007; Kawamura et al. 2011; Li-Marchetti et al. 2017a). In the course of these studies, genetic maps have been constructed in rose using a range of markers in several diploid and a few tetraploid populations. These maps will help to identify QTLs and candidate genes for rose breeding (Kirov et al. 2014b; Spiller et al. 2011; Vukosavljev et al. 2016). Recently, genome-wide association studies (GWAS) have been used to identify loci associated with anthocyanin and carotenoid concentrations in rose petals (Schulz et al. 2016a), loci associated with adventitious shoot regeneration in rose (Nguyen et al. 2017a) and loci influencing the number of petals and number of prickles on shoots (Hibrand Saint-Oyant et al. 2018).

In this study, adventitious root formation was investigated in a panel of 95 rose genotypes under both *in vitro* and *in vivo* conditions. Association mapping analysis was performed to identify SNP (single-

nucleotide polymorphism) markers and genomic regions that are significantly associated with these phenotypes, including SNPs from genes encoding orthologues of known factors involved in root formation.

Materials and methods

Plant materials and *in vitro* shoot culture

A panel of 95 rose cultivars described previously (Nguyen et al. 2017a; Schulz et al. 2016a) was used in this study (Table S1). Shoots were cultivated *in vitro* in proliferation medium consisting of MS (Murashige and Skoog, 1962) salts in which FeEDDHA (10 mg/l) replaced FeEDTA, 30 g/l sucrose, 2.21 µM BAP, 0.57 µM GA_3 and 8.5 g/l plant agar (Duchefa, Harlem, Netherlands). The pH was adjusted to 5.8, and the medium was autoclaved at 121°C for 20 minutes. The nodal segments were cultured under cool-white fluorescent light at a PPFD (photosynthetic photon flux density) of 40 µmol m^{-2} s^{-1}, at 23 ± 2°C with a 16 h photoperiod. Following culture initiation, the shoots that developed from the nodes were subcultured every 4-5 weeks onto fresh medium with the same composition to induce shoot multiplication.

Adventitious root induction *in vitro*

The *in vitro* shoots of all 95 rose genotypes were cultured in shoot proliferation medium for four weeks before being used in the rooting experiment. The shoots were cut to a length of 1-1.5 cm including the apical bud and four leaves and transferred to rooting medium (half-strength MS macro- and microelements, containing 20 g/l sucrose, 8 g/l plant agar, and 0.98 µM IBA at a pH of 5.8. The shoots were cultured in the same light and temperature conditions as indicated above for shoot multiplication. For each genotype, the experiment was repeated twice with five replicates (250 ml vessels containing 80 ml of medium and 6 shoots each). After four weeks, the following rooting data were recorded: the number of shoots exhibiting root formation, the root number per shoot and the total length of all roots per shoot. Root length was measured by scanning the washed root system using WinRhizo™ (Plant Image Analysis) software.

Adventitious root induction *in vivo*

In vivo root induction was conducted using the same 95 rose cultivars in a hydroponic system in the greenhouse. Three independent experiments were conducted using one cutting (10-15 cm) from each of three clones per genotype per experiment. Greenhouse conditions were semi-controlled, with a mean temperature of 20°C and a photoperiod of 16 h. Fresh cuttings were fixed in patterns consisting of 48 holes drilled into rectangular plastic plates. These plates were then transferred to black plastic trays and placed under a moist plastic tent to avoid evaporation. For the first three weeks, incubation of the cuttings was conducted with tap water, which was then replaced by nutrient solution (Table S2). Each tray was continuously aerated by fish tank pumps. The cuttings were randomized within three complete blocks represented by two trays each. Six weeks after the initiation of the rooting experiments, root numbers, the length of the longest root, and root dry mass were recorded. Root dry mass was measured after the roots had been cut off the stems and dried for four days at 80°C.

Statistical analysis

All data were statistically analysed with the R software package, version 3.2.5 (R Foundation for Statistical Computing, 2016). Differences between cultivars and replications with regard to the root traits were analysed with the Kruskal-Wallis test. The correlation between root traits was calculated employing Spearman's rank correlation coefficient.

SNP analysis and GWA mapping

SNPs were analysed with the Axiom WagRhSNP chip as described previously (Schulz et al. 2016, Nguyen et al. 2017); this chip contains 68.893 SNPs derived from cut and garden roses (Koning-Boucoiran et al. 2015b). The SNP dosage was determined by using fit Tetra (AAAA, AAAB, AABB, ABBB, and BBBB) (Voorrips et al. 2011b).

The association analysis was performed in TASSEL 3.0 (Bradbury et al. 2007a) using the phenotypic information of the 95 genotypes related to *in vitro* and *in vivo* adventitious root formation. For analysis in TASSEL, the SNP dosages of tetraploid rose cultivars were recoded as diploid values. For this purpose, homozygous genotypes were coded as A:A or B:B, and all possible heterozygous genotypes (AAAB, AABB, and ABBB) were coded as A:B. The mixed linear model (MLM, +K model) was used to search for associations between markers and phenotypic traits with the minor allele frequency (MAF) set at 0.05. The Q matrix was obtained from STRUCTURE 2.3 (Hubisz et al. 2009a) based on a subset of markers as described by Schulz et al. (2016). The K matrix was calculated by using SPAGeDi software (Hardy and Vekemans 2002a). Association analysis was performed for each trait. Correction for multiple testing was defined by using the Bonferroni method, and the threshold for the association between traits and markers was set at –logp10 >6.7. The allelic class effect was obtained from the TASSEL output.

For visualization in so-called Manhattan Plots, the significant SNPs were compared to the Old Blush rose genome sequence (Hibrand Saint-Oyant et al. 2018b) to search for the corresponding annotated genes in rose. Orthologues of published candidate genes were located by conducting a homology search via local BLAST analysis using BioEdit (Hall et al. 1999).

Results

Adventitious root formation

In vitro adventitious root formation

Adventitious roots formed to some extent in all genotypes studied. They were observed to regenerate at the base of the shoot, sometimes associated with callus formation. However, significant differences in the number and length of roots that formed were found depending on the genotype (Fig. 1).

Fig. 1: Example of *in vitro* adventitious root formation in selected rose cultivars after 4 weeks of culture in rooting medium.

The rooting percentage ranged from 5% for cv. Blue Parfum to 100% for the majority of the cultivars (Fig. 2A). The average root number per shoot in the genotypes ranged from 0.12 (cv. Magenta) to 18.8 (cv. Lavender Lassie; Fig. 2B), and the average total root length varied between 0.02 cm for cv. Blue Parfum to 25.26 cm for cv. Heidetraum (Fig. 2C); both parameters also showed significant differences between genotypes.

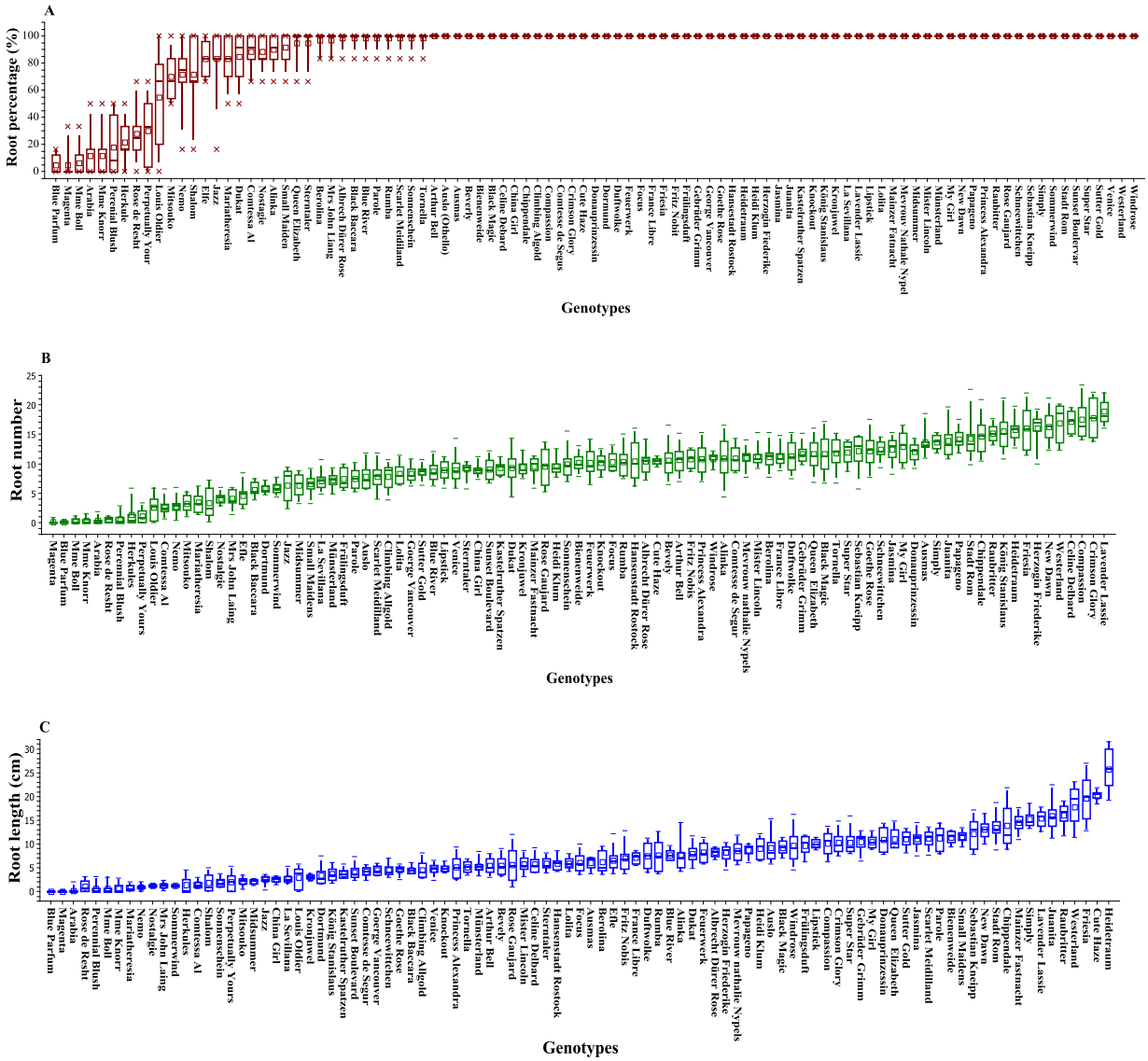


Fig. 2: *In vitro* rooting responses of 95 rose genotypes based on two independent experiments with six biological replicates (with 6 shoots each). Small square = mean; continuous line = median; asterisk = minimum, maximum; box = 1st and 3rd quartiles; and whiskers = standard deviation. A: *In vitro* rooting percentage, B: mean number of roots per *in vitro* shoot, C: average total *in vitro* root length per shoot.

Statistical analysis of the data for rooting percentage, root number and root length revealed significant differences between genotypes at p = 0.05 by the Kruskal Wallis test. The results of Tukey's test showed no significant differences (at p = 0.05) between the three repeated experiments for any of the parameters.

In vivo adventitious root formation

In vivo adventitious root formation was studied using a hydroponic system in the greenhouse (Fig. S1). Under these conditions, only 90 of the 95 genotypes were able to form roots. Again, significant differences were observed among genotypes, with the average rooting percentage ranging from 0 to 100% (Fig. 3A). Five genotypes that did not form roots under these conditions were Climbing Allgold, Mariatheresia, Mme Boll, Mme Knorr, Nemo and Venice. The average *in vivo* root number varied from

0 to 16.67, and the average length of the longest root ranged from 0 to 16.61 cm (Fig. 3B, C). The maximum root dry mass was 0.12 g for cv. Westerland (Fig. 3D).

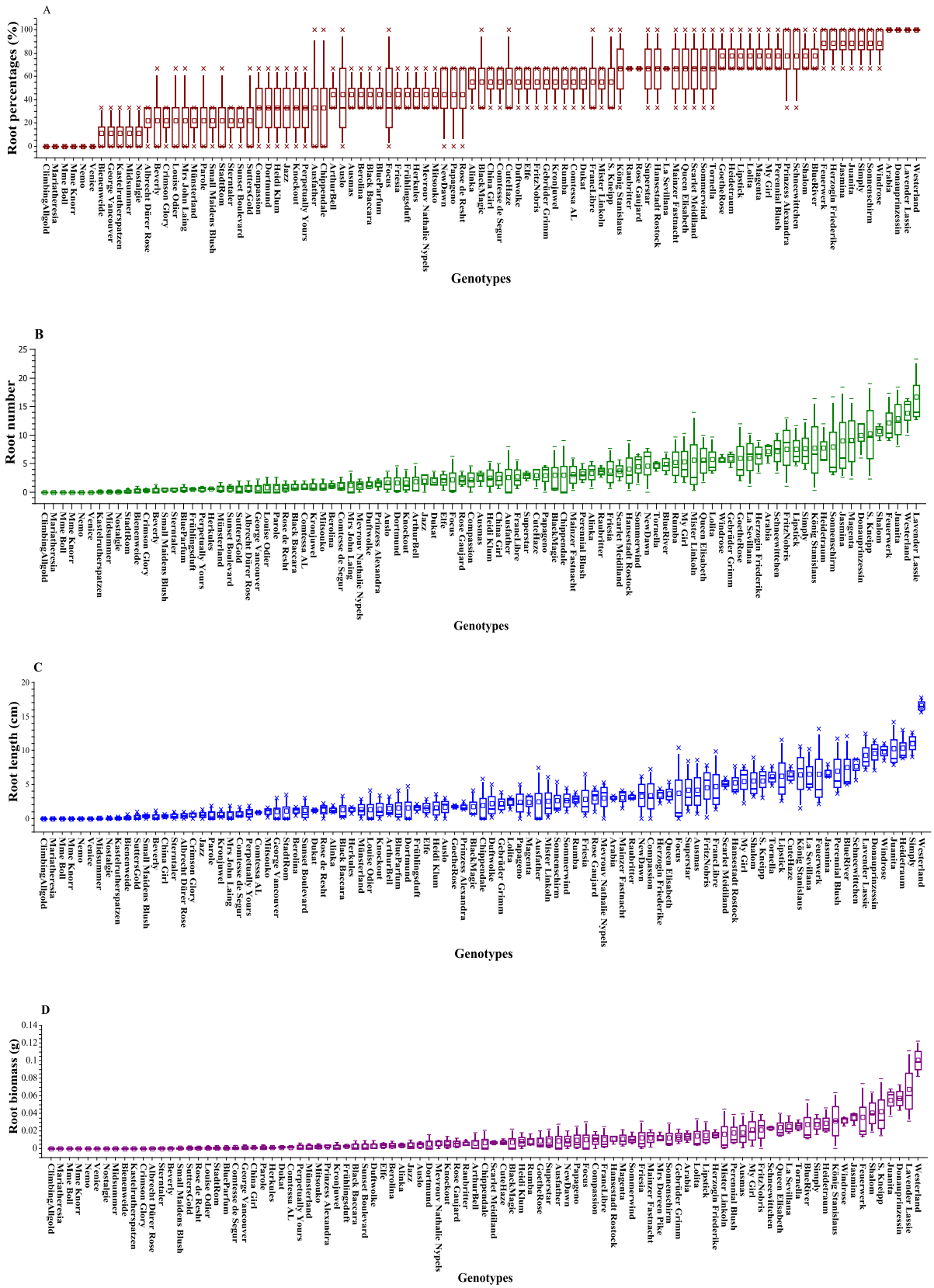


Fig. 3: *In vivo* rooting response of 95 rose genotypes based on three independent experiments with three biological replicates (with 3 cuttings each). Small square = mean; continuous line = median; asterisk = minimum, maximum; box = 1st and 3rd quartiles; and whiskers = standard deviation. A: *In vivo* rooting percentage, B: number of roots per cutting, C: average of the total *in vitro* root length per shoot, D: dry biomass of *in vivo* roots.

Statistical analysis of the root percentage, root number, root length and root biomass showed significant differences between genotypes at p = 0.05, while no significant differences (at p = 0.05) between the three repeat experiments were detected for all parameters.

The parameters measured in both the *in vivo* and *in vitro* experiments were analysed for correlations (Fig. 4). High and significant correlations were observed within the *in vitro* parameters (root number and total root length: 0.7) as well as within the *in vivo* parameters (root number, length of the longest root and root dry mass: 0.8-0.89). In contrast, the *in vitro* root number and *in vivo* root number exhibited only a weak correlation of 0.37. Although root length was slightly greater under *in vitro* conditions, it was significantly correlated with *in vivo* root length (0.52).

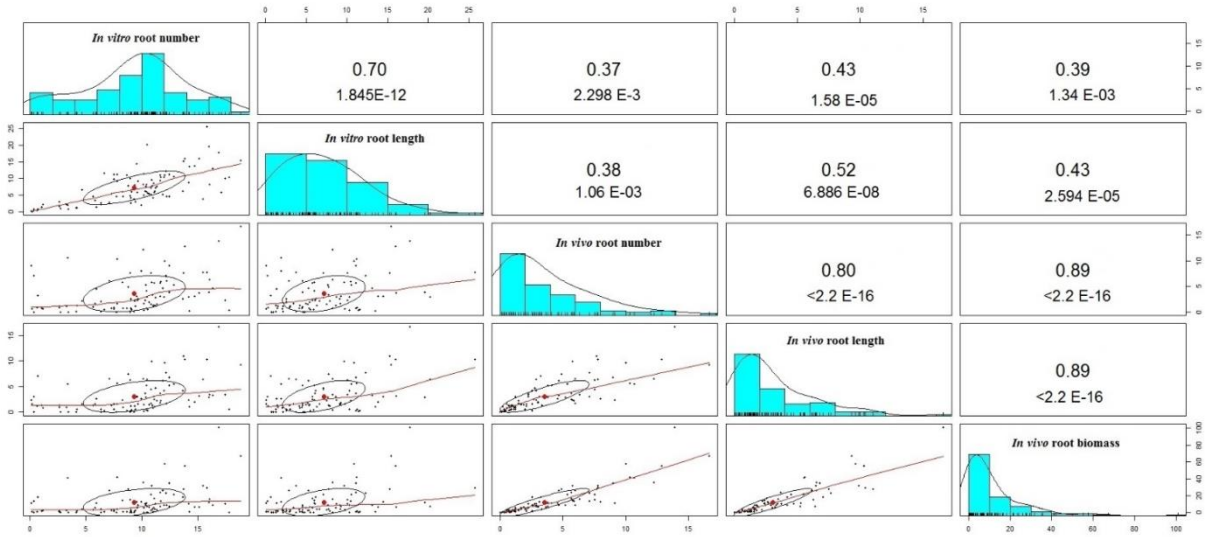


Fig. 4: Spearman's correlation coefficients of rooting traits under both *in vitro* and *in vivo* conditions at p given under the correlation value.

Marker-trait association analysis

Association mapping was performed for all the rooting traits to identify and locate genetic factors involved in AR formation under both *in vitro* and *in vivo* conditions.

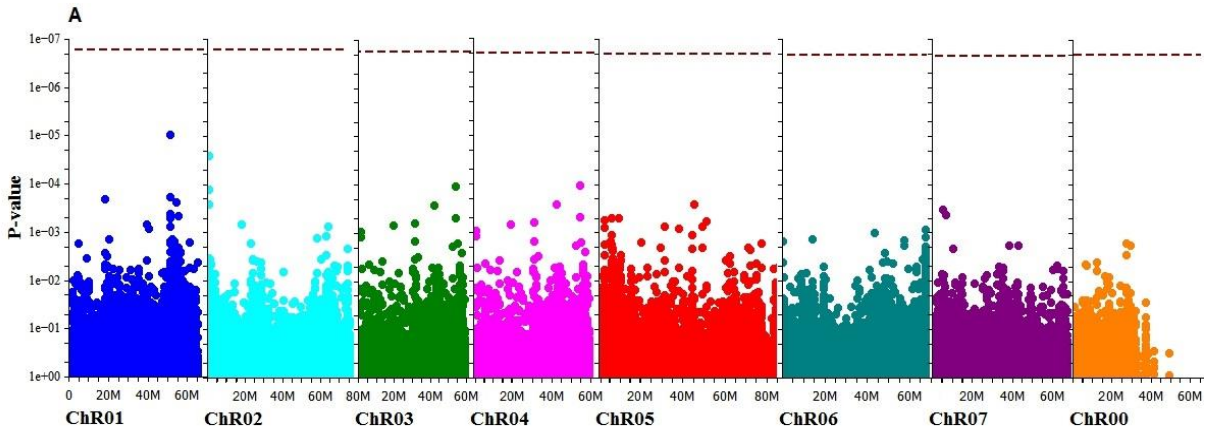
Under *in vitro* rooting, no significant SNP markers were found to be associated with root number (Fig. 5A). In contrast, we found 49 genes associated with total root length (Table S3; Fig. 5B). These markers formed five clusters on four of the seven rose chromosomes. Two clusters on chromosome 2 were located approximately at positions 25 Mb and 60 Mb. The latter group co-localized with the position of a candidate gene with similarity to a scarecrow-like gene. The third group was located on chromosome 3 at 45 Mb. This group co-localized with putative orthologues of the scarecrow gene, SCR. One of the SNPs in this group was generated from an EST with similarity to the WUSCHEL-related homeobox 8-like gene (Rh12GR_31633_585Q) at position 43622122 on chromosome 3. Furthermore, this group comprised a marker at position 36.877.701 that was associated with the lowest P-value found for this trait of 1.5E-61 (marker RhK5_11526_616P, with similarity to a mitochondrial inner membrane protease). The fourth cluster was found at the end of the chromosome

at approximately 60 Mb. At this position, a Wox3-like candidate was also observed. The fifth group of markers, located on chromosome 6 at 60 Mb, contained one SNP that was derived from an EST for a plant hormone response protein (ethylene-insensitive 3 like, marker RhK5_944_1305Q at position 18760323, Fig. 7). Some SNPs displayed strong effects on total root length when they were analysed in more detail as individual markers (Table 1), such as Rh12GR_16555_479Q (uncharacterized LOC101315363) (Fig. 7) at position 74912414 with a p-value of 3.71E-11 on chromosome 2, RhMCRND_63_4939Q (protein ROS1) (p-value: 2.53E-18) at position 320982 on chromosome 3 and RhMCRND_16904_622P (deoxynucleoside triphosphate triphosphohydrolase SAMHD1 homologue) with a p-value of 3.27E-07 at position 56571750 on chromosome 4 (Table 1).

In total, 98 SNPs were found to be associated with root numbers under *in vivo* conditions. Several highly significant markers formed clusters on chromosomes 1 to 4 (Fig. 6A, Table S4). A highly significant SNP (p = 3.17E-28 at position 10578014) was located within a cluster on chromosome 4; this SNP was derived from an EST for auxin response factor 19 (Fig. 8). A cluster at the end of chromosome 3 comprised the region with the SCR gene, and a cluster at the end of chromosome 2 co-localized with the ABCB19 gene. In addition, we analysed a number of SNPs individually and found 15 SNPs with good effects (Table. 2). On chromosome 5, we observed that RhK5_7321_779 (gene Histone H4 transcription factor (HiNF-P) (probable)) at position 73824400 presented strong effects (Fig. 8).

A total of 218 SNPs were found to be associated with *in vivo* root length (Table S5, Fig. 6B), with the lowest p-value of 6.40E-132 being detected for RhMCRND_26527_151P. Despite the large number of associated SNPs, these SNPs did not form distinct clusters, although some of the markers were accumulated at the ends of chromosomes 3 and 4, similar to the associations described for the other traits above. Among the significantly associated SNPs, one SNP was found to have putative functions related to organ development: marker RhK5_2637_676P from an EST annotated as Protein _AUXIN_RESPONSE_4 with a p-value of 2.08E-10 on chromosome 3 at position 42480660. In addition, 28 SNPs exhibited good effects (Table. 3). Strong effects were found for SNPs RhK5_252_3720Q (gene TATA-binding protein-associated factor 172 (TAF-172) and Rh12GR_3250_1751Q (Cell_division-protease-ftsH_homologue, chloroplastic, Precursor_ (similar to)) (Fig. 9).

Only four SNPs were significantly associated with *in vivo* root biomass, although some distinct clusters of markers that remained below the threshold value were formed (Table 4 Fig. 6C). These clusters were located at the beginning of chromosome 2, at the end of chromosome 3, in the middle of chromosome 5 and on chromosome 7 (Fig. 6C). The SNPs that displayed strong effects on *in vivo* root biomass were RhK5_5624_317Q (UPF0326_protein_At4g17486_ (putative)) and Rh12GR 3887_643Q (hypothetical protein) (Fig. 10).



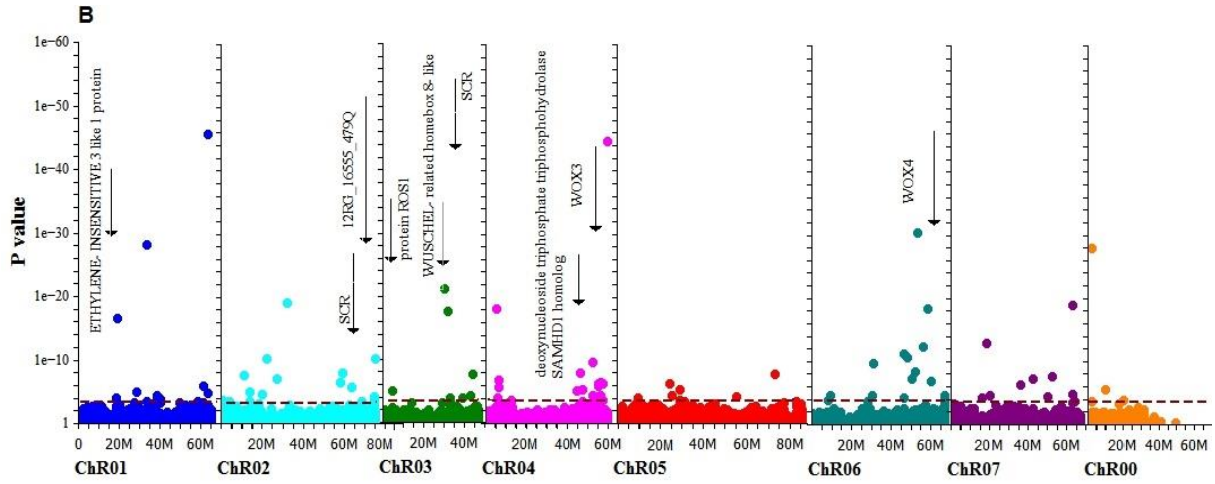
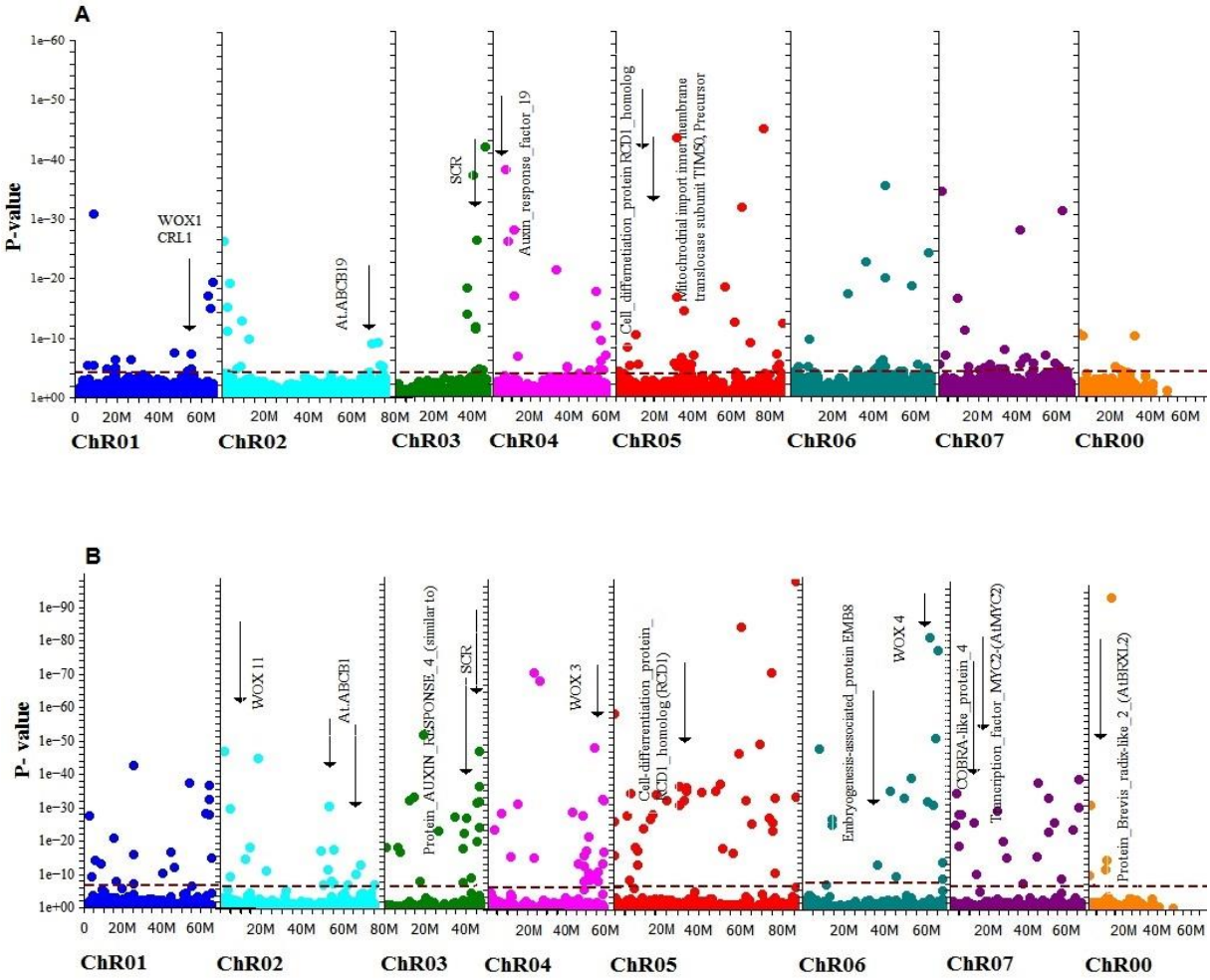


Fig. 5: Manhattan plots of *in vitro* root number (A) and total root length (B). The red dashed line represents the Bonferroni threshold of the adjusted significance level - [log10] = 6.7.



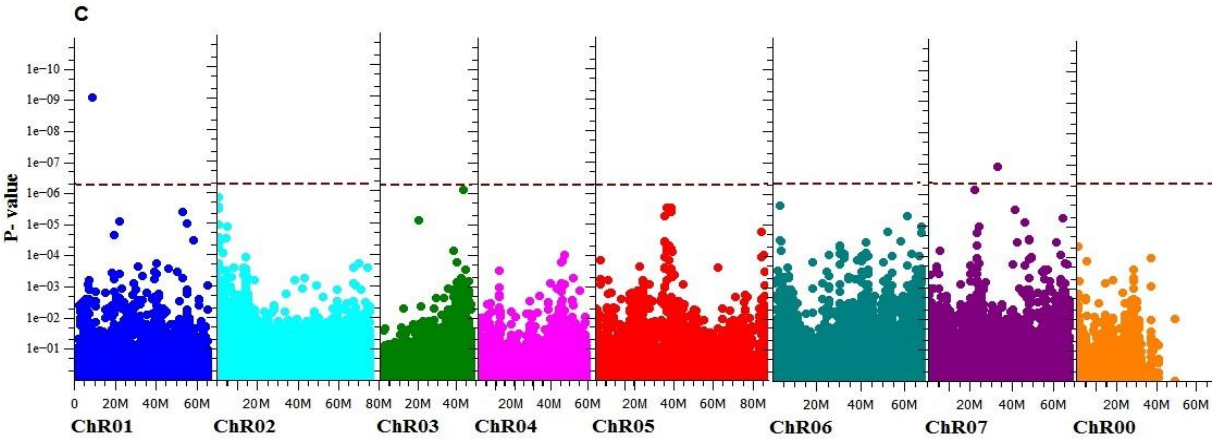


Fig. 6: Manhattan plots of *in vivo* root number (A), root length (B) and root biomass (C). The dashed line represents the Bonferroni threshold of the adjusted significance level - [log10] = 6.7.

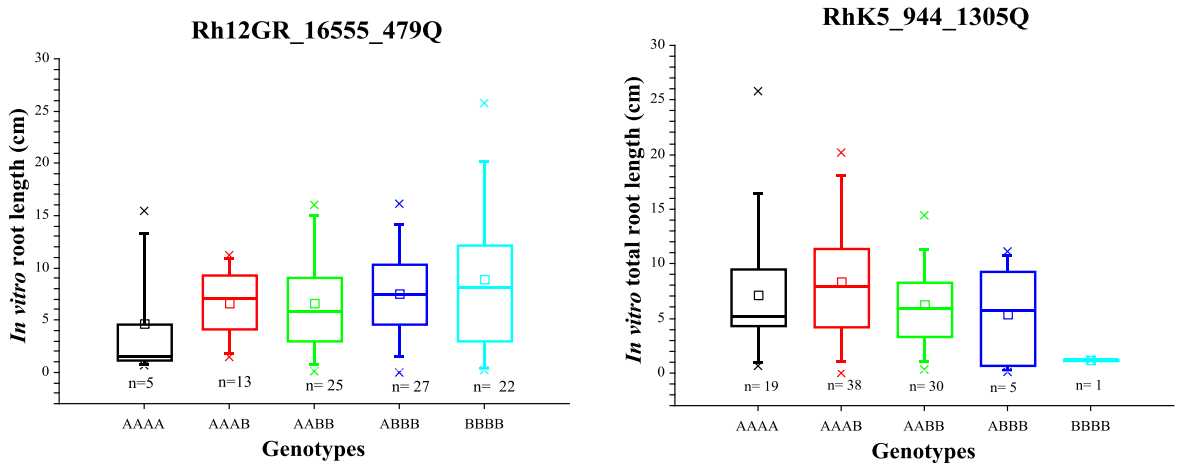


Fig. 7: Genotypic effects of SNP markers Rh12RG_16555_479Q (uncharacterized LOC101315363) and RhK5_944_1305Q (ETHYLENE INSENSITIVE 3-like 1 protein) on *in vitro* root length. (Small square = mean; continuous line = median; asterisk = minimum, maximum; box = 1st and 3rd quartiles; and whiskers = standard deviation).

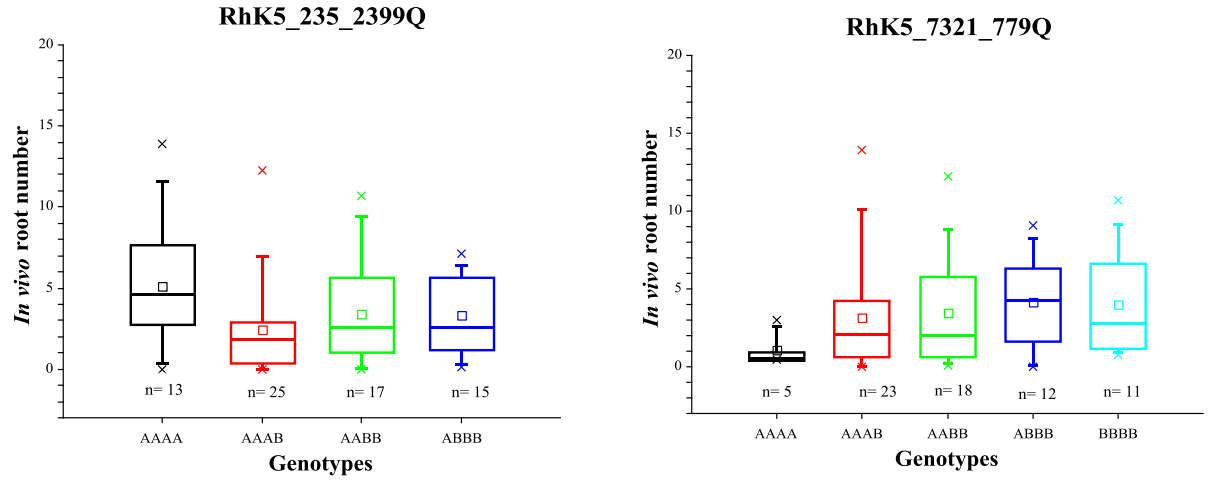


Fig. 8: Genotypic effects of SNP markers RhK5_235_2399Q (gene Auxin_response_factor_19) and RhK5_7321_779Q (gene Histone H4 transcription factor (HiNF-P)) on *in vivo* root number. (Small

square = mean; continuous line = median; asterisk = minimum, maximum; box = 1st and 3rd quartiles; and whiskers = standard deviation).

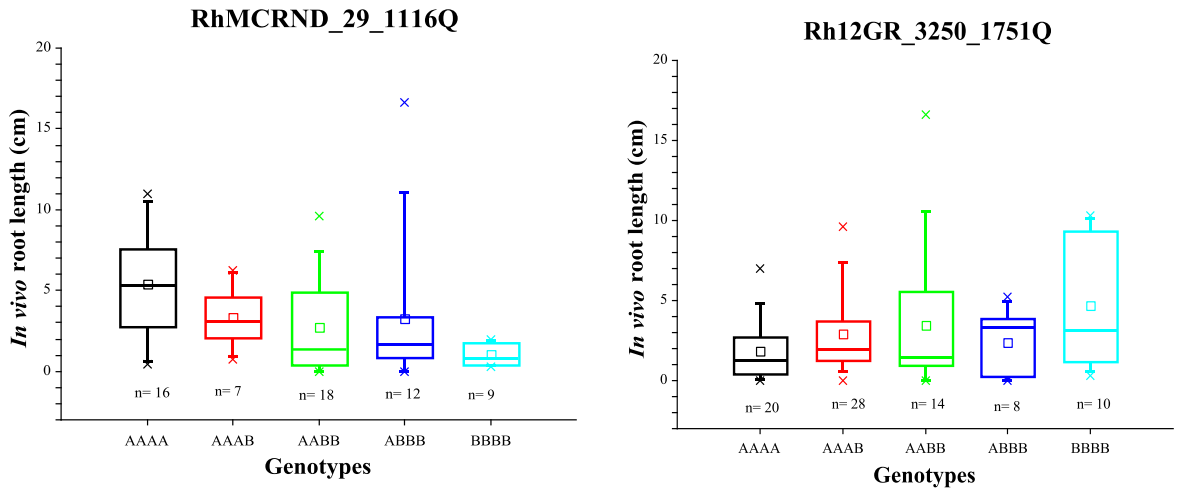


Fig. 9: Genotypic effects of SNP markers RhMCRND_29_1116Q (gene TATA-binding_protein-associated_factor_172 (TAF-172) (Probable)) and Rh12GR_3250_1751Q (gene Cell_division_protease_ftsH_homologue, chloroplastic, Precursor_ (similar to)) on *in vivo* root length. (Small square = mean; continuous line = median; asterisk = minimum, maximum; box = 1st and 3rd quartiles; and whiskers = standard deviation).

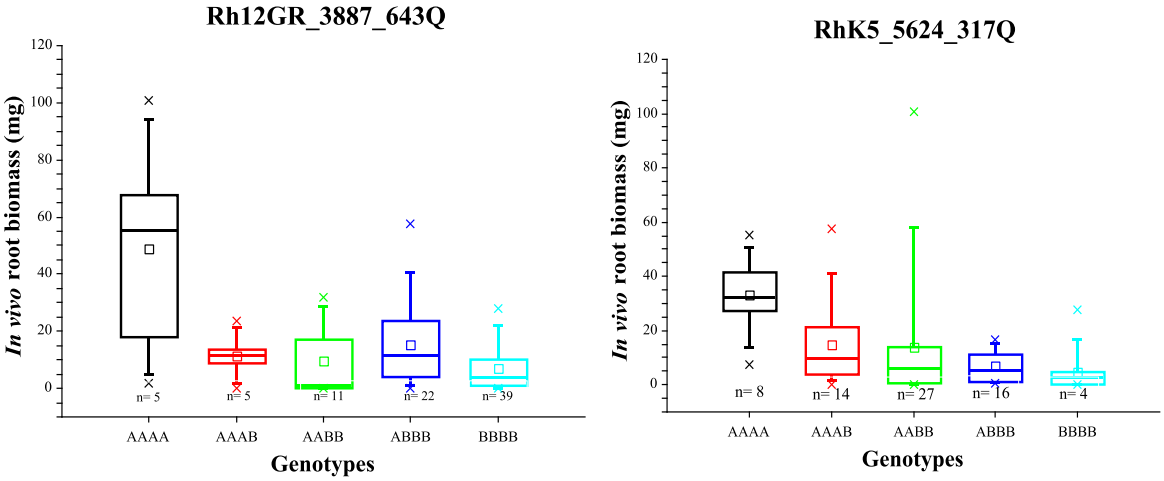


Fig. 10: Genotypic effects of SNP markers RhK5_5624_317Q (UPF0326_protein_At4g17486_ (putative)) and Rh12GR 3887_643Q (hypothetical protein) on *in vivo* root biomass. (Small square = mean; continuous line = median; asterisk = minimum, maximum; box = 1st and 3rd quartiles; and whiskers = standard deviation).

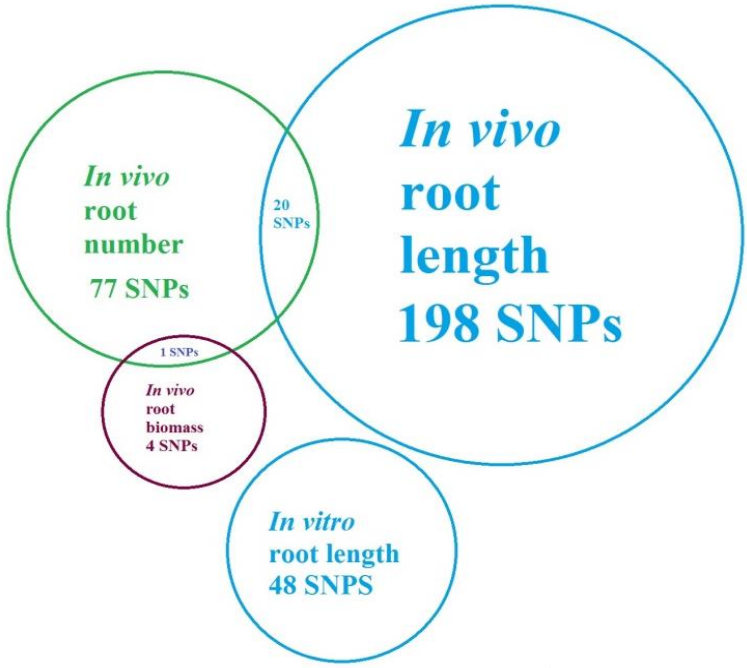


Fig. 11: Venn diagram for SNPs associated with rooting traits.

Among the total identified SNPs associated with rooting traits, there were 20 SNPs that overlapped between *in vivo* root number and *in vivo* root length. Only 1 overlapping SNP was found between *in vivo* root number and *in vivo* root biomass. There were no SNPs that overlapped between *in vivo* root biomass and *in vivo* root length or between *in vitro* root length and *in vivo* root traits (Fig. 11).

Table 1: Significant SNPs associated with *in vitro* total root length displaying the largest effects and the corresponding sequence similarity to known candidate genes

Marker	P	Chr	Position	Allele effect			Gene Prediction
				A:A	A:B	B:B	
RhK5_11526_616P	1.51E-61	Chr03	36877701	16.81	0	-	Mitochondrial inner membrane protease
RhMCRND_63_4939Q	2.53E-18	Chr03	32098241	10.42	0	-	protein ROS1 (LOC101306354)
RhK5_944_1305Q	2.72E-17	Chr01	18760323	-9.92	-8.01	0	ETHYLENE INSENSITIVE 3-like 1 protein
Rh12GR_16555_479Q	3.71E-11	Chr02	74912414	-8.00	0	-	uncharacterized LOC101315363
Rh12GR_31633_585Q	2.09E-08	Chr03	43622122	5.13	0	-	WUSCHEL-related homeobox 8-like
RhMCRND_16904_622P	3.27E-07	Chr04	56571750	1.97	12.42	0	deoxynucleoside triphosphate triphosphohydrolase SAMHD1 homolog
RhMCRND_3689_1357Q	4.25E-07	NA		18.12	0	-	aspartic proteinase A1-like (LOC101296033)

Table 2: Significant SNPs associated with *in vivo* root number displaying the largest effects and the corresponding sequence similarity to known candidate genes

Marker	P	Chr	Position	Allele effect			Gene Prediction
				A:A	A:B	B:B	
RhK5_317_1419Q	1.11E-131	Chr03	40212914	-6.92	-5,340		Protein transport protein Sec24-like At3g07100 (putative)
RhK5_7321_779Q	5.97E-100	Chr05	73824400	-3.3	-3.720		Histone H4 transcription factor (HiNF-P) (probable)
RhK5_8899_1285Q	2.83E-72	Chr05	45698363	-	4.48	0	Mitochondrial import inner membrane translocase subunit TIM50,_Precursor_ (similar to)
RhK5_4056_658Q	6.63E-53	Chr01	2184630	-	0	-9.67	Alcohol_dehydrogenase-like_1_ (probable)
RhK5_2555_767P	8.09E-53	Chr07	66059732		-0.440		Coatomer_subunit_alpha-1 (Alpha-COP_1) (similar to)
RhK5_235_2399Q	3.17E-28	Chr04	10578014		-3.670		Auxin_response_factor_19_(similar to)
RhMCRND_4332_1059P	2.00E-17	Chr04	10916131		5.06	0	F-box_protein_At5g07610_(probable)
RhMCRND_10708_222Q	4.28E-13	Chr05	61078364		2.46	0	Sentrin-specific_protease_8_(probable)
Rh12GR_1663_1052P	3.68E-08	Chr01	54603419		0	-6.62	Centrosomal_protein_of_290_kDa_ (Cep290)_(probable)
RhK5_2621_1523P	1.09E-09	Chr05	69433322	-0.15	0	2.93	Phospholipase_C_4,_Precursor _(probable)
RhMCRND_11628_825Q	1.21E-09	Chr02	68679645	0	5.46	1.36	Lamin-like_protein,_Precursor_ (similar to)
Rh12GR_49528_182P	1.52E-08	Chr07	33153851	0	-7.65	-8.24	NA
RhK5_9842_811P	1.21E-07	Chr05	40107884	0	-6.38	-	OTU_domain-containing_protein_5 (probable)
RhK5_13091_426P	1.30E-07	Chr07	53810245	-	0	-4.35	LisH_domain-containing_protein_C1711.05_ (probable)
Rh12GR_70672_85P	2.65E-07	Chr05	34124381	0	-3.90	-4.33	Cell_differentiation_protein RCD1_homolog (Rcd-1) (similar to)

Table 3: Significant SNPs associated with *in vivo* root length displaying the largest effects and the corresponding sequence similarity to candidate genes

Marker	P	Chr	Position	Allele effect			Gene Prediction
				A:A	A:B	B:B	
RhK5_6730_852Q	4.25E-36	Chr05	7630738	-	0	0.29	60S_ribosomal_protein_L11_(similar to)
RhK5_252_3720Q	4.05E-35	Chr05	85836695	-	0	0.31	TATA-binding_protein-associated_factor_172 (TAF-172)_(probable)
RhK5_14646_481Q	3.72E-34	Chr05	61846265	0.42	0	0.13	Mitogen-activated_protein_kinase_homolog NTF6 (similar to)
Rh88_10303_228Q	2.89E-33	Chr03	45770281	-1.11	0	-1.41	NA
RhK5_16105_273Q	4.33E-30	Chr07	4331459	-	4.09	0	COBRA-like_protein_4,_Precursor (similar to)
Rh12GR_11509_501Q	4.84E-30	Chr07	5547407	0	-1.40	2.87	gene F-box/LRR-repeat_protein_4_ (AtFBL4) (probable)

RhK5_41_5365P	1.50E-28	Chr05	17449063	-	-0.32	0	Dedicator_of_cytokinesis_protein_8_ (probable)
RhK5_4056_658Q	2.58E-28	Chr01	2184630	-	0	-3.00	Alcohol_dehydrogenase-like_1_(probable)
RhK5_15035_566P	1.72E-27	Chr05	74656672	-	0	0.30	Regulator_of_ribonuclease-like_protein_3(putative)
RhK5_2377_1023Q	8.82E-22	Chr07	26945579	0	-	-3.88	Transcription_factor_MYC2_(AtMYC2) (putative)
RhMCRND_1033_2408Q	2.36E-14	Chr02	68171595	3.57	0	0.78	Chaperone_protein_clpB_2_(similar to)
RhK5_2259_398P	6.69E-12	Chr06	29715438	0	-0.26	-	Embryogenesis-associated_protein EMB8 (probable)
Rh12GR_21320_86P	3.39E-11	Chr01	39723188	-	0	-2.76	Zinc_finger_protein_1_(probable)
RhK5_2637_676P	2.08E-10	Chr03	42480660	0.57	0.84	0	Protein_AUXIN_RESPONSE_4_(similar to)
Rh12GR_34039_714Q	3.05E-10	Chr06	66838972	-3.21	-2.71	0	Selenoprotein_H_(SelH)_(probable)
RhMCRND_903_1621P	8.11E-10	Chr05	7182076	-3.21	0	-3.32	Protein_SCAR3_(AtSCAR3)_(probable)
RhMCRND_28921_223P	1.74E-09	Chr06	31784013	-	-0.97	0	NA
RhK5_13480_2046P	3.89E-08	Chr02	62389538	-3.20	0	-4.12	Exosome_complex_exonuclease_rrp6 (probable)
RhK5_6397_539Q	4.59E-08	Chr05	75709769	0	-2.93	-3.16	Calcineurin_B-like_protein_3_(similar to)
RhMCRND_29_1116Q	8.14E-08	Chr05	85843901	-5.33	0	-3.17	TATA-binding_protein-associated_factor_ 172 (TAF-172) (probable)
Rh12GR_54107_458P	1.87E-07	Chr00	18120816	0	0.07	2.89	Transmembrane_protein_87B, Precursor_ (probable)
Rh12GR_2206_1423P	2.00E-07	Chr01	25358900	0	2.57	-	DEAD-box_ATP-dependent_ RNA_helicase_32 (similar to)

Table 4: Significant SNPs associated with *in vivo* root biomass

Marker	P	Chr	Position	Allele effect A:A	A:B	B:B	Gene Prediction
RhMCRND_27823_1500P	7.60E-10	Chr01	8666053	-	0	-48.39	Histidinol-phosphate _minotransferase,_ chloroplastic,_Precursor_(putative)
Rh12GR_49528_182P	1.22E-07	Chr07	33153851	0	-35.91	-35.43	NA
RhK5_5624_317Q	6.88E-07	Chr07	22194573	0	25.55	-1.09	UPF0326_protein_At4g17486_ (putative)
Rh12GR_3887_643Q	8.85E-07	Chr03	42667240	0	31.4	-4,71	hypothetical protein

Discussion

In this study, we present data on the genetic variation of the ability of 95 rose genotypes to form adventitious roots under both *in vitro* and *in vivo* conditions. In addition to phenotypic characterization of the rooting response in this panel, we identified genomic regions associated with adventitious root formation ability and located putative candidate genes with known functions in plant rooting.

Genotypic differences in adventitious root formation under *in vitro* and *in vivo* conditions

Pronounced genotypic differences in rooting ability were observed, especially in the cuttings grown under *in vivo* conditions (Fig. 5-9) but also to a lesser extent in the *in vitro* experiments (Fig. 1-4). In both the *in vitro* and *in vivo* experiments, adventitious roots regenerated at the base of the micro-shoot or the cutting within two to three weeks. Previous studies addressing the rooting of roses have focused

on either *in vitro* or *in vivo* rooting comparisons (Pati et al. 2010; Z.A. Rather and Tsewang Tamchos 2017)). Dubois and de Vries (1991) reported that the rooting percentages of softwood cuttings from 50 miniature rose genotypes to vary between 0 and 100%. These authors demonstrated the dependence of adventitious root formation on the leaf area. Our comprehensive dataset allowed a detailed comparison of rooting under the two conditions described in the largest set of genotypes analysed thus far. Our data indicate that the majority of the genotypes analysed formed roots to some extent under both conditions tested but that rooting occurred at higher rates *in vitro* than *in vivo*. The relatively low correlation of the rooting traits observed under *in vitro* conditions with those under *in vivo* conditions (Fig. 4) was most likely due to the application of the auxin IBA in our *in vitro* experiments, in contrast to rooting without the addition of rooting growth regulators under *in vivo* conditions. It can be assumed that the data would have been better correlated if either the *in vitro* tests were performed in plant growth regulator-free medium or the cuttings were also treated with IBA. Another important factor was the difference in the environmental conditions for rooting between the *in vitro* and the greenhouse experiments. Furthermore, the genotypic differences with regard to growth and proliferation under *in vitro* conditions might have influenced the rooting response, since shoots of slightly different sizes were subjected to the analyses. The correlation coefficient between the *in vitro* root number and *in vitro* root length was high (0.70), suggesting that these parameters are controlled by the same genetic factors. The same holds true for the traits related to rooting recorded under *in vivo* conditions. Therefore, our analyses reflect genotypic variation among the genotypes of the association panel that comprises partially non-overlapping genetic factors responsible for root development under the applied environmental conditions.

Markers associated with rooting traits

Marker-trait associations for rooting traits have been analysed in a number of plants, such as wheat (Beyer et al. 2018; Maccaferri et al. 2016), rice (Li et al. 2017; Phung et al. 2016; Wang et al. 2018), sorghum (Parra-Londono et al. 2018), cow pea (Burridge et al. 2017), maize (Bray and Topp 2018; Zaidi et al. 2016) and *Arabidopsis thaliana* (Lachowiec et al. 2015). In rose, two GWAS have been published thus far, one on anthocyanin and carotenoid contents in rose petals (Schulz et al. 2016a) and one on shoot organogenesis (Nguyen et al. 2017a). In this study, we utilized the same association panel and genotypic data published by (Schulz et al. 2016a)) and (Nguyen et al. 2017a)), except that 95 instead of 96 genotypes were analysed.

Markers associated with *in vitro* rooting traits

We did not detect any significant SNPs associated with *in vitro* root number, although a peak beneath the significance threshold on chromosome 1 occurred at a similar position to the cluster of markers associated with *in vivo* root number, which co-localized with the position of putative orthologues of the WOX 1 and CRL1 (Crown rootless) genes. All other rooting traits recorded in this analysis showed significantly associated markers at this position, which could be an indication that one or both of these genes may play a functional role in root formation or growth. CRL1 has been shown to be an auxin-inducible gene in rice and has a putative function in adventitious and lateral root induction that is directly regulated by ARFs (Guan et al., 2015). Rc WOX 1, characterized in *Rosa canina,* has recently been reported to be a factor involved in auxin-induced formation (Gao et al., 2014).

In contrast to the lack of SNPs associated with the number of roots *in vitro,* the total root length was associated with 49 SNPs. Among the associated markers, one marker (RhK5_944_1305Q) was derived from an EST encoding an ETHYLENE-INSENSITIVE 3-like 1 protein on chromosome 1 that has been reported to be involved in root formation in plants (Clark et al. 1999). Another marker derived from a putative candidate gene was Rh12GR_31633_585Q, which is derived from a gene encoding a WUSCHEL-related homeobox 8-like protein that is also known to be involved in root formation (Liu and Xu 2018). In addition to these SNPs representing candidate genes, three clusters of significantly

associated markers fell within regions that carry genes with known functions in root development. At the end of chromosome 3, one cluster co-localized with the SCR gene encoding the Scarecrow protein. SCR expression is auxin dependent and serves as a marker of endodermal development (Guan et al. 2016). This position on chromosome 3 contained significantly associated markers for *in vivo* traits as well, making it a very likely position for a QTL with an effect on rooting in roses. Determination of whether SCR is the causal gene will require further functional analysis in roses. Another identified region was a cluster at the end of chromosome 4 that also appeared to be important for the *in vivo* traits. This cluster contained homologues of the WOX3 gene; although this gene has not been directly shown to be related to root formation (Liu and Xu 2018), it might be involved in other developmental processes contributing indirectly to AR formation in roses. A similar case was found in the fourth cluster on chromosome 6, which comprised a homologue of the WOX 4 gene; together with WOX3, this gene is located in the clade of WC-WOX genes with roles in plant stem cell function (Xu. 2018).

The analysis of individual markers for *in vitro* root length confirmed significant, but small effects (Fig. 7) for the individual markers. This might be due to the action of several genes among which the tagged loci only make a small contribution or to a lack of linkage between the markers and the causal gene. As the Axiom WagRhSNP chip comprises 68893 SNPs, the reason is more likely to be that several genes each contribute small effects to rooting traits in roses.

Markers associated with *in vivo* rooting traits

The analysis of *in vivo* root numbers revealed 98 associated SNPs and SNP clusters at very similar positions to those observed for *in vitro* root length, including clusters on chromosomes 1, 2, 3 and 4 and more widely distributed markers on the other chromosomes. While the cluster at the end of chromosome 3 was at a similar position to the clusters for *in vitro* root length containing the SCR candidate gene, a group of markers at the end of chromosome 1 was close to the position of WOX 1 and CRL1 homologues. CLR1 is an auxin-inducible gene associated with lateral root induction and lateral root numbers in rice (Inukai et al. 2005). Furthermore, a cluster at the end of chromosome 2 co-localized with the ABCB19 gene, which encodes an auxin efflux gene putatively involved in adventitious rooting (Xu 2018). An additional cluster was found at the beginning of chromosome 4, in which one of the significant SNPs was derived from a gene encoding a homologue of auxin response factor 19 (Fig.8). Auxin response factor 19 belongs to a gene family that regulates auxin-mediated transcriptional activation/repression in lateral root formation (Li et al. 2006; Okushima et al. 2005). Furthermore, an EST encoding a gene annotated as Protein auxin response 4 (similar to) on chromosome 3 was associated with *in vivo* root length. The gene encoding the Protein auxin response 4 is involved in root formation in American ginseng, *Panax quinquefolium* (Chen et al. 2008).

Among the significantly associated SNPs for *in vivo* root number, we found overlap between 21 markers and the 218 markers associated with *in vivo* root length, confirming the observation of similar cluster positions and indicating that common processes might be associated with these two rooting parameters. In addition, we found two genes that may play a role in root elongation, Protein Brevis radix-like 2 (AtBRXL2), encoded by Rh12GR_4624_1250P, and COBRA-like _protein_4, Precursor on chromosome 7, encoded by RhK5_16105_273Q. The gene *BREVIS RADIX* was shown to be a major regulator of root growth in *Arabidopsis* (Mouchel et al. 2004), while the function of the BRX-like genes has not yet been resolved. *COBRA* loss-of-function mutants exhibit strong phenotypes involving stunted roots since the *COBRA* gene is involved in cellulose deposition in the cell wall and, thus, in cell expansion (Ko et al. 2006).

Although *in vivo* root length showed a more dispersed distribution of significantly associated markers, groups of markers clustered at similar positions on chromosomes 1, 2, 3, 4 and 6 to the markers for the traits discussed above. This finding further supports the idea that common processes lead to

clusters of markers tagging the same QTL regions. The low correlation between the traits can be explained by the small effects of the significant SNPs detected here on the traits and the contribution of additional undetected QTLs to the observed phenotypic variation.

Considering the marker-trait associations of all measured traits in our dataset, it is very likely that allelic variation of some of the known genetic factors with relevance to adventitious root formation (e.g., several WOX-related genes, SCR and CRL1) has a significant effect on rooting in roses. Previous analyses conducted with the same association panel using the same genotypic data revealed major factors, such as the number of petals or the content of carotenoids in petals that displayed much more pronounced marker-trait associations (Schulz, et al. 2016, Hibrand Saint-Oyant et al. 2018). As no comparable effect was detected in the present study, we can conclude that quantitative variation in rooting is based on a larger number of factors with smaller effects of individual QTLs compared to those traits mentioned above. This conclusion seems to be reasonable also because the time point at which we monitored adventitious root formation was rather late. Thus, the measured parameters are a result of a number of molecular processes involved in dedifferentiation, induction, initiation, elongation and lateral root formation. Further dissection of the different phases of adventitious root formation should be considered in future studies to identify genes with greater contributions to single processes.

Conclusion

In the present study, we investigated different rooting traits under both *in vitro* and *in vivo* conditions. We observed great variation in rooting traits between genotypes under both conditions. A GWAS identified a number of markers that were significantly associated with rooting parameters, although with relatively small effects on the traits. The lack of a strong correlation between rooting traits observed under contrasting conditions and the small effects of the associated markers indicate that a larger number of QTLs, each with small effects, influence rooting in roses. The results provide the first insights into the genetic architecture of rooting ability in roses, and this genetic information could potentially be useful for further functional studies of candidate genes for rooting traits in roses.

Acknowledgements

This study was carried out with financial support from the Vietnamese Government and the unit of Molecular Plant Breeding, Institute of Plant Genetics, Leibniz University Hannover, Germany. We would like to thank Klaus Dreier and his team from the unit of Molecular Plant Breeding for assistance in the greenhouse experiment.

Supplements

Table S1: List of the association panel of rose genotypes was used in the study

DNA Code	Cultivar	Code	Breeder	Country	Bred in (Y)	Type/habit	Flower	Polyploid
1	Parole	PR	W. Kordes&Söhne	GER	1991	Hybrid Tea	Pink	tetraploid
2	Queen Elizabeth	QE	Lammerts	USA	1954	Grandiflora, shrub	Pink	tetraploid
3	Schneewittchen[1)]	SC	W. Kordes&Söhne	GER	1958	Floribunda, shrub	White	triploid
4	Nemo	NE	Noack Rosen	GER	2001	Floribunda, ground cover	White	tetraploid
5	Super Star[1)]	SS	Rosen Tantau	GER	1960	Hybrid Tea	salmon pink	triploid
6	Small Maid. Blush	SM	Unknown	UK	1797	Alba, shrub	light pink	tetraploid
10	Chippendale	CP	Rosen Tantau	GER	2005	Hybrid Tea	Orange	tetraploid
11	Climbing Allgold	CG	Douglas L. Gandy	UK	1961	Floribunda, climber	Yellow	tetraploid
12	Blue Parfum	BP	Rosen Tantau	GER	1978	bedding	Violet	tetraploid
13	Feuerwerk	FE	Rosen Tantau	GER	1962	shrub	orange, red	tetraploid
14	Gebrüder Grimm	GG	W. Kordes&Söhne	GER	2007	Floribunda, bedding	Orange	tetraploid
15	George Vancouver	GV	Ag Can	CAN	1983	Hybrid Kordesii, shrub	Red	tetraploid
16	König Stanislaus	KS	Rosen Tantau	GER	1998	shrub	Yellow	tetraploid
17	Heidi Klum	HK	Rosen Tantau	GER	1999	Floribunda, bedding	Violet	tetraploid
18	Jasmina	JA	W. Kordes&Söhne	GER	1996	climber	Pink	tetraploid
20	Sonnenschirm	SO	Rosen Tantau	GER	1993	Floribunda, ground cover	Yellow	tetraploid
24	Heidetraum[1)]	HT	Noack Rosen	GER	1988	ground cover	carmine-pink	triploid
26	Nostalgie	NO	Rosen Tantau	GER	1995	Hybrid Tea	white, pink	tetraploid
27	Sommerwind[1)]	SW	W. Kordes&Söhne	GER	1985	bedding	light pink	triploid
28	New Dawn[1)]	ND	Somerset Rose Nurs.	USA	1930	climber	light pink	triploid n
32	Mevrouw N. Nypels[2)]	MN	Mathias Leenders	NL	1919	Polyantha, shrub	pink	Diploid
35	Mitsouko	MI	Delbard	F	1970	Hybrid Tea	yellow	tetraploid
36	Black Baccara	BB	Meilland	F	2000	Hybrid Tea	red	tetraploid
37	Alinka	AL	Patrick Dickson	UK	1971	Hybrid Tea	red	tetraploid
38	Auslo (=Othello)	AU	David Austin Roses	UK	1986	shrub	red	tetraploid
39	Ausmas (=Graham Thomas)	AM	David Austin Roses	UK	1983	shrub	yellow	tetraploid

40	Shalom	SH	PoulsenRoser A/S	DAN	1972	Floribunda, shrub	red	tetraploid
41	La Sevillana	LA	Meilland	F	1978	Floribunda, shrub	red	tetraploid
42	Mister Lincoln	ML	Swim & Weeks	USA	1964	Hybrid Tea	red	tetraploid
43	Rumba	RU	PoulsenRoser A/S	DAN	1958	Floribunda, bedding	orange	tetraploid
44	Arthur Bell	AB	Sam McGredy Roses	NZ	1965	Floribunda, shrub	yellow	tetraploid
46	Comtesse de Ségur	CS	Delbard	F	1992	Floribunda, shrub	pink	tetraploid
47	Mme Boll	MB	Daniel Boll	USA	1858	Portland, shrub	red	tetraploid
49	Compassion	CO	Harkness & Co Ltd.	UK	1972	climber	salmon-pink	tetraploid
50	Sutters Gold	SG	Herbert C. Swim	USA	1950	Hybrid Tea	yellow	tetraploid
51	Scarlet Meidilland	SMD	Meilland	F	1987	shrub, ground cover	red	tetraploid
52	Rose de Resht	RR		Persia	1900	Damask, shrub	red	tetraploid
53	Celine Delbard*	CD	Delbard	F	1986	Floribunda, shrub	salmon-pink	tetraploid
54	Louise Odier	LO	Jules Margottin Père & Fils	F	1851	Bourbon, shrub	deep pink	tetraploid
55	Ausfather (=Charles Austin)**	AF(Ma et al. 2016)	David Austin Roses	UK	1973	shrub	apricot	tetraploid
56	Perpetually Yours	PY	Harkness & Co Ltd.	UK	1999	climber	light yellow	tetraploid
57	Mme Knorr	MK	Viktor Verdier	F	1855	Portland, shrub	pink	tetraploid
58	Papageno	PG	Sam McGredy Roses	NZL	1989	Hybrid Tea	red bled, stripes	tetraploid
59	France Libre	FL	Delbard	F	1981	Hybrid Tea	orange	tetraploid
61	Princess Alexandra	PA	PoulsenRoser A/S	DK	1988	Hybrid Tea	violet	tetraploid
62	Mrs John Laing	MJ	Henry Bennet	UK	1885	Hybrid Perpetual, shrub	deep pink	tetraploid
66	Black Magic	BM	Rosen Tantau	GER	1995	Hybrid Tea	dark red	tetraploid
67	China Girl	CG	Mehring/ Tantau	GER	2005	Floribunda, bedding	yellow	Tetraploid
68	Perennial Blush	PB	Henry Bennet	UK	2007	climber/rambler	white, light pink	Tetraploid
69	Comtessa AL	CA	Rosen Tantau	GER	2006	Hybrid Tea	yellow, white	Tetraploid
70	Lipstick	LS	Rosen Tantau	GER	2001	ground cover	pink	Tetraploid
71	Midsummer	MS	Rosen Tantau	GER	2007	Floribunda, bedding	orange-red	Tetraploid
72	Arabia	AR	Rosen Tantau	GER	2001	shrub	orange blend	Tetraploid
73	Hansestd. Rostock	HR	Rosen Tantau	GER	2004	Floribunda, bedding	apricot	Tetraploid

74	Kastelrut. Spatzen	KA	Rosen Tantau	GER	2011	ground cover	white	Tetraploid
75	Elfe	EF	Rosen Tantau	GER	2000	climber	yellow	Tetraploid
77	Jazz	JA	Rosen Tantau	GER	2003	ground cover	copper-orange	Tetraploid
78	MainzerFastnacht	MF	Rosen Tantau	GER	1964	Hybrid Tea	violet	Tetraploid
79	Dukat	DU	Rosen Tantau	GER	2010	Floribunda, climber	yellow	Tetraploid
80	My Girl	MG	Rosen Tantau	GER	2006	Hybrid Tea	white, yellow center	Tetraploid
81	Mariatheresia	MT	Rosen Tantau	GER	2003	Floribunda, bedding	light pink	Tetraploid
84	Knockout[1)]	KO	Radler	USA	1988	shrub	red	triploid
85	Berolina	BE	W. Kordes&Söhne	GER	1984	Hybrid Tea	yellow	Tetraploid
89	Westerland	WL	W. Kordes&Söhne	GER	1969	shrub	orange	Tetraploid
92	Frühlingsduft	FD	W. Kordes&Söhne	GER	1949	shrub	white, pink shading	Tetraploid
93	Sebastian Kneipp	SK	W. Kordes&Söhne	GER	1997	Hybrid Tea	white, pink center	tetraploid
94	Lavender Lassie[1)]	LL	W. Kordes&Söhne	GER	1960	shrub	violet	triploid
95	Dortmund	DO	W. Kordes&Söhne	GER	1955	climber	red	tetraploid
96	Friesia	FR	W. Kordes&Söhne	GER	1973	Floribunda, bedding	yellow	tetraploid
97	Sterntaler	ST	W. Kordes&Söhne	GER	1995	shrub	yellow	tetraploid
99	Raubritter[1)]	RA	W. Kordes&Söhne	GER	1936	climber	light pink	triploid
100	Herkules	HE	W. Kordes&Söhne	GER	2006	shrub	pink, light lavender	tetraploid
103	Fritz Nobis	FN	W. Kordes&Söhne	GER	1940	shrub	rose-pink	Tetraploid
104	Beverly	BV	W. Kordes&Söhne	GER	1999	Hybrid Tea	pink	Tetraploid
105	Juanita	JU	W. Kordes&Söhne	GER	1996	mini-shrub	light pink	tetraploid
110	Windrose	WR	Noack Rosen	GER	1993	ground cover	pink	tetraploid
111	Donauprinzessin	DN	Noack Rosen	GER	1994	Floribunda, bedding	salmon-pink	tetraploid
112	Münsterland	MU	Noack Rosen	GER	1986	Floribunda, shrub	light pink	tetraploid
114	Venice	VE	Noack Rosen	GER	2003	Floribunda, ground cover	white	tetraploid
115	Focus	FO	Noack Rosen	GER	1997	Hybrid Tea	light pink	tetraploid
116	Simply	SI	Noack Rosen	GER	2003	ground cover	pink	Tetraploid
118	Kronjuwel	KR	Noack Rosen	GER	1997	Floribunda, bedding	red	Tetraploid
119	Tornella	TO	Noack Rosen	GER	2005	shrub	red	Tetraploid

120	Herzogin Friederike	HF	Noack Rosen	GER	2002	shrub	pink	Tetraploid
122	Blue River	BR	W. Kordes&Söhne	GER	1984	Hybrid Tea	magenta	Tetraploid
131	Cute Haze	CH	Rosen Tantau	GER	2010	ground cover, shrub	white	Tetraploid
132	Duftwolke	DW	Rosen Tantau	GER	1963	bedding	red	Tetraploid
133	Goethe Rose	GR	Rosen Tantau	GER	2004	Hybrid Tea	red	Tetraploid
134	Albrecht Dürer Rose	AD	Rosen Tantau	GER	1996	Hybrid Tea	orange	Tetraploid
135	Stadt Rom	SR	Rosen Tantau	GER	2000	ground cover	carmine-pink	Tetraploid
136	Bienenweide	BI	Rosen Tantau	UK	2011	mini-shrub	red	Tetraploid
137	Lolita	LT	W. Kordes&Söhne	GER	1972	Hybrid Tea	apricot	Tetraploid
138	Magenta	MA	W. Kordes&Söhne	GER	1954	Floribunda, shrub	violet	Tetraploid
139	Rose Gaujard	RG	Jean-Marie Gaujard	F	1957	Hybrid Tea	cherry-red	Tetraploid
140	Crimson Glory	CR	W. Kordes&Söhne	GER	1935	Hybrid Tea	purple, crimson	Tetraploid
141	Sunset Boulevard	SB	Harkness & Co Ltd.	UK	1997	Floribunda, shrub	salmon-pink	Tetraploid

Note: * is missed in *in vivo* **is missed in *in vitro* experiment

Table S2: Composition of the nutrient solution used for the hydroponic rooting experiments in vivo

Minerals	Amount (g/L)
NH_4NO_3	12
KH_2PO_4	16.28
$MgSO_4$ x 7 H_2O	7.12
KNO_3	17.4
$Mg(NO_3)_2$ x 6 H_2O	48.7
NaCl	2.55
$Ca(NO_3)_2$ x 4 H_2O	86.1
$ZnSO_4$ x 7 H_2O	0.24
Fe EDTA (Fetrilon 5 % Fe)	1.2
$MnSO_4$ x H_2O	0.19
$CuSO_4$ x 5 H_2O	0.036
H_3BO_3	0.19
Na_2MoO_4	0.016

Table S3*: In vitro* rooting traits

No	Genotypes	Root number. mean	Root number. Sd	Total length. mean	Total length.sd
1	Albrecht Dürer Rose	10.42	3.80	8.23	3.44
2	Alinka	10.95	5.48	7.77	5.85
3	Arabia	0.33	0.95	0.25	1.09
4	Arthur Bell	10.65	4.63	5.46	3.24
5	Ausfather				
6	Auslo	7.78	4.03	9.22	4.99
7	Ausmas	13.68	6.62	6.12	4.80
8	Berolina	11.15	4.81	6.27	3.67
9	Bevely	10.53	4.82	5.486	2.44
10	Bienenweide	10.20	3.81	11.44	4.69
11	Black Baccara	5.65	2.63	4.57	1.94
12	Black Magic	11.65	4.26	9.293	2.82
13	Blue Parfum	0.22	0.74	0.053	0.20
14	Blue River	8.76	3.60	7.72	3.37
15	Celine Delbard	17.13	4.08	5.78	2.30
16	China Girl	9	3.37	2.56	1.32
17	Chippendale	14.73	6.67	13.84	8.46
18	Climbing Allgold	7.87	3.60	10.68	10.24
19	Compassion	17.48	5.83	10.09	4.36
20	Comtessa Al	2.62	2.03	1.52	1.47
21	Comtesse de Segur	10.97	4.45	4.24	2.35
22	Crimson Glory	17.82	5.33	10.32	3.82
23	Cute Haze	10.45	2.40	19.13	4.04
24	Donauprinzessin	12.07	3.51	10.90	4.04
25	Dortmund	5.8	2.15	3.39	2.80
26	Duftwolke	11.33	3.94	7.506	4.96
27	Dukat	9.37	6.38	7.942	5.67
28	Efle	4.63	3.70	6.59	4.76
29	Feuerwerk	10.13	4.96	8.22	4.19
30	Focus	10.28	3.24	2.81	1.83
31	France Libre	11.2	3.16	7.117	2.01
32	Friesia	16.02	5. 90	18.08	11.25
33	Fritz Nobis	10.79	3.80	6.19	3.09
34	Frülingsduft	7.483	3.42	9.73	4.51
35	Gebrüder Grimm	11.67	3.68	10.46	4.24
36	Goerge Vancouver	8.3	3.47	4.50	1.93
37	Goethe Rose	12.23	4.71	4.56	2.06
38	Hansenstadt Rostock	10.4	6.14	5.97	3.10
39	Heidetraum	15.83	3.99	25.26	9.22
40	Heidi Klum	9.63	2.99	9.14	5.91

41	Herkules	2.087	3.57	2.93	4.79
42	Herzogin Friederike	16.07	5.71	8.25	3.08
43	Jasmina	12.47	4.24	11.11	4.44
44	Jazz	6.31	4.94	2.51	1.94
45	Juanita	15.17	5.85	17.34	9.97
46	Kastelruther Spatzen	9.48	2.94	3.81	1.93
47	Knockout	10.15	2.72	4.91	1.33
48	König Stanislaus	15.48	5.94	3.75	2.33
49	Kronjuwel	9.4	3.49	3.04	1.12
50	La Sevillana	7.12	3.86	2.85	2.06
51	Lavender Lassie	18.87	5.96	18.96	29.03
52	Lipstick	8.93	2.97	10.04	3.69
53	Lolita	8.27	4.79	6.02	4.37
54	Louis Oldier	2.6	3.13	2.98	3.65
55	Magenta	0.17	0.64	0.115	0.47
56	Mainzer Fastnacht	9.53	3.47	14.478	5.94
57	Mariatheresia	3.47	2.98	0.77	1.12
58	Mevrouw nathalie Nypels	11.03	3.79	8.60	3.93
59	Midsummer	6.35	3.65	1.54	1.68
60	Mister Lincoln	11.13	3.55	5.74	2.37
61	Mitsouko	3.27	3.27	2.12	2.68
62	Mme Boll	0.3	1.25	0.68	2.84
63	Mme Knorr	0.4	1.03	0.79	2.16
64	Mrs John Laing	4.15	2.58	1.25	1.27
65	Münsterland	7.23	3.19	5.38	2.85
66	My Girl	12.88	4.58	10.55	3.44
67	Nemo	2.7	2.69	0.95	1.23
68	New Dawn	16.33	4.82	13.10	4.33
69	Nostalgie	4.1	2.42	1.24	0.79
70	Papageno	14.2	3.75	8.73	2.36
71	Parole	7.5	3.48	11.27	5.10
72	Perennial Blush	0.65	1.57	0.66	1.74
73	Perpetually Yours	1.22	2.03	2.13	3.58
74	Princess Alexandra	10.82	4.08	5.19	2.98
75	Queen Elizabeth	11.63	5.03	11.07	5. 40
76	Raubritter	15.15	5.99	16.09	7.43
77	Rose de Resht	0.53	1.06	0.30	0.76
78	Rose Gaujard	9.57	5.18	5.71	4.77
79	Rumba	10.35	4.63	7.68	5.27
80	Scarlet Meidilland	7.8	3.65	11.19	3.98
81	Schneewittchen	12.45	3.89	4.56	2.20
82	Sebastian Kneipp	12.13	5.00	12.27	5.83
83	Shalom	3.35	3.40	1.936	2.72
84	Simply	13.73	4.91	15.30	5.27

85	Small Maidens	6.6	3.28	11.60	5.58
86	Sommerwind	5.95	2.85	1.319	1.04
87	Sonnenschein	9.97	4.68	1.94	1.42
88	Stadt Rom	14.25	5.65	13.52	5.79
89	Sterntaler	8.25	6.06	6.91	5.19
90	Sunset Boulevard	9.14	3.11	4.30	2.17
91	Super Star	11.98	3.59	10.34	4.15
92	Sutter Gold	8.63	3.08	11.09	4.26
93	Tornella	11.85	4.57	5.23	2.16
94	Venice	8.93	4.09	4.784	2.57
95	Westerland	16.87	6.79	17.77	8.95
96	Windrose	10.85	4.19	9.63	5.28

Talble S4: *In vivo* rooting traits

N°	Genotypes	Root number	Root number SD	Root length	Root length SD	Root Biomass	Root Biomass SD
1	Albrecht Dürer Rose	0.78	0.21	0.444	1.014	4.2	0.467
2	Alinka	3.33	0.48	1.289	1.518	36.3	4.033
3	Arabia	7.11	0.38	3.022	1.748	125.4	13.933
4	ArthurBell	1.89	0.44	1.533	2.939	59.6	6.622
5	Ausfather	2.67	0.524	2.467	3.893	83.7	9.3
6	Auslo	1.67	0.304	1.678	2.431	39.4	4.378
7	Ausmas	2.56	3.60	4.2	6.164	164.2	18.244
8	Berolina	1.22	2.04	1.156	1.721	36.1	4.011
9	Beverly	0.44	0.88	0.367	0.843	5.5	0.611
10	Bienenweide	0.33	1.00	0.189	0.567	1.6	0.1778
11	Black Baccara	1.00	1.41	1.289	2.133	30.8	3.422
12	BlackMagic	3.00	4.63	1.811	2.067	69	7.667
13	BlueParfum	0.56	1.01	1.567	2.976	12.1	1.344
14	BlueRiver	4.89	2.84	7.478	4.434	248.5	27.611
15	China Girl	2.56	4.16	0.378	0.386	13.6	1.511
16	Chippendale	3	4.61	1.944	3.517	62.6	6.956
17	ClimbingAllgold	0	0	0	0	0	0
18	Compassion	2.22	3.67	3.411	5.24	94.2	10.467
19	Comtessa AL	1.11	1.167	0.911	1.17	18	2
20	Comtesse de Segur	1.22	2.28	0.778	1.302	12.4	1.3778
21	Crimson Glory	0.33	0.70	0.511	1.025	2.7	0.3
22	CuteHaze	2.89	3.65	6.422	6.75	68.5	7.611
23	Donauprinzessin	10.00	5.07	9.6	3.35	519.5	57.722
24	Dortmund	1.67	3.64	1.578	4.371	50	5.556
25	Duftwolke	1.44	1.81	2.2	3.175	32.7	3.633
26	Dukat	2.11	3.59	1.2	1.452	16.9	1.878
27	Elfe	2.11	2.71	1.589	2.59	35.9	3.989
28	Feuerwerk	12.22	7.17	6.522	5.72	323.2	35.91
29	Focus	2.22	3.45	3.733	5.152	93.6	10.4
30	FrancLibre	2.78	3.56	4.711	5.185	94.8	10.533
31	Friesia	3.78	5.31	2.878	4.706	112.4	12.489
32	FritzNobris	7.76	9.81	4.522	6.348	193.9	21.544
33	Frühlingsduft	0.56	0.72	1.578	2.319	27.5	3.056
34	Gebrüder Grimm	5.89	8.35	2.233	3.278	121.6	13.51
35	George Vancouver	0.78	2.33	1.022	3.067	12.9	1.43
36	GoetheRose	6.00	8.39	1.756	1.785	81	9
37	Hansestadt Rostock	4.11	4.62	5.256	5.402	99.1	11.011
38	Heidetraum	7.78	7.01	10.367	6.99	265.4	29.489
39	Heidi Klum	2.56	3.88	1.622	2.477	78.2	8.689
40	Herkules	0.67	0.87	1.289	2.005	15.4	1.711

41	Herzogin Friederike	6.33	4.24	3.489	2.801	146.6	16.289
42	Jasmina	9.00	8.47	6.756	5.674	316.1	35.122
43	Jazz	2.00	3.27	0.611	1.296	37.2	4.133
44	Juanita	12.89	9.04	10.289	5.38	497.1	55.233
45	Kastelrutherspatzen	0.11	0.33	0.078	0.233	1.9	0.211
46	Knockout	1.67	2.69	1.456	2.463	54.4	6.044
47	König Stanislaus	7.78	9.83	6.467	5.996	286.4	31.822
48	Kronjuwel	1.11	1.61	0.633	0.820	24.1	2.677
49	La Sevillana	6.00	5.5	6.511	5.686	233.4	25.93
50	Lavender Lassie	16.67	7.83	9.322	5.116	607.4	67.489
51	Lipstick	7.56	5.68	6.278	5.505	139.7	15.522
52	Lolita	5.78	5.86	2.378	2.420	131.1	14.567
53	Louise Odier	0.89	2.03	1.356	2.701	10	1.111
54	Magenta	9.11	7.55	2.389	2.518	102.1	11.344
55	Mainzer Fastnacht	3.11	4.31	3.1	4.496	114.1	12.678
56	Mariatheresia	0	0	0	0	0	0
57	Mevrouv Nathalie Nypels	1.22	2.04	3.011	4.045	52.8	5.867
58	Midsummer	0.11	0.33	0.033	0.1	1	0.111
59	Mister Linkoln	5.67	6.87	2.567	3.805	150.7	16.744
60	Mitsouko	1.11	1.69	1	1.598	23.6	2.622
61	Mme Boll	0	0	0	0	0	0
62	Mme Knorr	0	0	0	0	0	0
63	Mrs John Laing	1.22	2.73	0.633	1.269	13.8	1.533
64	Münsterland	0.67	1.66	1.333	2.926	20.9	2.322
65	My Girl	5.33	4.58	5.478	4.746	192.4	21.378
66	Nemo	0	0	0	0	0	0
67	NewDawn	4.67	4.71	3.333	3.84	84.1	9.344
68	Nostalgie	0.11	0.33	0.044	0.133	0.1	0.011
69	Papageno	2.89	3.62	2.378	3.583	92.4	10.267
70	Parole	0.89	1.83	0.622	1.654	14.1	1.567
71	Perennial Blush	3.22	3.63	7.011	7.332	163.7	18.189
72	Perpetually Yours	0.55	1.01	0.856	1.82	20.7	2.3
73	Prinzess Alexandra	1.44	1.33	1.767	1.648	23.8	2.644
74	Queen Elisabeth	5.67	8.20	3.667	5.297	217.7	24.189
75	Raubritter	3.66	3.24	3.189	3.457	57	6.333
76	Rose de Resht	0.89	1.61	1.267	1.656	8.9	0.9889
77	Rose Gaujard	2.22	2.33	3	4.177	55.5	6.167
78	Rumba	5.22	9.03	2.878	3.377	79.9	8.878
79	S. Kneipp	10.33	12.40	5.578	6.39	378.2	42.022
80	Scarlet Meidiland	3.78	5,19	5.211	5.867	65.7	7.3
81	Schneewittchen	7.11	5.37	7.667	5.309	211.2	23.467
82	Shalom	10.67	10.81	5.511	5.988	369.2	41.022
83	Simply	7.67	8.17	11	7.864	252.3	28.033
84	Small Maidens Blush	0.44	0.88	0.344	0.6876	7	0.778

85	Sommerwind	4.56	4.90	2.689	2.327	102.6	11.4
86	Sonnenschirm	8.00	7.26	2.667	2.326	116.3	12.922
87	StadtRom	0.22	0.44	1.144	2.278	10.6	1.178
88	Sterntaler	0.44	0.88	0.422	1.002	4.4	0.489
89	Sunset Boulevard	0.67	1.41	1.1889	2.983	31.5	3.5
90	Superstar	2.89	2.93	4.189	5.847	83.4	9.267
91	SuttersGold	0.67	1.66	0.311	0.619	8.2	0.911
92	Tornella	4.67	3.35	6.1667	5.285	235.5	26.167
93	Venice	0	0	0	0	0	0
94	Westerland	13.89	3.35	16.61	2.653	908.1	100.9
95	Windrose	5.78	3.42	9.889	5.753	286.4	31.82

Table S5: Significant SNPs associated with total length of root *in vitro*

Trait	Marker	Site	p	Chr	Position	Contig	Gene Prediction
Total_length	RhK5_11526_616P	240	1.51E-61	Chr03	36877701	Contig11526	Mitochondrial inner membrane protease
Total_length	Rh12GR_19014_1492P	1988	2.29E-46	Chr01	62766764	Contig19014	Zinc finger CCCH domain-containing protein 13
Total_length	Rh12GR_68348_93P	8118	3.24E-46	Chr02	19323335	Contig68348	NA
Total_length	Rh12GR_41613_4841Q	2825	1.90E-45	Chr04	58522900	Contig41613	Sacsin
Total_length	Rh12GR_1759_1129Q	4046	3.61E-32	NA		Contig1759	uncharacterized LOC101295475
Total_length	RhMCRND_30310_241Q	876	3.99E-31	Chr06	52689755	Contig30310	Methyl-CpG-binding domain-containing protein 4-like
Total_length	RhK5_2973_1284Q	14187	6.26E-29	Chr01	33009925	Contig2973	Protein TRIGALACTOSYLDIACYLGLYCEROL4, chloroplastic
Total_length	RhK5_1033_1351Q	4819	1.03E-28	Chr00	1711166	Contig1033	Probable transcription factor PosF21
Total_length	RhMCRND_30298_168P	2943	5.20E-22	Chr03	30083245	Contig30298	alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase
Total_length	RhMCRND_10809_238Q	6578	6.49E-20	Chr02	32212953	Contig10809	uncharacterized protein At1g10890-like
Total_length	RhMCRND_10451_371P	1362	9.43E-20	Chr07	63372275	Contig10451	melanoma-associated antigen G1 (LOC101312638), mRNA
Total_length	RhK5_3203_939Q	756	4.25E-19	Chr06	57855275	Contig3203	serpin-ZX
Total_length	RhK5_19633_736P	5382	7.04E-19	Chr04	5240389	Contig19633	uncharacterized LOC101302253 (LOC101302253), mRNA
Total_length	RhMCRND_63_4939Q	6551	2.53E-18	Chr03	32098241	Contig63	protein ROS1 (LOC101306354)
Total_length	RhK5_944_1305Q	13460	2.72E-17	Chr01	18760323	Contig944	ETHYLENE INSENSITIVE 3-like 1 protein
Total_length	RhK5_1075_1815Q	2349	5.20E-16	NA		Contig1075	Sterol 3-beta-glucosyltransferase UGT80A2
Total_length	RhMCRND_7400_1010P	1736	1.07E-13	Chr07	18095084	Contig7400	Probable sugar phosphate/phosphate translocator
Total_length	RhK5_241_3965P	7935	3.53E-13	Chr06	55538408	Contig241	U2 snRNP-associated SURP motif-containing protein

Total_length	RhK5_19295_2075P	3879	4.57E-12	Chr06	46262701	Contig19295	CLIP-associated protein (LOC101314039)
Total_length	RhMCRND_18648_265P	4515	2.22E-11	Chr06	47688050	Contig18648	Probable LRR receptor-like serine/threonine-protein kinase
Total_length	Rh12GR_16555_479Q	6269	3.71E-11	Chr02	74912414	Contig16555	uncharacterized LOC101315363
Total_length	RhK5_12504_308Q	2253	5.32E-11	Chr02	22044061	Contig12504	rRNA methyltransferase 3A, mitochondria
Total_length	Rh12GR_11463_1038P	6132	1.39E-10	Chr04	51761364	Contig11463	Uridine-cytidine kinase C
Total_length	RhMCRND_3946_1307Q	1532	1.80E-10	Chr06	30847645	Contig3946	Benzyl alcohol O-benzoyltransferase
Total_length	RhK5_52_5511Q	2333	7.35E-10	NA		Contig52	Proteasome activator subunit 4
Total_length	RhK5_250_1345P	425	9.86E-10	NA		Contig250	DNA damage-binding protein 1
Total_length	RhK5_4525_452Q	3966	3.09E-09	Chr06	51866554	Contig4525	cyclin-P3-1
Total_length	RhK5_376_1591P	6711	4.01E-09	NA		Contig376	probable UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase SEC
Total_length	RhK5_1677_3917P	1175	4.21E-09	Chr06	52085819	Contig1677	probable UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase
Total_length	Rh12GR_13508_635P	3493	7.06E-09	Chr02	59490087	Contig13508	NA
Total_length	RhK5_383_2371Q	3732	9.82E-09	Chr04	46493362	Contig383	calmodulin-binding transcription activator 4
Total_length	RhK5_305_2049P	3496	1.29E-08	Chr05	72592326	Contig305	protein TIC110, chloroplastic
Total_length	RhMCRND_26121_1222Q	10112	1.81E-08	Chr02	10683164	Contig26121	CSC1-like protein
Total_length	Rh12GR_34593_954Q	7812	1.89E-08	Chr07	52209344	Contig34593	vinorine synthase-like
Total_length	Rh12GR_31633_585Q	3309	2.09E-08	Chr03	43622122	Contig31633	WUSCHEL-related homeobox 8-like
Total_length	Rh12GR_63352_283Q	7615	4.48E-08	Chr07	42339184	Contig63352	NA
Total_length	Rh12GR_11502_284P	10415	5.51E-08	Chr06	49952389	Contig11502	putative pentatricopeptide repeat-containing protein
Total_length	RhK5_2313_797Q	1354	5.98E-08	Chr02	27273564	Contig2313	Bifunctional aspartate aminotransferase and glutamate/aspartate-prephenate aminotransferase
Total_length	RhMCRND_8676_969P	5838	1.20E-07	Chr04	5904855	Contig8676	Putative protein phosphatase 2C-like protein 44

Total_length	RhMCRND_14995_204Q	6224	1.27E-07	Chr06	59923851	Contig14995	Avium protein YeeZ (LOC110771525), mRNA
							Lupinus angustifolius cultivar Tanjil
Total_length	RhMCRND_14747_229P	6624	2.53E-07	Chr02	58369406	Contig14747	chromosome LG-09
Total_length	RhK5_8416_661Q	5823	2.69E-07	Chr04	56216659	Contig8416	uncharacterized
Total_length	RhK5_9999_562Q	1464	3.04E-07	NA		Contig9999	isocitrate dehydrogenase [NADP]-like
Total_length	RhK5_3262_482Q	6253	3.26E-07	Chr04	54852262	Contig3262	uncharacterized LOC101299178
							deoxynucleoside triphosphate triphosphohydrolase
Total_length	RhMCRND_16904_622P	8216	3.27E-07	Chr04	56571750	Contig16904	SAMHD1 homolog
Total_length	Rh12GR_29211_289Q	8987	3.62E-07	Chr05	24402392	Contig29211	NA
Total_length	RhMCRND_3689_1357Q	1196	4.25E-07	NA		Contig3689	aspartic proteinase A1-like (LOC101296033)
Total_length	RhK5_3688_940Q	13778	4.70E-07	Chr07	35922442	Contig3688	Histone deacetylase 9
							Ferredoxin-dependent glutamate synthase,
Total_length	RhK5_71_1934Q	3129	7.65E-07	NA		Contig71	Chloroplastic

Table S6: Significant SNPs associated with root number in *vivo*

Trait	Marker	Site	p	Chr	Position	Contig	Gene prediction
RN	RhK5_317_1419Q	5771	1.11E-131	Chr03	40212914	Contig317	Protein transport protein Sec24-like At3g07100 (putative)
RN	RhK5_4957_957Q	13387	2.50E-100	Chr07	28916459	Contig4957	Eukaryotic translation initiation factor 3 subunit J (eIF3j) (probable)
RN	RhK5_7321_779Q	4393	5.97E-100	Chr05	73824400	Contig7321	Histone H4 transcription factor (HiNF-P) (probable)
RN	RhMCRND_26896_126P	4457	1.32E-96	Chr05	76016030	Contig26896	F-box/kelch-repeat protein At3g06240 (probable)
RN	RhK5_3083_188Q	4394	2.14E-88	Chr06	17479191	Contig3083	Probable 1-acyl-sn-glycerol-3-phosphate acyltransferase 5 (putative)
RN	RhK5_8899_1285Q	13105	2.83E-72	Chr05	45698363	Contig8899	Mitochondrial import inner membrane translocase subunit TIM50,_Precursor_(similar to)
RN	RhK5_141_1630P	2841	1.14E-68	Chr00	12346488	Contig141	Probable serine/threonine-protein kinase DDB G0272254
RN	RhK5_4056_658Q	5850	6.63E-53	Chr01	2184630	Contig4056	Alcohol_dehydrogenase-like_1_ (probable)
RN	RhK5_2555_767P	2307	8.09E-53	Chr07	66059732	Contig2555	Coatomer_subunit_alpha-1 (Alpha-COP_1)(similar to)
RN	Rh12GR_47780_467P	3032	5.97E-51	Chr04	2905467	Contig47780	Cellulose_synthase-like_protein _G3_(AtCslG3)(probable)
RN	RhK5_1789_1730Q	3127	3.43E-47	Chr05	24604831	Contig1789	RING_finger_protein_44_(probable)
RN	RhMCRND_8150_446Q	3194	3.14E-46	NA		Contig8150	Mps_one_binder_kinase_ activator-like_1_(similar to)
RN	Rh12GR_27884_1243Q	1624	1.20E-45	NA		Contig27884	SNF1-related_protein_kinase _regulatory subunit_gamma_1 _(AKIN_gamma-1) (putative)
RN	RhK5_16723_83Q	4377	8.39E-45	Chr05	75969082	Contig16723	NA
RN	RhK5_1017_1265P	3864	1.71E-44	NA		Contig1017	Telomere-binding_protein_1 _(probable)
RN	RhMCRND_13500_687Q	6494	1.95E-43	Chr05	30908216	Contig13500	Anti-adapter_protein_iraM (probable)
RN	Rh88_10303_228Q	65	7.57E-42	Chr03	45770281	Contig10303	NA
RN	RhK5_15294_1220P	3538	3.56E-38	Chr04	6279161	Contig15294	Nuclease_sbcCD_subunit_C _(probable)
RN	RhK5_6697_1287Q	13262	3.65E-37	Chr03	40199825	Contig6697	gene FACT_complex_subunit _SPT16_(similar to)
RN	RhK5_107_2439P	2759	1.73E-35	Chr06	45387454	Contig107	E1A-binding_protein_p400 mDomino)(probable)
RN	RhK5_9050_472Q	877	1.80E-34	Chr07	1504967	Contig9050	Probable_ATP-dependent_RNA _helicase_DHX36
RN	RhMCRND_23130_1044P	3521	5.82E-32	Chr05	65498759	Contig14823	NA
RN	Rh12GR_14823_1243P	5627	9.65E-32	Chr07	63326173	Contig650	Protein_phosphatase_1_regulatory _subunit_pprA_(probable)
RN	RhK5_650_2680P	1197	2.10E-31	Chr07	63326173	Contig650	Protein_phosphatase_1_regulatory _subunit pprA_(probable)
RN	Rh12GR_28210_606Q	7031	6.96E-31	NA		Contig28210	Putative_ubiquitin_thioesterase _232R_(probable)

RN	RhK5_446_213P	1807	7.16E-31	NA		Contig446	Mitochondrial_Rho_GTPase_2 _(MIRO-2)_ (probable)
RN	Rh12GR_32282_726Q	5533	1.61E-30	NA		Contig32282	Suppressor/enhancer_of_lin-12 _protein_9,_Precursor_(probable)
RN	RhK5_235_2399Q	10012	3.17E-28	Chr04	10578014	Contig235	Auxin_response_factor_19 _(similar to)
RN	RhK5_5111_895P	3840	3.27E-28	Chr07	41046537	Contig5111	hypothetical_protein
RN	RhK5_69_2438Q	13360	1.84E-26	Chr02	691563	Contig69	hypothetical_protein
RN	RhMCRND_8232_1199Q	84	1.87E-26	Chr03	41907727	Contig8232	Putative_F-box_protein_ At3g52320_(probable)
RN	Rh12GR_82721_184P	5545	2.76E-26	Chr04	7476666	Contig82721	NA
RN	RhK5_7708_325P	7356	1.69E-24	Chr06	66120659	Contig7708	hypothetical protein
RN	RhK5_5284_752P	1635	5.30E-23	NA		Contig5284	Glucosamine-fructose-6-phosphate aminotransferase- isomerizing2 (GFAT_2) (putative)
RN	RhK5_16786_257Q	10620	5.55E-23	Chr06	36444765	Contig16786	mTERF_domain-containing_ protein_1,mitochondrial, Precursor_(probable)
RN	RhMCRND_1154_1032Q	2652	9.50E-22	Chr04	33189866	Contig1154	Serine/threonine-protein_kinase _WNK1 (AtWNK1) (probable)
RN	RhK5_15295_125Q	1675	2.48E-20	Chr06	45110951	Contig15295	E3_ubiquitin/ISG15_ligase_ TRIM25_(probable)
RN	RhK5_5772_666P	141	3.43E-20	Chr01	64574531	Contig5772	Inositol-tetrakisphosphate 1 -kinase_1_(Atltpk-1) (putative)
RN	RhK5_5215_773Q	13356	1.51E-19	Chr02	3360035	Contig5215	UPF0326_protein_At4g17486_ (similar to)
RN	RhK5_1138_459P	4244	4.99E-19	Chr06	57523947	Contig1138	hypothetical protein
RN	RhK5_901_960Q	12036	7.16E-19	Chr05	55941211	Contig901	Glucomannan_4-beta-mannosyltransferase 2 (AtCslA2) _(putative)
RN	RhMCRND_2712_1028Q	5767	9.20E-19	Chr03	36864250	Contig2712	Probable_LRR_receptor-like_serine/threonine-protein kinase_At1g56140,_Precursor
RN	RhK5_10911_184P	579	5.09E-18	Chr04	54036623	Contig10911	Histone_acetyltransferase_GCN5 (probable)
RN	Rh12GR_19014_122Q	6557	5.87E-18	Chr01	62764904	Contig19014	DNA_ligase_1_(probable)
RN	RhK5_4688_911Q	11733	1.20E-17	Chr06	27093425	Contig4688	GATA_transcription_factor_27 _(probable)
RN	RhMCRND_4332_1059P	2255	2.00E-17	Chr04	10916131	Contig4332	F-box_protein_At5g07610 _(probable)
RN	Rh12GR_6906_1490P	32	3.66E-17	NA		Contig6906	Putative_pre-mRNA-splicing Factor ATP- dependent_RNA_ helicase_DHX16 (probable)
RN	RhK5_1958_1219P	1638	4.37E-17	Chr05	31147273	Contig1958	Tryptophan_synthase_beta_chain _2,chloroplastic,_ Precursor_(putative)
RN	RhMCRND_16405_526P	4060	6.04E-17	Chr07	9342934	Contig16405	NA
RN	RhK5_131_1504Q	3909	8.53E-16	Chr01	63562483	Contig131	Neuroblastoma-amplified_sequence_(probable)
RN	RhK5_11161_872Q	327	9.32E-16	Chr02	1995190	Contig11161	Protein_SRG1_(AtSRG1) _(probable)
RN	Rh12GR_18217_614P	3483	1.37E-15	NA		Contig18217	NA
RN	RhK5_11428_96P	3622	5.88E-15	Chr05	34584307	Contig11428	NA

RN	RhMCRND_3891_1012P	3119	1.91E-14	Chr03	37157876	Contig3891	Acyl- coenzyme_A_oxidase_4, peroxisomal_(AOX_4) (putative)
RN	RhK5_209_887Q	10968	2.18E-13	Chr02	9051557	Contig209	Saccharopine_dehydrogenase_ (putative)
RN	RhMCRND_10708_222Q	3744	4.28E-13	Chr05	61078364	Contig10708	Sentrin-specific_protease_8_ (probable)
RN	RhK5_5553_284Q	5417	6.46E-13	Chr05	85745030	Contig5553	Exostosin-2_(probable)
RN	RhK5_10911_145Q	2663	1.74E-12	Chr04	54036662	Contig10911	Histone_acetyltransferase_GCN5 _(probable)
RN	Rh12GR_8601_183Q	7219	2.78E-12	Chr03	40881766	Contig8601	gene Nicotianamine_synthase_ (putative)
RN	RhK5_10236_362P	2220	5.17E-12	Chr03	40951159	Contig10236	GTP-binding_protein_SAR1A_ (similar to)
RN	RhK5_6704_408Q	13962	7.77E-12	Chr02	2484802	Contig6704	Beta-1,4-mannosyl-glycoprotein 4-beta -N-acetylglucosaminyltransferase (N-acetylglucosaminyltransferase_III) (probable)
RN	RhK5_5621_803Q	12411	9.25E-12	NA		Contig5621	Putative_hydrolase_C777.06c_ (probable)
RN	Rh12GR_48217_390Q	6067	9.35E-12	Chr07	13479885	Contig48217	NA
RN	RhK5_7272_77Q	3214	3.35E-11	Chr00	113922	Contig7272	Period_circadian_protein_homolog_2 (cPER1) (probable)
RN	RhK5_7897_890Q	12992	5.09E-11	Chr05	9802115	Contig7897	Calcyclin-binding_protein_(CacyBP) (probable)
RN	Rh12GR_13483_1270P	5315	7.24E-11	Chr00	31347219	Contig13483	Probable_rhamnose_biosynthetic _enzyme_1 (putative)
RN	RhK5_20947_367Q	9077	7.78E-11	Chr00	1689106	Contig20947	F-box/LRR-repeat_protein_3_ (probable)
RN	Rh12GR_36000_270P	5778	7.80E-11	NA		Contig36000	Annexin_D5_(putative)
RN	RhK5_6532_163Q	13660	9.81E-11	NA		Contig6532	Mps_one_binder_kinase_activator -like_1 (putative)
RN	RhK5_1705_891P	4668	1.92E-10	Chr02	11938868	Contig1705	Cytochrome_P450_90C1_ (similar to)
RN	RhK5_18945_1033Q	1391	2.53E-10	Chr04	56728317	Contig645	Probable_alpha,alpha-trehalose-phosphate synthase [UDP-forming]_10_(AtTPS10)_(putative)
RN	RhMCRND_645_325Q	2074	5.45E-10	Chr04	56728317	Contig645	Probable_alpha,alpha-trehalose-phosphate synthase [UDP-forming]_10_(AtTPS10)_ (putative)
RN	RhMCRND_9611_1032Q	5762	7.27E-10	Chr02	71641919	Contig9611	Quinone_oxidoreductase-like_ protein_ At1g23740, chloroplastic,_Precursor_ (similar to)
RN	RhK5_2621_1523P	4760	1.09E-09	Chr05	69433322	Contig2621	Phospholipase_C_4,_Precursor _(probable)
RN	RhMCRND_11628_825Q	6487	1.21E-09	Chr02	68679645	Contig11628	Lamin-like_protein,_Precursor _(similar to)
RN	RhMCRND_4183_989Q	5752	1.41E-09	NA		Contig4183	NA
RN	RhK5_1157_1890P	4159	4.94E-09	Chr05	5423615	Contig1157	DNA_polymerase_alpha-binding_ protein_ (probable)
RN	Rh12GR_49528_182P	5809	1.52E-08	Chr07	33153851	Contig49528	NA
RN	RhK5_10627_495Q	3263	2.76E-08	Chr01	46800903	Contig10627	Programmed_cell_death_ protein_7_(probable)

RN	Rh12GR_1663_1052P	10522	3.68E-08	Chr01	54603419	Contig1663	Centrosomal_protein_of_290_kDa _(Cep290)_(probable)
RN	RhK5_14297_200Q	2447	5.26E-08	NA		Contig14297	Seryl-tRNA_synthetase_(SerRS) _(probable)
RN	RhK5_43_4451P	3630	6.95E-08	Chr05	83182036	Contig43	Callose_synthase_9_(similar to)
RN	RhK5_3228_870Q	1860	8.61E-08	NA		Contig3228	E3_ubiquitin-protein_ligase _SINAT3_(similar to)
RN	RhK5_1049_2189P	1893	1.17E-07	NA		Contig1049	Conserved_oligomeric_Golgi _complex subunit_3 (COG_complex_subunit_3) (probable)
RN	RhK5_9842_811P	9017	1.21E-07	Chr05	40107884	Contig9842	OTU_domain-containing_protein_5 (probable)
RN	Rh12GR_41596_375P	6578	1.27E-07	NA		Contig41596	Sucrose_synthase_2_(putative)
RN	RhK5_13091_426P	3742	1.30E-07	Chr07	53810245	Contig13091	LisH_domain-containing_protein_C1711.05_ (probable)
RN	RhMCRND_3684_1281P	2016	1.40E-07	Chr07	3209451	Contig3684	Dof_zinc_finger_protein_DOF3.3 (AtDOF3.3) (probable)
RN	RhK5_9153_955P	10234	1.53E-07	Chr04	58782447	Contig9153	ATP-dependent_RNA_helicase_DBP7 (probable)
RN	RhK5_9467_586P	10435	2.16E-07	Chr04	12671207	Contig9467	UDP-N-acetylenolpyruvoylglucosamine reductase (probable)
RN	Rh12GR_70672_85P	5538	2.65E-07	Chr05	34124381	Contig70672	Cell_differentiation_protein RCD1 _homolog (Rcd-1) (similar to)
RN	RhK5_91_4238Q	2712	3.36E-07	Chr01	19376259	Contig91	Probable_serine/threonine-protein_kinase_vps15
RN	RhK5_13957_418Q	4469	3.42E-07	Chr07	44449592	Contig13957	GDSL_esterase/lipase_At1g33811, _Precursor (similar to)
RN	RhK5_4809_987Q	274	3.55E-07	Chr01	25607322	Contig4809	Probable_RING-H2_finger_protein _ATL5G
RN	RhK5_3436_577P	1894	4.88E-07	Chr07	42974450	Contig3436	Pinin_(DRS_protein)_(probable)
RN	RhMCRND_22170_662Q	1685	6.12E-07	Chr06	44264630	Contig22170	Putative_quinone- oxidoreductase _homolog, chloroplastic

Table S7: Significant SNPs associated with root length *in vivo*

Trait	Marker	Site	P	Chr	Position	Contig	Gene prediction
RL	RhMCRND_26527_151P	4471	6.40E-132	NA		Contig26527	Inositol_oxygenase_1_ (MI_oxygenase_1) (similar to)
RL	RhMCRND_435_2405Q	75	1.16E-114	NA		Contig435	Protein_EFR3_homolog_B _(probable)
RL	Rh12GR_4642_1250P	6003	4.36E-112	Chr00	12024446	Contig4642	Protein_Brevis_radix-like_2 _(AtBRXL2) (similar to)
RL	RhK5_827_547Q	5209	1.24E-108	Chr07	49255721	Contig827	Polyphosphoinositide_phosphatase (probable)
RL	RhK5_10522_1126Q	10035	7.25E-104	Chr03	15344675	Contig10522	DEAD-box_ATP-dependent _RNA_helicase 24 (putative)
RL	RhK5_1722_1991Q	8435	1.82E-101	Chr02	5074915	Contig1722	SNW_domain-containing_protein_1 (probable)
RL	Rh12GR_3250_1751Q	5270	2.25E-100	Chr05	85514722	Contig3250	Cell_division_protease_ftsH _homolog, chloroplastic, Precursor_(similar to)

RL	RhK5_6314_381Q	4200	3.52E-97	Chr00	12758419	Contig6314	Putative_lipase_ROG1_(probable)
RL	RhK5_13489_1363P	2417	7.62E-91	NA		Contig13489	Translocase_of_chloroplast_159, _chloroplastic (AtToc159) (probable)
RL	RhK5_1049_2189P	1893	2.59E-89	NA		Contig1049	Conserved_oligomeric_Golgi _complex subunit_3 (COG_complex_subunit_3) _(probable)
RL	RhK5_8904_317Q	2481	1.62E-86	Chr05	60329083	Contig8904	F-box/kelch-repeat_protein _At3g06240_(probable)
RL	RhK5_2191_1105P	2730	2.82E-85	NA	-	Contig2191	Ribonuclease_3_(RNase_III) _(probable)
RL	RhK5_6865_984P	2759	6.43E-83	Chr06	60786531	Contig6865	Tubulin_beta-6_chain_(similar to)
RL	RhK5_6281_425P	1717	6.54E-79	NA	-	Contig6281	Probable_E3_ubiquitin-protein_ligase_MGRN1
RL	RhK5_5005_818Q	13410	7.76E-79	Chr06	65003693	Contig5005	Arginine_N-methyltransferase_ 2_(probable)
RL	RhK5_4548_999P	536	5.07E-74	NA	-	Contig4548	Protein_TRANSPARENT_ TESTA_12 (probable)
RL	RhK5_3224_591P	2227	7.75E-73	Chr04	23386445	Contig3224	Microtubule-associated_protein TORTIFOLIA1 (putative)
RL	RhK5_7321_779Q	4393	1.07E-72	Chr05	73824400	Contig7321	Histone_H4_transcription_factor_ (HiNF-P) (probable)
RL	RhMCRND_12614_672P	6485	2.91E-70	Chr04	25968402	Contig12614	Putative_F-box/kelch-repeat_protein _At5g24040 (probable)
RL	RhMCRND_2019_2493Q	6483	1.92E-60	Chr05	138043	Contig2019	Glycosyltransferase_QUASIMODO1 (similar to)
RL	Rh12GR_2854_62Q	5889	2.15E-53	Chr03	19034871	Contig2854	Pentatricopeptide_repeat-containing _protein At2g13420,mitochondrial, _Precursor_(putative)
RL	RhK5_3663_1299P	3200	2.30E-52	Chr06	63835763	Contig3663	Patatin-05,_Precursor_(probable)
RL	RhK5_2621_1523P	4760	5.44E-51	Chr05	69433322	Contig2621	Phospholipase_C_4,_ Precursor_(probable)
RL	RhK5_3720_97P	3954	3.67E-50	Chr04	53982286	Contig3720	Protein_ycf2_(probable)
RL	RhMCRND_4784_585P	1662	6.12E-50	NA	-	Contig4784	WD_repeat-containing_ protein_70_(probable)
RL	RhMCRND_7614_440Q	1723	1.93E-49	Chr06	7621271	Contig7614	Double_homeobox_protein_4 _(probable)
RL	Rh12GR_22444_354Q	5471	1.96E-48	Chr02	1617279	Contig22444	Lactadherin_(MFG-E8),_ Precursor_(probable)
RL	RhK5_2492_1697P	4132	1.97E-48	Chr03	46237324	Contig2492	Protein TRIGALACTOSYLDIA-CYLGLYCEROL 3, chloroplastic _(ABC_transporter_ABCI.13), Precursor (similar to)
RL	RhMCRND_4698_674P	3566	3.24E-48	Chr05	59088391	Contig4698	Putative_serine/threonine-protein_ Kinase receptor (SRK), Precursor_(probable)
RL	RhK5_14950_196P	1051	1.40E-46	Chr02	17743395	Contig14950	F-box_protein_SKIP2_(putative)
RL	RhK5_4282_560Q	2236	1.21E-43	Chr01	25152041	Contig4282	SEC12-like_protein_1_(PHF-1) _(similar to)
RL	RhK5_4148_3177Q	4431	5.20E-42	NA	-	Contig4148	Serine-rich_adhesin_ for_platelets, Precursor (probable)
RL	RhK5_2133_1315P	5440	8.02E-41	NA	-	Contig2133	Probable_complex_I_intermediate-associated_protein_30
RL	Rh88_13156_160Q	8143	8.26E-41	Chr07	65823882	Contig13156	NA

RL	Rh12GR_27560_1424Q	7295	1.27E-40	NA	-	Contig27560	Lys-63-specific_deubiquitinase _BRCC36 (probable)
RL	Rh12GR_21115_177Q	7207	1.30E-40	Chr06	52082030	Contig21115	Cysteine-rich_receptor-like _protein_kinase 25 (Cysteine-rich_RLK25),_Precursor_(probable)
RL	RhK5_3415_1034P	1718	9.18E-40	Chr07	44581136	Contig3415	UPF0496_protein_4_(probable)
RL	Rh12GR_66630_246Q	904	3.57E-39	Chr05	49915140	Contig66630	DNA-directed_RNA_polymerases _I,_II _and_III subunit RPABC5 RNA_polymerases_I, II, and_III_subunit_ABC5) (similar to)
RL	Rh12GR_23202_2787P	7492	7.11E-39	NA	-	Contig23202	Pleiotropic_drug_resistance_protein _1 (putative)
RL	Rh88_40983_449P	8102	2.74E-38	Chr05	30980456	Contig40983	NA
RL	Rh12GR_18936_241P	5548	2.77E-38	Chr01	53584600	Contig18936	NA
RL	RhMCRND_31974_180Q	5419	3.35E-38	NA	-	Contig31974	D-2-hydroxyglutarate_ dehydrogenase, mitochondrial, Precursor_(similar to)
RL	Rh88_13485_715Q	8231	5.32E-38	Chr03	46174455	Contig13485	NA
RL	RhMCRND_17848_232Q	2068	5.85E-38	NA	-	Contig17848	Sentrin-specific_protease_2_(Axam) (probable)
RL	RhK5_8635_151P	9963	6.02E-38	Chr05	34406218	Contig8635	FERM,_RhoGEF_and_pleckstrin _domain-containing_protein_2 (FIR)_(probable)
RL	RhMCRND_21388_203P	5059	1.34E-37	Chr01	63771888	Contig21388	NA
RL	Rh12GR_46438_208Q	5366	2.78E-37	NA	-	Contig46438	NA
RL	Rh12GR_5432_826Q	1346	4.67E-37	Chr05	48413458	Contig5432	Glycoprotein-N-acetylgalactosamine _3-beta-galactosyltransferase_1 (Core_1_beta3-Gal-T) (probable)
RL	Rh12GR_70672_85P	5538	6.21E-37	Chr05	34124381	Contig70672	Cell_differentiation_protein_ RCD1_homolog (Rcd-1) (similar to)
RL	Rh12GR_30801_1108Q	8592	1.00E-36	Chr06	41789274	Contig30801	Peptide_chain_release_factor_1 _(RF-1)_(probable)
RL	RhK5_1151_2043Q	10127	2.01E-36	NA	-	Contig1151	Dynamin-like_protein_C_(probable)
RL	RhK5_21136_49Q	10571	2.05E-36	Chr05	41226247	Contig21136	Probable_CCR4-associated_ factor_1 homolog_11 (similar to)
RL	RhMCRND_3684_1281P	2016	2.38E-36	Chr07	3209451	Contig3684	Dof_zinc_finger_protein_DOF3.3 (AtDOF3.3) (probable)
RL	RhK5_6730_852Q	12901	4.25E-36	Chr05	7630738	Contig6730	60S_ribosomal_protein_L11 _(similar to)
RL	RhK5_1155_1593Q	9876	8.73E-36	NA	-	Contig1155	Protein_transport_protein _SEC23_(probable)
RL	Rh12GR_33881_1350P	4823	9.24E-36	Chr05	19817305	Contig33881	NA
RL	RhK5_6710_317Q	4624	3.53E-35	Chr07	50361694	Contig6710	Probable_esterase_At1g33990 _(similar to)
RL	RhK5_252_3720Q	10145	4.05E-35	Chr05	85836695	Contig252	TATA-binding_protein-associated _factor_172 (TAF-172)_(probable)
RL	RhK5_16723_83Q	4377	8.20E-35	Chr05	75969082	Contig16723	NA
RL	RhK5_4169_239P	5761	1.17E-34	Chr03	14141449	Contig4169	Protein_rolling_stone_(probable)
RL	RhMCRND_12252_231P	9614	1.48E-34	Chr04	57962133	Contig12252	NA
RL	RhMCRND_11874_191Q	1163	1.57E-34	Chr06	48549773	Contig11874	Serine/threonine-protein_kinase _STE20 (probable)
RL	RhK5_1017_1265P	3864	1.75E-34	NA	-	Contig1017	Telomere-binding_protein_1

							_(probable)
RL	RhK5_1661_1118P	6355	2.66E-34	NA	-	Contig1661	WD_repeat-containing_protein_26 _(probable)
RL	RhK5_14646_481Q	4152	3.72E-34	Chr05	61846265	Contig14646	Mitogen-activated_protein_kinase _homolog NTF6 (similar to)
RL	RhMCRND_2849_1053Q	6970	5.64E-34	Chr04	58783519	Contig2849	Lipoyl_synthase,_mitochondrial _(Lip-syn), Precursor (similar to)
RL	RhK5_1789_1730Q	3127	5.83E-34	Chr05	24604831	Contig1789	RING_finger_protein_44_(probable)
RL	RhMCRND_19047_1139Q	7567	6.49E-34	Chr05	32933600	Contig19047	Calcium-dependent_protein_ kinase_isoform_2 (CDPK_2 (putative)
RL	RhMCRND_10125_256P	3488	1.67E-33	Chr06	59901379	Contig10125	BEL1-like_homeodomain_ protein_11_(BEL1-like_protein_11) (similar to)
RL	RhK5_7371_243P	5776	1.76E-33	Chr03	11770810	Contig7371	Glutathione_S-transferase_ (similar to)
RL	RhK5_2271_883P	2193	2.22E-33	Chr01	63566192	Contig2271	Major_facilitator_superfamily _domain-containing_protein_5 (probable)
RL	Rh12GR_68844_266Q	5392	2.50E-33	Chr00	768213	Contig68844	Protein_translocase_subunit _secA(similar to)
RL	Rh88_10303_228Q	65	2.89E-33	Chr03	45770281	Contig10303	NA
RL	RhK5_1613_1045Q	5155	4.08E-33	Chr04	15401887	Contig1613	Lysyl-tRNA_synthetase_ (LysRS)_(similar to)
RL	Rh12GR_16137_133Q	6460	7.60E-33	Chr03	45099704	Contig16137	Peptidyl-prolyl_cis-trans_isomerase _CYP19-3 (PPIase_CYP19-3) _(similar to)
RL	Rh12GR_24671_671P	2280	1.38E-32	Chr06	63102707	Contig24671	Probable_E3_ubiquitin-protein _ligase_HERC3
RL	RhMCRND_13500_687Q	6494	1.68E-32	Chr05	30908216	Contig13500	Anti-adapter_protein_iraM _(probable)
RL	RhK5_13515_498P	3914	5.54E-32	NA	-	Contig13515	Oxysterol-binding_protein_5 _(OSBPe) (probable)
RL	RhK5_8678_89Q	1485	5.93E-32	NA	-	Contig8678	tRNA_guanosine-2'-O-methyltransferase_TRM13 homolog (probable)
RL	RhK5_395_2157P	6290	7.03E-32	Chr02	53214321	Contig395	Coatomer_subunit_beta'-2_ (Beta'-COP_2) (putative)
RL	RhMCRND_29771_219P	8071	1.56E-31	NA	-	Contig29771	NA
RL	Rh12GR_19394_1395P	6553	2.34E-31	Chr02	4266822	Contig19394	Transcription_factor_IIIA_(Factor_A) (probable)
RL	Rh12GR_74969_324P	6177	4.61E-31	Chr07	24138886	Contig74969	NA
RL	RhK5_1179_505Q	1248	1.30E-30	NA	-	Contig1179	Serine/threonine-protein_kinase_ 38-like (probable)
RL	RhMCRND_31878_220Q	1392	1.66E-30	Chr04	42793039	Contig31878	NA
RL	RhK5_15294_1220P	3538	2.75E-30	Chr04	6279161	Contig15294	Nuclease_sbcCD_subunit_C (probable)
RL	RhK5_16105_273Q	2	4.33E-30	Chr07	4331459	Contig16105	COBRA-like_protein_4,_ Precursor (similar to)
RL	Rh12GR_11509_501Q	4969	4.84E-30	Chr07	5547407	Contig11509	gene F-box/LRR-repeat_protein_4 _(AtFBL4) (probable)
RL	RhMCRND_16969_158Q	5044	7.11E-30	Chr05	17833939	Contig16969	Transcription_termination_factor, mitochondrial (mTERF), Precursor_(probable)

RL	RhK5_231_1356P	3706	1.65E-29	Chr04	47959473	Contig231	Putative_leucine-rich_repeat-containing_protein_DDB_G0290503 (probable)
RL	RhK5_8909_679Q	3088	2.41E-29	Chr05	5977185	Contig8909	Zinc_finger_CCCH_domain-containing_protein_27_(AtC3H27) (probable)
RL	RhMCRND_25855_153P	4567	3.34E-29	Chr01	61970795	Contig25855	NA
RL	RhK5_3385_731Q	13961	4.50E-29	NA	-	Contig3385	Probable_S-acyltransferase_ At3g51390 (similar to)
RL	RhMCRND_12523_3338P	5411	5.94E-29	Chr01	64352231	Contig12523	Guanosine-3',5'-bis(diphosphate)_ 3'-pyrophosphohydrolase ((ppGpp)ase)_(probable)
RL	RhMCRND_27267_284Q	7392	1.02E-28	Chr03	33561159	Contig27267	NA
RL	RhK5_21708_310P	7677	1.03E-28	Chr05	73056511	Contig21707	NA
RL	RhK5_41_5365P	2441	1.50E-28	Chr05	17449063	Contig41	Dedicator_of_cytokinesis protein_8_(probable)
RL	RhK5_4056_658Q	5850	2.58E-28	Chr01	2184630	Contig4056	Alcohol_dehydrogenase-like _1_(probable)
RL	RhMCRND_13229_184Q	4834	3.22E-28	Chr03	39956877	Contig13229	hypothetical protein
RL	RhMCRND_17583_425Q	7209	3.29E-28	NA	-	Contig17583	U3_small_nucleolar ribonucleo protein protein IMP4 (U3_snoRNP_protein_IMP4) _(probable)
RL	RhMCRND_6179_1232P	7724	4.48E-28	Chr06	13851135	Contig6179	WW_domain-binding_protein_4 _ (WBP-4) (probable)
RL	Rh12GR_63552_69Q	5911	8.93E-28	Chr05	221991	Contig63552	NA
RL	RhMCRND_36104_481Q	1908	1.59E-27	Chr07	52976822	Contig36104	NA
RL	RhK5_4940_1947Q	5053	1.68E-27	Chr07	11647214	Contig4940	Filaggrin_(probable)
RL	RhK5_15035_566P	2243	1.72E-27	Chr05	74656672	Contig15035	Regulator_of_ribonuclease-like_protein_3 (putative)
RL	RhMCRND_23130_1044P	3521	6.59E-27	Chr05	65498759	Contig23130	F-box_protein_At3g07870_ (probable)
RL	RhMCRND_9294_463P	8669	7.13E-27	Chr07	2491288	Contig9294	NADH-ubiquinone_ oxidoreductase_chain_5 (probable)
RL	RhK5_7068_1984Q	13362	1.40E-26	Chr06	14341730	Contig7068	Far_upstream_element-binding _protein_3 (FUSE-binding_ protein_3) (probable)
RL	RhK5_446_213Q	4124	5.65E-26	NA	-	Contig446	Mitochondrial_Rho_GTPase_2 _(MIRO-2) (probable)
RL	RhK5_10321_598P	103	1.29E-25	Chr03	46008712	Contig10321	NA
RL	Rh12GR_20266_421P	5974	1.48E-25	Chr05	14288260	Contig20266	Transcription_initiation_factor _TFIID subunit_3 (probable)
RL	RhK5_650_2680P	1197	1.59E-25	Chr07	63326173	Contig650	Protein_phosphatase_1_regulatory subunit_pprA (probable)
RL	Rh12GR_47780_467P	3032	3.29E-25	Chr04	2905467	Contig47780	Cellulose_synthase-like_protein _G3 (AtCslG3)_(probable)
RL	Rh88_37659_249Q	8078	8.31E-25	Chr07	50274438	Contig37659	NA
RL	RhK5_15035_147Q	10149	9.31E-25	Chr05	74657091	Contig15035	Regulator_of_ribonuclease-like _protein_3 (putative)
RL	RhK5_27_6960Q	3213	2.73E-24	Chr03	25584477	Contig27	Protein_virilizer_(probable)
RL	RhK5_8557_583P	3126	7.58E-24	NA	-	Contig8557	Putative_Holliday_junction_ resolvase (probable)

RL	RhK5_13580_328P	2854	1.25E-23	Chr03	39482368	Contig13580	Methenyltetrahydrofolate_ Cyclohydrolase (similar to)
RL	RhK5_5957_263P	2152	5.60E-23	Chr04	51055490	Contig5957	Allene_oxide_cyclase_3,_ chloroplastic, Precursor (putative)
RL	RhK5_2377_1023Q	4696	8.82E-22	Chr07	26945579	Contig2377	Transcription_factor_MYC2_ (AtMYC2) (putative)
RL	RhK5_2894_1269Q	12667	1.27E-21	Chr01	14621092	Contig2894	Secologanin_synthase_(SLS)_ (similar to)
RL	RhMCRND_20976_279Q	5808	2.43E-21	Chr03	45346468	Contig20976	UvrABC_system_protein_C (Protein_uvrC)_ (probable)
RL	RhMCRND_3928_2035Q	8674	8.01E-21	NA	-	Contig3928	hypothetical protein
RL	RhMCRND_3066_1036Q	10456	2.24E-20	Chr07	3891906	Contig3066	UDP-glucose_6-dehydrogenase_ (UDP-Glc_dehydrogenase)_ (putative)
RL	RhK5_446_213P	1807	4.35E-20	NA	-	Contig446	Mitochondrial_Rho_GTPase_2_ (MIRO-2) (probable)
RL	RhK5_11977_99P	2716	5.42E-20	Chr05	9849302	Contig11977	Chaperone_protein_dnaK_(probable)
RL	Rh12GR_44358_178P	3353	1.38E-19	Chr05	51287973	Contig44358	NA
RL	RhMCRND_21373_198P	7884	1.65E-19	Chr02	14478437	Contig21373	NA
RL	RhMCRND_20557_255Q	1254	1.95E-19	Chr03	370416	Contig20557	NA
RL	RhK5_943_556P	363	2.21E-19	Chr03	6164747	Contig943	Exosome_component_10_ (PM/Scl-100) (probable)
RL	RhK5_446_1434Q	14030	3.98E-19	Chr03	38370827	Contig446	Mitochondrial_Rho_GTPase_2_ (MIRO-2) (probable)
RL	RhK5_2983_1422Q	13784	6.15E-19	Chr02	54837137	Contig2983	hypothetical protein
RL	RhMCRND_18936_424Q	1905	1.00E-18	Chr04	50253479	Contig18936	Probable_inactive_receptor_kinase At3g08680, Precursor
RL	RhMCRND_5622_1363P	7909	1.15E-18	Chr05	10615356	Contig5622	Putative_F-box/LRR-repeat_protein _23 (similar to)
RL	RhK5_374_2490P	3783	1.32E-18	Chr04	58542242	Contig374	ATP-dependent_Clp_protease _proteolytic subunit_2, mitochondrial, Precursor_(similar to)
RL	RhMCRND_4841_856Q	278	1.38E-18	Chr02	48504478	Contig4841	3-isopropylmalate_dehydrogenase _2,_chloroplastic (3-IPM-DH_2), Precursor_(putative)
RL	RhK5_439_742P	3230	1.80E-18	NA	-	Contig439	Coatomer_subunit_beta-1_(Beta-COP_1)_(putative)
RL	RhMCRND_17527_614P	2013	2.31E-18	NA	-	Contig17527	Lectin-domain_containing_receptor _kinase _A4.3, Precursor_(probable)
RL	RhMCRND_9419_1018Q	521	3.56E-18	Chr03	7593887	Contig9419	Wiskott-Aldrich_syndrome_protein homolog_1 (similar to)
RL	RhK5_947_454P	9991	6.11E-18	Chr05	56101223	Contig947	ABC_transporter_G_family_ member_3 (ABC_transporter_ABCG.3) _(similar to)
RL	RhK5_583_1899Q	3422	1.54E-17	Chr01	43842329	Contig583	Multiple_C2_and_transmembrane _domain-containing_protein_1 (probable)
RL	RhK5_53_366P	2723	1.76E-17	Chr05	272822	Contig53	Sister_chromatid_cohesion_protein_ PDS5_homolog_B-B (probable)
RL	Rh12GR_33170_1025Q	85	2.67E-17	NA	-	Contig33170	NA
RL	RhK5_20607_155P	3676	2.90E-17	Chr07	44208395	Contig20607	mRNA_turnover_protein_4_homolog (probable)
RL	Rh12GR_67678_173P	6616	3.18E-17	Chr04	48694348	Contig67678	NA

RL	RhMCRND_14257_535Q	968	4.42E-17	Chr04	10888146	Contig14257	F-box_protein_At5g07610_(probable)
RL	RhK5_16262_65P	2172	5.34E-17	Chr01	24659683	Contig16262	NA
RL	RhMCRND_21326_123P	6490	5.79E-17	NA	-	Contig21326	NA
RL	RhK5_4957_957Q	13387	7.50E-17	Chr07	28916459	Contig4957	Eukaryotic_translation_initiation _factor_3 subunit_J (eIF3j) (probable)
RL	RhK5_3224_591Q	5583	9.35E-17	Chr04	23386445	Contig3224	Microtubule-associated protein TORTIFOLIA1 (putative)
RL	RhK5_4441_1157Q	3885	9.95E-17	Chr04	8821526	Contig4441	DNA_cross-link_repair_1B_protein (chSNM1B) (probable)
RL	RhK5_4643_636Q	1161	1.59E-16	Chr06	48654359	Contig4634	mRNA-decapping_enzyme-like _protein (probable)
RL	RhK5_13009_56P	2736	2.44E-16	Chr01	58630894	Contig13009	Isoaspartyl_peptidase/L-asparaginase _3 subunit _beta, Precursor_(similar to)
RL	RhMCRND_18945_243P	9527	4.52E-16	Chr00	9867275	Contig18945	Probable_beta-D-xylosidase_5 _(AtBXL5), Precursor_(putative)
RL	RhK5_3253_1339P	91	5.72E-16	Chr01	64719518	Contig3253	Surface_presentation_of_antigens _protein spaS (probable)
RL	RhK5_8746_245Q	4215	8.06E-16	Chr02	12386417	Contig8746	Mitochondrial_chaperone_ BCS1_(probable)
RL	RhK5_519_4026P	395	2.19E-15	Chr04	36497619	Contig519	Tetratricopeptide_repeat_protein_13 (TPR_repeat_protein_13)_(probable)
RL	Rh12GR_8924_1115P	6617	3.77E-15	Chr01	5109837	Contig8924	Methyltransferase-like_protein_13 (probable)
RL	RhMCRND_7129_545P	8049	3.78E-15	Chr06	65852222	Contig1729	Pantothenate_kinase_2_(similar to)
RL	Rh12GR_67729_1618P	7563	7.30E-15	Chr04	-	Contig67729	TPR_repeat-containing_protein _MJ1345_(probable)
RL	RhK5_20843_574P	3876	8.93E-15	Chr04	46343796	Congtig20843	NA
RL	RhK5_916_589Q	163	1.34E-14	Chr03	19436734	Contig916	Vignain,_Precursor_(putative)
RL	RhK5_16951_264Q	1134	2.14E-14	Chr05	10652582	Contig16951	BAH_and_coiled-coil_domain-containing_protein_1 (BAH_domain-containing_protein_2)_(probable)
RL	RhMCRND_433_3179P	7565	2.29E-14	Chr06	36046341	Contig433	Serine_incorporator_3_(probable)
RL	RhMCRND_1033_2408Q	8843	2.36E-14	Chr02	68171595	Contig1033	Chaperone_protein_clpB_2 _(similar to)
RL	Rh12GR_14308_325Q	6486	2.52E-14	Chr04	49161057	Contig14308	SWI/SNF_complex_subunit_ SMARCC1_(probable)
RL	Rh12GR_14823_1243P	5627	4.52E-14	Chr01	8415135	Contig14823	Copper-containing_nitrite_reductase, Precursor_(probable)
RL	Rh12GR_4203_863P	8246	4.72E-14	Chr02	55163054	Contig4203	Ankyrin_repeat-containing_protein _At3g12360_(probable)
RL	Rh12GR_61639_731P	6443	3.39E-13	NA	NA	Contig61639	NA
RL	RhK5_20937_1145Q	9826	3.92E-13	Chr01	46487420	Contig20937	Cell_division_cycle_2-related protein kinase_7 (CDC2-related_ protein_kinase_7)_(probable)
RL	RhK5_838_2471P	3938	3.94E-13	Chr00	2761172	Contig838	NADP-specific_glutamate_ dehydrogenase (NADP-GDH) (probable)
RL	RhMCRND_10274_234Q	1029	1.01E-12	Chr02	51832287	Contig10274	Acyl-protein_thioesterase_2 _(APT-2) (probable)
RL	Rh12GR_1391_2256Q	6459	2.40E-12	Chr04	56120598	Contig1391	Protein_VAC14_homolog_(probable)
RL	Rh12GR_36603_2450P	670	2.60E-12	Chr02	22073340	Contig36603	Probable_1-deoxy-D-xylulose-5-phosphate_synthase, chloroplastic (1-deoxyxylulose-5-phosphate_synthase),

							Precursor_(similar to)
RL	RhK5_488_2494P	2182	6.05E-12	Chr04	50854414	Contig488	Serine/threonine-protein_kinase_ PRP4 homolog (probable)
RL	RhMCRND_2865_677P	2681	6.45E-12	Chr07	12998520	Contig2865	hypothetical protein
RL	RhK5_2259_398P	1016	6.69E-12	Chr06	29715438	Contig2259	Embryogenesis-associated_protein EMB8_(probable)
RL	RhK5_7272_77Q	3214	1.47E-11	Chr00	112414	Contig7272	Period_circadian_protein_homolog_2 (cPER1) (probable)
RL	RhMCRND_29438_1013Q	7300	1.81E-11	Chr01	22415157	Contig29438	Probable_receptor-like_protein_ kinase At5g39030, Precursor
RL	RhK5_9322_473P	9125	2.85E-11	Chr02	65814732	Contig9322	Allene_oxide_cyclase_4,_ chloroplastic,_Precursor (similar to)
RL	Rh12GR_21320_86P	10093	3.39E-11	Chr01	39723188	Contig21320	Zinc_finger_protein_1_(probable)
RL	RhK5_15295_125Q	1675	4.28E-11	Chr06	45111110	Contig15295	E3_ubiquitin/ISG15_ligase_TRIM25 (probable)
RL	RhMCRND_8227_1081P	8555	1.06E-10	Chr02	4708217	Contig8227	Serine/arginine_repetitive_matrix _protein_1 (SRm160) (probable)
RL	RhMCRND_5730_1253Q	2217	1.54E-10	Chr04	50978112	Contig5730	RB1-inducible_coiled-coil_protein_1_(probable)
RL	RhMCRND_28932_598Q	9134	2.06E-10	Chr07	57486348	Contig28932	NA
RL	RhK5_2637_676P	4602	2.08E-10	Chr03	42480660	Contig2637	Protein_AUXIN_RESPONSE_4_ (similar to)
RL	RhK5_10814_115Q	9629	2.14E-10	Chr04	50828122	Contig10814	Autophagy-related_protein_8i_ (Protein_autophagy_8i) (putative)
RL	Rh12GR_34039_714Q	4840	3.05E-10	Chr06	66838972	Contig34039	Selenoprotein_H_(SelH)_(probable)
RL	RhK5_1348_1854P	4247	3.30E-10	Chr05	24885326	Contig1348	Probable_6-phosphogluconolactonase _1 (6PGL_1) (similar to)
RL	Rh12GR_22268_1075P	6407	3.38E-10	Chr01	3643188	Contig22268	K(+)/H(+)_antiporter_13_ (AtCHX13)_(probable)
RL	RhK5_761_400P	3358	8.10E-10	Chr04	52164796	Contig761	Phosphatidylinositol_4-kinase _type_2-beta (PI4KII-BETA) _(probable)
RL	RhMCRND_903_1621P	8990	8.11E-10	Chr05	7182076	Contig903	Protein_SCAR3_(AtSCAR3) _(probable)
RL	RhK5_1717_2065P	1525	1.61E-09	Chr04	49400089	Contig1717	Alpha-galactosidase,_ Precursor_(probable)
RL	RhMCRND_28921_223P	1408	1.74E-09	Chr06	31784013	Contig28921	NA
RL	RhK5_17292_131P	3103	1.94E-09	Chr04	55710725	Contig17292	Cyclin-SDS_(probable)
RL	RhK5_1934_1519Q	9113	2.67E-09	Chr02	54144948	Contig1934	Serpin_B10_(probable)
RL	RhK5_8836_402P	11700	4.07E-09	Chr03	17327867	Contig8836	UPF0182_protein_CKL_0015 _(probable)
RL	RhK5_7691_676Q	2043	6.37E-09	Chr07	36590454	Contig7691	Armadillo_repeat-containing _protein_7_(probable)
RL	RhK5_88_1678Q	14140	7.15E-09	Chr03	38171009	Contig88	Regulatory-associated_protein _of_mTOR (Raptor)_(similar to)
RL	RhK5_8736_697Q	4392	7.75E-09	Chr01	15854984	Contig8736	Cysteine_proteinase_inhibitor_6 _(AtCYS-6), Precursor_(similar to)
RL	RhK5_4152_1380P	1137	1.53E-08	Chr02	54774011	Contig4152	Acyltransferase_mdmB_(probable)
RL	RhK5_105_1333P	4785	2.27E-08	Chr06	11459664	Contig105	Filament-like_plant_protein_7 _(AtFPP7) (probable)
RL	RhMCRND_2657_1926P	6778	2.64E-08	Chr02	49797633	Contig2657	Protein_FAR1-ELATED_ SEQUENCE_6 (similar to)
RL	RhK5_13480_2046P	5299	3.89E-08	Chr02	62389538	Contig13480	Exosome_complex_exonuclease_ rrp6 (probable)

RL	Rh12GR_15815_779P	68	4.06E-08	Chr01	45656391	Contig15815	Ubiquitin-conjugating_enzyme_ E2-23_kDa (similar to)
RL	RhK5_6397_539Q	5816	4.59E-08	Chr05	75709769	Contig6397	Calcineurin_B-like_protein_3 _(similar to)
RL	Rh12GR_62393_260P	5570	4.67E-08	NA	NA	Contig62393	NA
RL	RhK5_13727_512Q	9034	5.95E-08	Chr01	25221825	Contig13727	Dr1-associated_corepressor_(NC2-alpha)_ (similar to)
RL	RhMCRND_29_1116Q	4840	8.14E-08	Chr05	85843901	Contig29	TATA-binding_protein-associated_factor_172 (TAF-172) (probable)
RL	RhK5_811_2469Q	13211	9.75E-08	Chr04	43014174	Contig811	Capsid_protein_(probable)
RL	RhK5_9753_424P	1444	1.47E-07	Chr05	9534044	Contig9753	Phospho-N-acetylmuramoyl-pentapeptide-transferase_homolog (probable)
RL	Rh12GR_61961_1098Q	7600	1.71E-07	NA	NA	Contig61961	Kinesin-4_(probable)
RL	Rh12GR_54107_458P	6829	1.87E-07	Chr00	18120816	Contig54107	Transmembrane_protein_87B, Precursor_(probable)
RL	RhMCRND_3277_1392Q	10059	1.88E-07	Chr01	54849507	Contig3277	Pumilio_homolog_2_ (Pumilio-2)_(probable)
RL	Rh12GR_2206_1423P	2955	2.00E-07	Chr01	25358900	Contig2206	DEAD-box_ATP-dependent_RNA_helicase_32 (similar to)
RL	Rh12GR_67678_173Q	6529	5.25E-07	NA	NA	Contig67678	NA
RL	Rh12GR_49561_165Q	6823	8.70E-07	Chr06	32126987	Contig49561	NA
RL	RhK5_5215_773P	2783	8.83E-07	Chr02	3361324	Contig5215	UPF0326_protein_At4g17486 _(similar to)

Fig S1: *In vivo* adventitious root formation of selected rose cultivars after 3 weeks culture in the rooting solution.

1: Mariatheresia, 2: Westerland, 3: Nostagie, 4: Herkule, 5: Nemo, 6: Midsummer, 7: Parole, 8: Lavender Lassie, 9: Beverly, 10: Shalom, 11 Auslo, 12: China Girl, 14: Munsterland, 15: Goerge Vancouver, 16: Arhtur Bell

4. Additional results

4.1 Genetic analysis of callus induction and shoot proliferation in a diversity panel of 96 rose genotypes

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Contribution to the article: Planned and performed the experiments, completed the statistical analysis and wrote most of the manuscript.

Contribution of other authors: Traud Winkelmann contributed to the experimental setup and wrote part of the manuscript.

Thomas Debener was involved in planning the experiments and wrote parts of the manuscript.

Status of publication: Prepare to submit to Plant tissue and organ culture

Genetic analysis of callus induction and shoot proliferation in a diversity panel of 96 rose genotypes

Abstract

In a diversity panel of 96 rose genotypes, callus induction and shoot proliferation were induced *in vitro* to investigate the variation and perform a genome-wide association study (GWAS) to identify genetic factors associated with callus size and shoot multiplication rate. Callus was induced from *in vitro* leaf explants on two media differing in their plant growth regulator composition. Significant differences in callus size on the first callus-inducing medium (CIM1) was observed on a 0–4 scale as well as on a second callus inducing medium (CIM2) from 0.82–4. Significant variation in the shoot multiplication rate was observed with variations from 0.5–4.24 among genotypes. GWAS analysis with 68,000 SNPs for callus size induced on either CIM1 or CIM2 led to the identification of 26 and 13 significantly associated SNPs, respectively. Among these, we found SNPs in genes encoding the *Rosa chinensis* transmembrane E3 ubiquitin-protein ligase 1 and the *Rosa chinensis* lysophospholipase BODYGUARD associated with callus size on CIM1 possessing good effects between alleles. Two SNPs, RhK5_4734_773P (*Rosa chinensis* protein_transport_protein_Sec24-like_CEF) and Rh12GR_37799_568Q (NA), were associated with callus size on CIM2 with good effect sizes. Among 6 SNPs that were found significantly associated with shoot multiplication rate, RhMCRND_5043_1547Q was located in a gene encoding a *Rosa chinensis* plasma membrane-type ATPase 10, and RhK5_4734_773P was located in a gene *Rosa chinensis* cytochrome P450 71A1-like. Both SNPs showed conspicuous effects. These markers need to be validated in additional plant populations followed by functional analyses.

Introduction

The genus Rosa comprises hundreds of species and roses are one of the most popular and economically important horticultural crops. Roses are used for many different purposes, such as ornamental plants in the form of cut flowers, potted plants and garden plants, as well as for the food, pharmaceutical and perfumes industries (Leus et al. 2018a). Nowadays, there are roughly 30,000–35,000 known cultivated rose varieties, most of which are tetraploids, of complex hybrid origin, highly heterozygous or of a wide phenotypic variability (Bendahmane et al. 2013; Kirov et al. 2014a). That being said, roses propagated by seeds may not fall true-to-type, vegetative propagation by cuttings, layering, budding and grafting may be time-consuming and there may be a limitation in stock plants (Marchant et al. 1996a). *In vitro* propagation of roses allows rapid multiplication, the production of disease-free plants and the application of genetic engineering to test gene functions and speed up breeding programs. However, the high input of labour and strong genotypic differences in propagation and rooting efficiency make the *in vitro* propagation of roses economically infeasible for most genotypes.

When aiming at genetic engineering, *in vitro* regeneration is a prerequisite and regeneration via organogenesis and somatic embryogenesis often involves callus, undifferentiated and proliferating cells, as the first step (Taimori et al. 2016). Furthermore, callus formation is important to seal wounds, prevent water loss and provide cellular sources for vasculature differentiation (Ikeuchi et al. 2017). The most frequently-used growth regulators for callus induction are auxins and cytokinins. Incubating various plant explants on rich auxin callus-inducing media (CIM) can induce callus formation. Recent studies have demonstrated callus formation using various plant explants on CIM (Ikeuchi et al. 2013; Xu et al. 2018). Callus induction occurs when plant cells dedifferentiate and proliferate. It is controlled by many factors, particularly by the interplay of the plant

hormones auxin and cytokinin, and it requires *PASTICCINO* (*PAS*) genes for coordinating cell division and differentiation of plant cells during development (Harrar 2003). During callus development, many up-regulated genes have been found to be involved in response to stress (Che et al. 2006). The gene *ENHANCER OF SHOOT REGENERATION1* was directly up-regulated by WOUND INDUCED DEDIFFERENTIATION1, an Apetala 2 /Ethylene response factors transcription factor in *Arabidopsis thaliana* that stimulates callus formation and shoot regeneration (Iwase et al. 2017). The reactivation of core cell cycle regulators *CYCLIN (CYC)* and *CYCLIN-DEPENDENT KINASES (CDK)* leads to callus formation and organ regeneration (Cheng et al. 2015; Inzé and Veylder 2006). The genes *ETHYLENE RESPONSE FACTOR 115*, *PLETHORA3*, *PLETHORA5* and *PLETHORA7* have been recently identified as factors involved in callus generation (Ikeuchi et al. 2017). *In vitro* shoot multiplication via axillary shoots is a method for the rapid propagation of many horticultural plants (Aygun and Dumanoglu 2015; Gutiérrez-Quintana et al. 2018; Litwińczuk 2013; Phillips et al. 2013). Plant growth regulators play a central role in the shoot multiplication of tissue cultures, especially cytokinins (Girgžde 2017; Grzegorczyk-Karolak et al. 2015; Tanaka et al. 2006). Cytokinin can promotes shoot branching by activating axillary buds (Müller and Leyser 2011b). Strigolactones, a group of sesquiterpene lactones derived from carotenoids, also promotes shoot branching, but only inhibits shoot branching in the presence of a competing auxin source (Crawford et al. 2010b; Shinohara et al. 2013). The multiple pathways that converge on common integrators are most probably involved in growing shoots, and numerous factors (such as TEOSINTE BRANCHED1, CYCLOIDEA, PCF transcription factor TB1/BRC1 and the polar auxin transport stream in the stem) are integrated at the bud and plant levels to determine the numbers of growing shoots (Aguilar-Martínez et al. 2007; Rameau et al. 2014a). The gene *supershoot* controls axillary bud initiation, which is characterized by a massive shoot proliferation in *Arabidopsis* (Tantikanjana et al. 2001). The *SHORT INTERNODES-like* gene is one of a 10-member *SHIRELATED SEQUENCE* gene family that regulates shoot growth and xylem proliferation (Zawaski et al. 2011). The *PHOTOPERIOD RESPONSE1-like* genes enhance shoot and root growth as well as starch accumulation (Zawaski et al. 2012). Although physiological and molecular studies dealing with underlying genes for callus induction and shoot proliferation have been carried out in recent years, the molecular mechanisms and the integration of environmental and endogenous signals are quite complex and not fully understood.

Several past studies dealing with callus induction and shoot proliferation of roses have been performed (Canli 2003; Evans 1990; Hsia and Korban 1996; Khosh-Khui and Sink 1982b; Noriega and Söndahl 1991; Shamsiah et al. 2011; Zakizadeh et al. 2010). Despite this, the genes involved in callus formation and shoot proliferation of roses have not yet been identified. In recent years, genome-wide association studies (GWASs) have been found to be an effective strategy for discovering underlying complex genetic traits (Chen et al. 2017). In roses, GWAS has been used to determine the loci and genes associated with anthocyanin and carotenoid concentration in petals (Schulz et al. 2016b), with adventitious shoot regeneration (Nguyen et al. 2017), the number of petals and the number of prickles on the shoot (Hibrand et al. 2018). These are the basis for identifying quantitative trait loci (QTLs) and the discovery of genes and markers for complex traits of roses.

In this study, we investigated the callus induction and shoot proliferation of 96 rose genotypes in a diversity panel. Based on 68,000 SNPs from the Axiom WagRhSNP analysis (Koning-Boucoiran et al. 2015), the variation of callus induction and shoot proliferation for 96 rose genotypes were analysed using GWAS. The aim of this study was to identify the SNP markers and chromosome (ChR) regions as well as candidate genes associated with callus induction and shoot proliferation.

Materials and methods

Plant material and *in vitro* establishment

The nodal stem segments of 96 rose genotypes close to the apical meristem were collected from healthy plants in the greenhouse of the Federal Plant Variety Office in Hannover, Germany (Nguyen et al. 2017; Schulz et al. 2016)The stem segments were surface disinfected for 1 min in 70% ethanol, then for 10 min in 1% sodium hypochlorite solution and finally rinsed 4 times in sterile deionized water. The culture medium for shoot proliferation consisted of MS basal salts (Murashige and Skoog 1962) with ferric ethylenediamine di-2-hydroxylphenyl acetate (instead of ferric ethylenediaminetetraacetic acid), 30 g L^{-1} sucrose, 8 g L^{-1} plant agar, 2.22 µM benzylaminopurine (BAP) and 0.58 µM gibberellin acid (Duchefa, Harlem, Netherlands). After two weeks, the shoots emerging from the axillary buds were excised and transferred to fresh medium to promote shoot growth and proliferation.

Callus induction

Leaves of the top third of the vigorously growing *in vitro* shoots were used to prepare explants for callus induction. The petioles of single leaflets were removed and three cuts were incised on the adaxial surface. All leaflet explants were placed with the adaxial surface in contact with the medium. Two media, CIM1 and CIM2 (Table 1), that had been used previously to induce embryogenic calluses in roses (Dohm et al. 2001) and cyclamen (Prange et al. 2010) were compared.

Table 1: Composition of callus induction media CIM1 and CIM2

Media	Salts and vitamins	Plant growth regulators	Carbon source	Solidifying agent
CIM1	Full-strength MS basal salts and vitamins	NAA (10.7 µM)	30 g L^{-1}glucose	4.0 g L^{-1} Gelrite
CIM2	Full-strength MS basal salts and vitamins	2.4D (4.5 µM) 2iP (2 µM)	30 g L^{-1} glucose	4.0 g L^{-1} Gelrite

For each rose genotype, 10 leaflet explants were cultured in the Petri dish with 9 cm diameter with 5 replicates, and the experiment was repeated three times. The explants were incubated in darkness for four weeks at 24°±2C. Callus development was scored based on the proportion of callus covering the leaflet using a 0–4 point scale, where 0 indicated no callus formation, 1 indicated less than 25% of the leaflet covered by callus, 2 represented 25–50% coverage, 3 indicated 50–75% coverage, and 4 signalled more than 75% of the leaflet being covered by callus (Tuskan et al. 2018a). A callus size was calculated as:

Callus size = n x G/N with n as the number of explants initiating of callus, G as the scale of callus rating for each explant and N being the total number of explants.

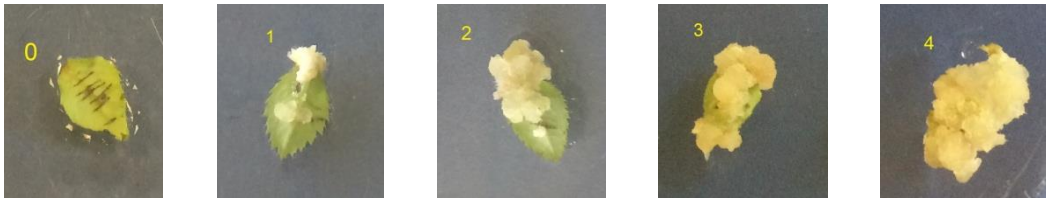

Fig 1: Example of the rating of callus size. The rating of callus size is given by the numbers at the top of each picture.

Shoot proliferation

In vitro shoots of the 96 rose genotypes (1.2–1.5 cm) were placed vertically in the shoot proliferation medium with 10 explants per 250 ml plastic vessel and three replicates in each. After a four-week culture period under cool-white fluorescent light at a photosynthetic photon flux density of 40 µmol m^{-2} s^{-1}, a temperature of 23 ± 2°C and a 16 h photoperiod, the shoot multiplication rate was recorded by dividing the total number of shoots obtained from one vessel by the initial number of shoots. Data were taken from three subsequent culture passages, representing three repetitions.

Statistical analyses

Data was analysed for differences between genotypes and repetitions of both experiments (callus induction and shoot proliferation) with the Kruskal–Wallis test. Normal distribution of the traits was tested using a quasibinomial model. The correlation coefficient between callus traits and shoot proliferation was calculated with Spearman's rank correlation. All statistical analyses were performed with the R software package, version 3.2.5 (The R-foundation for statistical computing 2016).

Association mapping

SNPs were analysed with the Axiom WagRhSNP chip, which comprises 68,000 SNPs derived from cut and garden roses (Koning-Boucoiran et al. 2015). The SNP dosage was estimated as for each of the five allelic classes by fit Tetra (AAAA, AAAB, AABB, ABBB and BBBB) (Voorrips et al. 2011).

The association analysis was performed in TASSEL, version 3.0 (Bradbury et al. 2007b), using information from the 96 genotypes for callus induction, shoot proliferation and genotypic data comprising 68,000 SNPs. To investigate associations between SNPs with callus induction and shoot proliferation traits, a linear mixed model was used with a minor allele frequency of 0.05. The Q matrix was obtained using STRUCTURE, version 2.3 (Hubisz et al. 2009), based on a subset of markers. The K matrix was calculated with SPAGeDi 1.3 software (Hardy and Vekemans 2002). Association analysis was performed for each trait. The significance between traits and markers in the association was defined with the Bonferroni method using a threshold set to –log p10 > 6.7. The allelic class effects were obtained directly from the TASSEL output.

To visualise the associations, significant SNPs were used to blast against the *Rosa chinensis* 'Old Blush' genome (Hibrand Saint-Oyant et al. 2018a) for localized SNP searching in the rose ChR from Bio Edit (Hall 1999). A homology search via a BLAST analysis on https://blast.ncbi.nlm.nih.gov/Blast.cgi was performed to locate the genes associated with the traits.

Results

Callus induction

Callus formation started from the cut edges of the leaf explants and gradually grew to completely cover the explants after 28 days in case of some genotypes (Fig. 1, 2). The amount of callus, expressed on a callus scale of 0 to 4, varied considerably among genotypes (Table S1, Fig. 2). On CIM1, 95 of 96 genotypes showed callus formation, with only leaflets of the Jazz cultivar failing to form calluses (Fig. 2A). On the CIM2, leaflets of all genotypes formed calluses with callus size falling between 0.8 and 4 (Fig. 2B). Overall, the size were higher than those recorded on CIM1. Interestingly, in both media, the lowest callus size were observed for the same group of genotypes, including Jazz, Ausfather, Blue Perfume, Perennial Blush, Comtessa Al, Feuerwerk, Magenta and Herkules (Fig. 3).

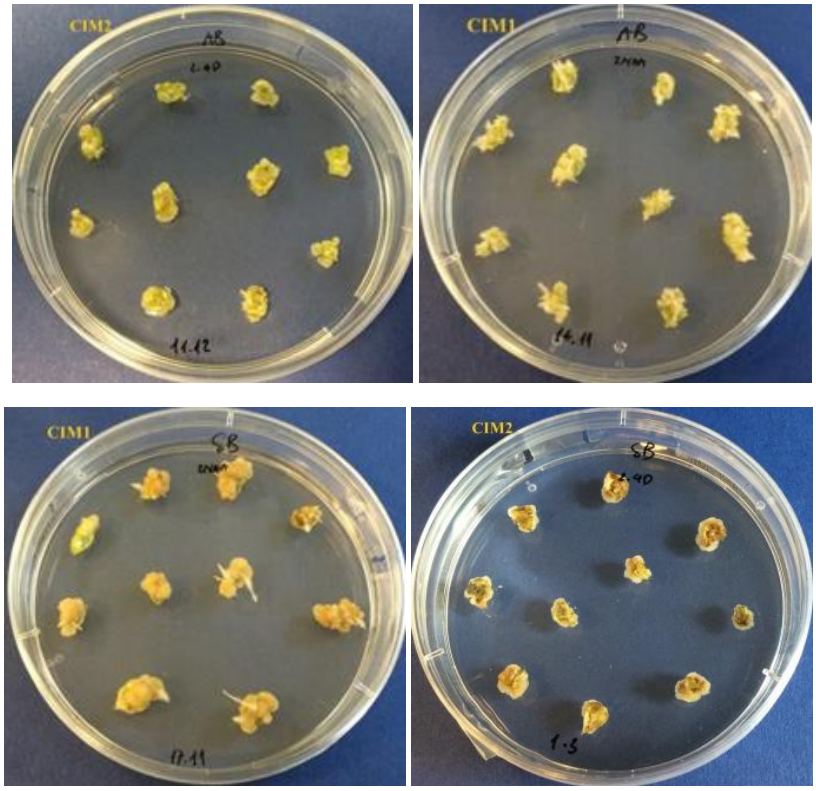

Fig 2: Callus formation of genotype Arthur Bell (AB) and Sunset Boulevard (SB) on CIM1 and CIM2. The diameter of the petri dishes is 94 mm. Average of callus size for AB on CIM1 is 3.18 and on CIM2 is 2.91 and for SB on CIM1 is 3.94 and on CIM2 is 3.53

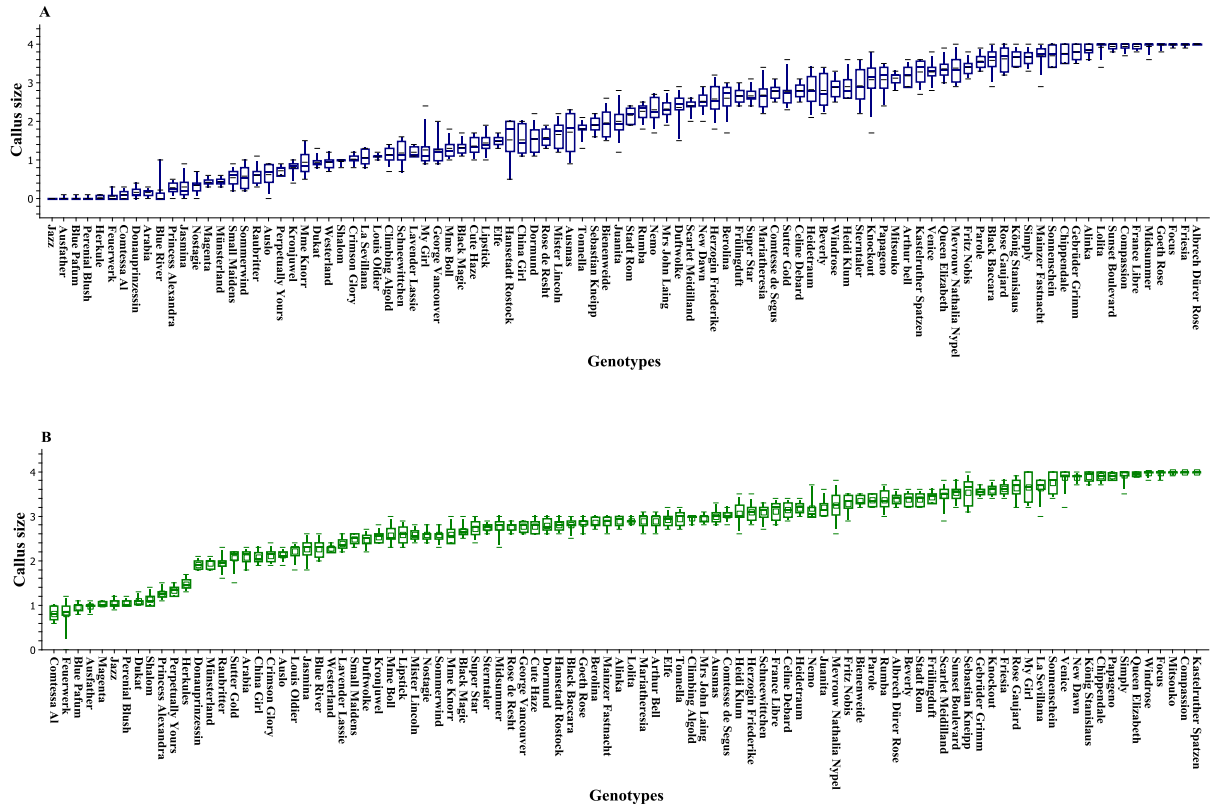

Fig. 3: Callus size of the 96 rose genotypes after four weeks of culture on CIM1 (A) and CIM2 (B), based on three independent experiments using five biological replicates (with 10 explants each). Small square = mean; horizontal line = median; asterisk = minimum, maximum; box = 1st and 3rd quartiles; and whisker = standard deviation.

Statistical analysis of the data for callus induction on both CIM1 and CIM2 revealed significant differences between genotypes at p = 0.05 using a Kruskal–Wallis test, whereas no significant differences were revealed between the repeat experiments for the callus size (under Tukey's test).

Shoot proliferation

Regarding multiplication via axillary shoots, the 96 rose genotypes showed pronounced differences (see Table S1, Fig. 4 and Fig. 5). Some genotypes, such as Bienenweide and Herzogin Friederike, had high multiplication rates of 4.24 and 3.74, respectively. In contrast, multiplication was not possible for some genotypes and the death of some shoots led to multiplication rates of less than 1 (e.g. Ausfather, Perennial Blush and Blue Perfume with propagation rates of 0.5, 0.72 and 0.74, respectively. See Fig. 4).

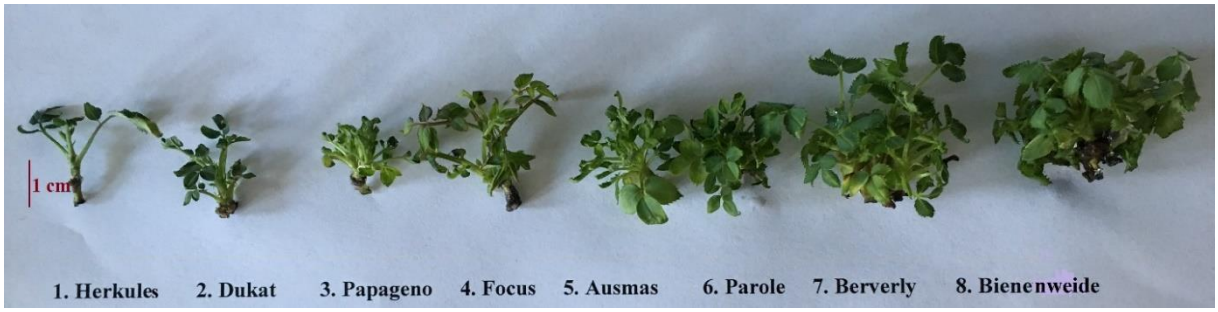

Fig. 4: Shoot multiplication of some genotype after four weeks of culture.

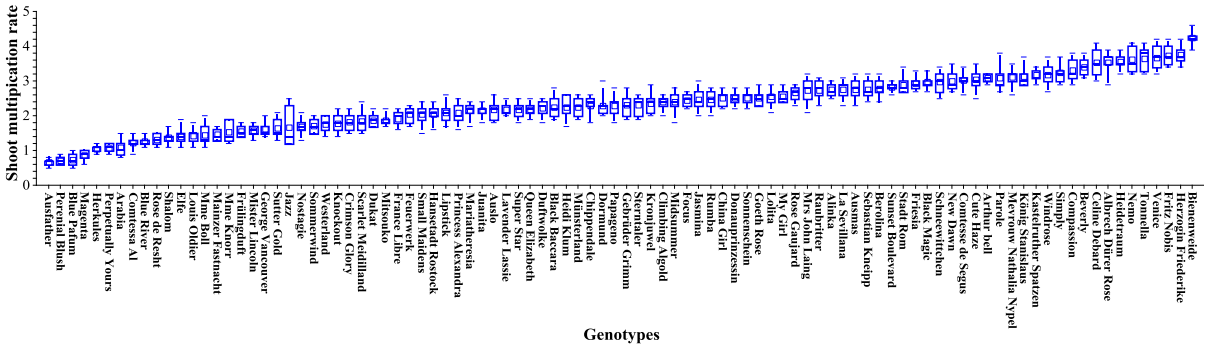

Fig. 5: Shoot multiplication rates of 96 rose genotypes after four weeks of culture based on three culture passages using three biological replicates (with 10 shoots each). Small square = mean; horizontal line = median; asterisk = minimum, maximum; box = 1st and 3rd quartiles; and whisker = standard deviation.

The multiplication rate differed significantly between genotypes at p = 0.05, while no significant differences between the three culture passages were detected for this parameter.

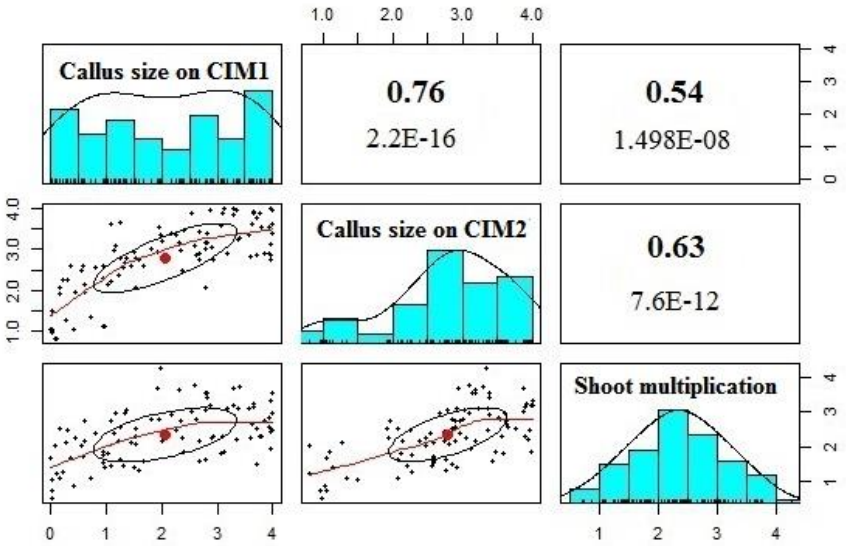

Fig. 6: Pearson's correlation coefficients of callus size and shoot multiplication rates with the p value given under the correlation index.

The different parameters measured for callus induction and shoot proliferation were analysed for correlations (Fig. 6). A high correlation was found between callus size for CIM1 and CIM2 (0.76), whereas

slightly weaker correlations were observed between the shoot propagation rate and callus size (CIM1: 0.54 and CIM2: 0.63).

Marker-traits association analysis

GWAS was performed with the data for the callus size and shoot propagation rates of the 96 rose genotypes of the panel to identify and localize the genetic factors associated with these traits. For callus induction on CIM1, 26 SNPs associated with the callus size were found (Table 2, Fig. 7A). Almost all SPNs co-located on ChR 3 and formed one large conspicuous cluster. Only 3 SNPs were found on ChR 0 forming a second cluster. Some SNPs had large effects, such as Rh12GR_12098_1092Q (*Rosa chinensis* uncharacterized LOC112192505 (Fig. 9), transcript variant X4, misc._RNA) at position 370111 on ChR 3, Rh12GR_6077_815P (*Rosa chinensis* probable lysophospholipase BODYGUARD 4 (LOC112192624), transcript variant X1, mRNA) at position 5193454, Rh12GR_86832_276 (U-box_domain-containing_protein_4_(probable)) and RhMCRND_2903_1233Q (*Rosa chinensis* pentatricopeptide repeat-containing protein At5g15010, mitochondrial-like (LOC112192673), transcript variant X1, mRNA) (Fig. 9) at position 25447590 on ChR 3.

GWAS analyses of the callus size on CIM2 revealed 13 significantly associated SNPs (Table 3, Fig. 7B). Among them, 3 SNPs were located on ChR02, 6 SNPs were on ChR03, 1 SNP was on ChR04 and 2 SNPs were on ChR06. Some SNPs showed good effects, such as Rh12GR_37799_568Q (NA) at position 6468674 on ChR 3 (Fig. 10) and RhK5_ 5473_763Q, RhK5_ 5473_763P (*Rosa chinensis* S-formylglutathione hydrolase) (Fig. 10) at position 18402920 on ChR 3.

For shoot multiplication rates, only 6 SNPs were found associated with the trait below the threshold of 1E-6 although some marker clusters could be identified below the thresholds (Table 4, Fig. 8). Those were RhMCRND_5403_1547Q (*Rosa chinensis* ATPase 10, plasma membrane-type (LOC112187313), transcript variant X2, mRNA), RhK5_4734_773P (*Rosa chinensis* protein_transport_protein_Sec24-like_CEF (LOC112197354), transcript variant X3, mRNA) on ChR 4, RhK5_7015_457P (*Rosa chinensis* pentatricopeptide repeat-containing protein At3g62470, mitochondrial-like (LOC112180940), mRNA) at position 66696517 on ChR07. 3 SNPs were found on ChR00, namely RhMCRND_6488_1056Q (*Rosa chinensis* uncharacterized LOC112188011) at position 37102205, RhK5_4373_1158Q (*Rosa chinensis* linoleate 13S-lipoxygenase 3-1, chloroplastic) at position 17892320 and RhK5_4373_1158Q (*Rosa chinensis* cytochrome P450 71A1-like) at position 17824124. Strong effects were determined for RhMCRND_5043_1547Q (*Rosa chinensis* ATPase 10, plasma membrane-type (LOC112187313), transcript variant X2) and RhK5_4734_773P (*Rosa chinensis* cytochrome P450 71A1-like (LOC112187937) (Fig. 11).

Of all SNPs associated with the callus size, 2 SNPs overlapped between CIM1 and CIM2. They were RhK5_4750_1179Q (*Rosa chinensis* uncharacterized CRM domain-containing protein At3g25440, chloroplastic (LOC112193599), transcript variant X2) and Rh12RG_37799_568Q (NA), whereas no overlaps were found for SNPs associated with shoot multiplication rates.

Table 2: Significant SNPs associated with callus size induced on CIM1

Marker	Site	p-value	ChR	Position	Contig	Gene
Rh12GR_27683_2069P	8791	1.21E-09	3	10166387	Contig27683	*Rosa chinensis* probable fructokinase-6, chloroplastic (LOC112194730), mRNA
Rh12GR_27683_2069Q	8842	1.47E-08	3	10166387	Contig27683	*Rosa chinensis* probable fructokinase-6, chloroplastic (LOC112194730), mRNA
Rh12GR_4846_920P	8713	2.62E-08	3	8790885	Contig4846	*Rosa chinensis* DEAD-box ATP-dependent RNA helicase 13 (LOC112193330), mRNA
Rh12GR_59753_1764Q	8826	3.63E-08	NA	NA	Contig59753	TATA_element_modulatory _factor_(TMF)_(probable)

Rh12GR_25423_3834P	8426	4.84E-08	3	9153717	Contig25423	*Rosa chinensis* spliceosome-associated protein 130 A
RhK5_4750_1179Q	12293	1.20E-07	3	7868346	Contig4750	*Rosa chinensis* uncharacterized CRM domain-containing protein At3g25440, chloroplastic (LOC112193599), transcript variant X2
Rh12GR_13539_496P	8528	1.62E-07	0	2687062	Contig13539	*Vitis vinifera* E3 ubiquitin-protein ligase Arkadia (LOC100248215), mRNA
Rh12GR_25423_3834Q	8460	1.65E-07	3	9153717	Contig25423	*Rosa chinensis* spliceosome-associated protein 130 A (LOC112193025), mRNA
Rh12GR_13539_496Q	8555	3.70E-07	3	2687062	Contig13539	Vitis vinifera E3 ubiquitin-protein ligase Arkadia (LOC100248215), mRNA
RhMCRND_13074_681P	674	5.45E-07	3	9613831	Contig13074	*Rosa chinensis* protein C2-DOMAIN ABA-RELATED 5-like (LOC112192906), mRNA
RhMCRND_9915_389Q	7477	5.69E-07	3	9758183	Contig9915	*Rosa chinensis* glutathione S-transferase DHAR3, chloroplastic (LOC112195020), mRNA
Rh12GR_59259_108P	5018	6.19E-07	3		Contig59259	NA
RhMCRND_9892_919P	3550	7.11E-07	3	9165684	Contig9892	*Rosa chinensis* uncharacterized LOC112193027 (LOC112193027), transcript variant X1, mRNA
RhMCRND_9915_389P	7472	7.17E-07	3		Contig9915	*Rosa chinensis* glutathione S-transferase DHAR3, chloroplastic (LOC112195020), mRNA
Rh12GR_12098_1092Q	8115	1.30E-06	3	370111	Contig12098	*Rosa chinensis* uncharacterized LOC112192505, transcript variant X4, misc_RNA
RhK5_6755_333P	6319	1.33E-06	3	11931437	Contig6755	*Rosa chinensis* transcription termination factor MTERF4, chloroplastic (LOC112193459), mRNA
RhK5_6755_333Q	2182	1.34E-06	3	11931437	Contig6755	*Rosa chinensis* transcription termination factor MTERF4, chloroplastic (LOC112193459), mRNA
Rh12GR_6077_815P	1628	1.37E-06	3	5193454	Contig6077	*Rosa chinensis* probable lysophospholipase BODYGUARD 4 (LOC112192624), transcript variant X1, mRNA
RhMCRND_11099_934P	3766	1.50E-06	3	9179620	Contig11099	*Rosa chinensis* psbP domain-containing protein 6, chloropla stic (LOC112191588), transcript variant X1, mRNA
Rh12GR_37799_568Q	8415	2.50E-06	3	6468674	Contig37799	NA
RhMCRND_20513_1468P	575	2.51E-06	3	5667332	Contig20513	*Rosa chinensis* putative pentatricopeptide repeat-containing protein At5g08490 (LOC112193021), transcript variant X2, mRNA
Rh12GR_19029_1911P	8186	2.60E-06	0	25447725	Contig19029	*Rosa chinensis* pentatricopeptide repeat-containing protein At5g15010, mitochondrial-like (LOC112192673), transcript variant X1, mRNA
Rh12GR_86832_276P	4847	2.67E-06	NA	NA	Contig86832	U-box_domain-containing_protein_4_(probable)
Rh12GR_81252_184Q	4848	2.80E-06	NA	NA	Contig81252	NA
RhMCRND_2903_1233Q	7842	3.00E-06	0	25447590	Contig2903	*Rosa chinensis* pentatricopeptide repeat-containing protein At5g15010, mitochondrial-like (LOC112192673), transcript variant X1, mRNA

Rh12GR_54251_670P	4841	3.05E-06	3	5665174	Contig54251	*Rosa chinensis* putative pentatricopeptide repeat-containing protein t5g08490(LOC112193021), transcript variant X2, mRNA

Table 3: Significant SNPs associated with callus induction on CIM2 (callus size)

Marker	Site	p-value	ChR	Position	Contig	Gene prediction
RhK5_107_2439P	2759	2.24E-18	6	45395443	Contig107	*Rosa chinensis* chromatin modification-related protein EAF1 B-like (LOC112172241), transcript variant X2, mRNA
RhMCRND_6130_146Q	2510	3.2E-12	2	68676139	Contig6130	*Rosa chinensis* chorismate mutase 1, chloroplastic (LOC112188602), mRNA
RhMCRND_10042_489P	2209	1.60E-09	6	62167206	Contig10042	*Rosa chinensis* 54S ribosomal protein L24, mitochondrial (LOC112174756), mRNA
RhK5_4750_1179Q	12293	1.26E-08	3	7868346	Contig4750	*Rosa chinensis* uncharacterized CRM domain-containing protein At3g25440, chloroplastic (LOC112193599), transcript variant X1, mRNA
RhK5_12450_841P	2954	1.6E-07	2	38349478	Contig12450	*Rosa chinensis* transmembrane E3 ubiquitin-protein ligase 1 (LOC112188470), transcript variant X1, mRNA
RhMCRND_4377_105P	3460	5.3E-07	2	31990763	Contig4377	*Rosa chinensis* aspartic proteinase Asp1 (LOC112190217), mRNA
RhK5_5473_763P	4438	6.12E-07	3	18402920	Contig5473	*Rosa chinensis* S-formylglutathione hydrolase (LOC112191850), mRNA
RhK5_5473_763Q	5119	6.62E-07	3	18402920	Contig5473	*Rosa chinensis* S-formylglutathione hydrolase (LOC112191850), mRNA
RhK5_12078_99Q	4369	1.00E-06	3	17475978	Contig12078	*Rosa chinensis* folylpolyglutamate synthase (LOC112194857), transcript variant X5, mRNA
RhK5_6079_150Q	161	2.70E-06	4	9719228	Contig6079	*Rosa chinensis* protein SULFUR DEFICIENCY-INDUCED 2 (LOC112201022), mRNA
Rh12GR_37799_568Q	8415	2.79E-06	3	6468674	Contig37799	NA
Rh12GR_3363_1266Q	9059	3.20E-06	3	13761410	Contig3363	*Rosa chinensis* pectinesterase-like (LOC112191366), mRNA
Rh12GR_3363_1266P	9067	3.20E-06	3	13761410	Contig3363	*Rosa chinensis* pectinesterase-like (LOC112191366), mRNA

Table 4: Significant SNPs associated with shoot propagation rates.

Marker	Site	P	ChR	Position	Contig	Gene
RhMCRND_5043_1547Q	9866	7.68E-08	2	57046683	Contig5043	*Rosa chinensis* ATPase 10, plasma membrane-type (LOC112187313), transcript variant X2, mRNA
RhK5_7015_457P	4973	2.99E-07	7	66696517	Contig7015	*Rosa chinensis* pentatricopeptide repeat-containing protein At3g62470, mitochondrial-like (LOC112180940), mRNA
RhMCRND_6488_1056Q	8724	5.50E-07	0	37102205	Contig6488	*Rosa chinensis* uncharacterized LOC112188011, mRNA
RhK5_4373_1158Q	5028	8.55E-07	0	17824124	Contig4373	*Rosa chinensis* cytochrome P450 71A1-like

						(LOC112187937), mRNA
RhK5_5062_1235P	3948	1.91E-06	0	17892320	Contig5062	*Rosa chinensis* linoleate 13S-lipoxygenase 3-1, chloroplastic (LOC112186516), mRNA
RhK5_4734_773P	3564	3.14E-06	4	57793640	Contig4734	*Rosa chinensis* protein_transport_protein_Sec24-like_CEF (LOC112197354), transcript variant X3, mRNA

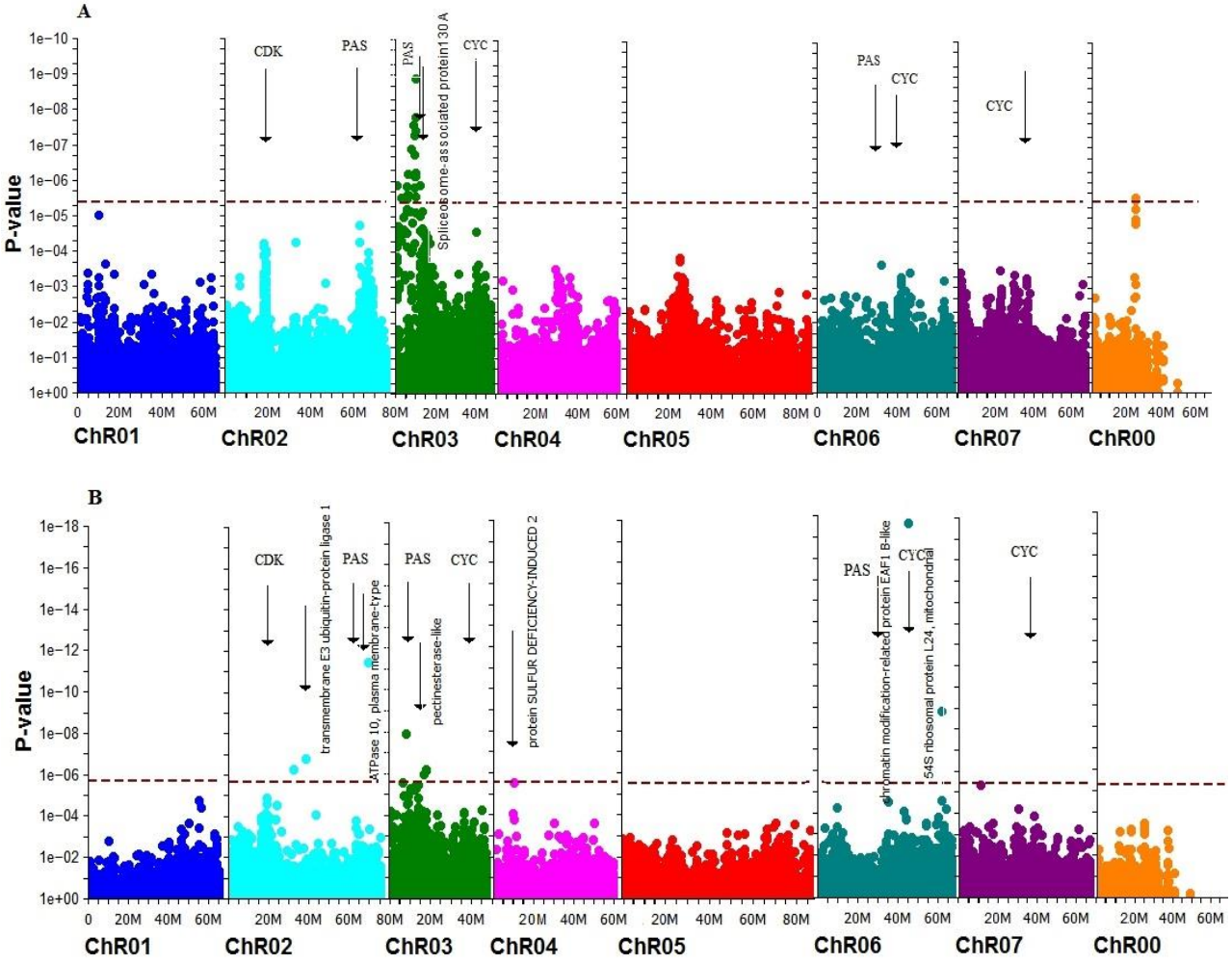

Fig. 7: Manhattan plot of callus size induced on CIM1 (A) and CIM2 (B) PAS: *PASTICCINO*, *CYC: CYCLIN*, CDK: *CYCLIN- DEPENDENT KINASES*. The red dashed line represents the Bonferroni threshold of the adjusted significance level - [log10] = 6.7 The subdivision of the x-axis is by chromosome (ChR01-ChR00) including Chromosome 0 with contigs not assigned to a precise location yet. Each scale bar of the x-axis represents 5 Mb.

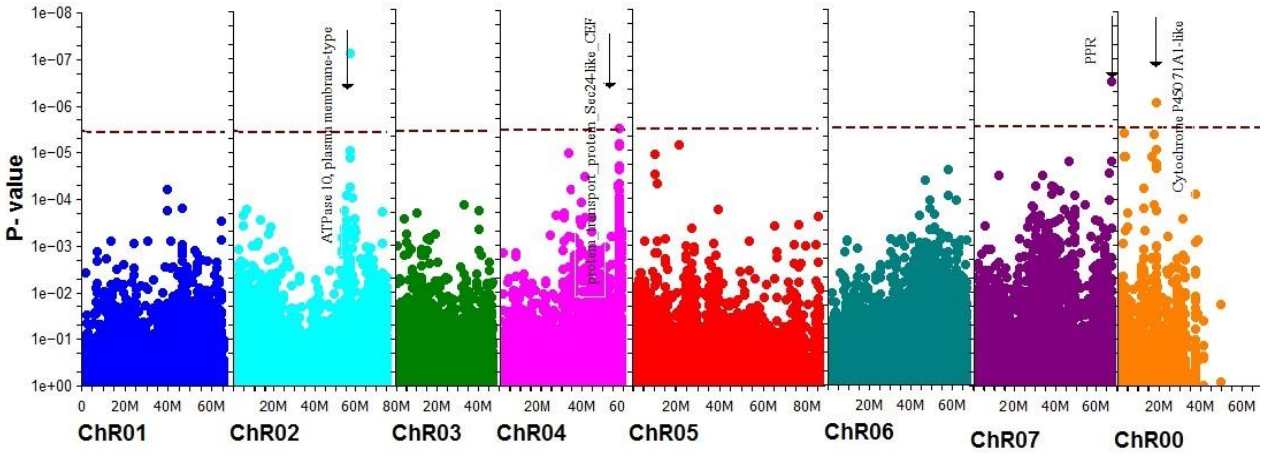

Fig. 8: Manhattan plot of shoot multiplication rates. The red dashed line represents the Bonferroni threshold of the adjusted significance level - [log10] = 6.7. The subdivision of the x-axis is by chromosome (ChR01-ChR00) including Chromosome 0 with contigs not assigned to a precise location yet. Each scale bar of the x-axis represents 5 Mb. Abbreviation: PPR: pentatricopeptide repeat-containing protein At3g62470, mitochondrial-like.

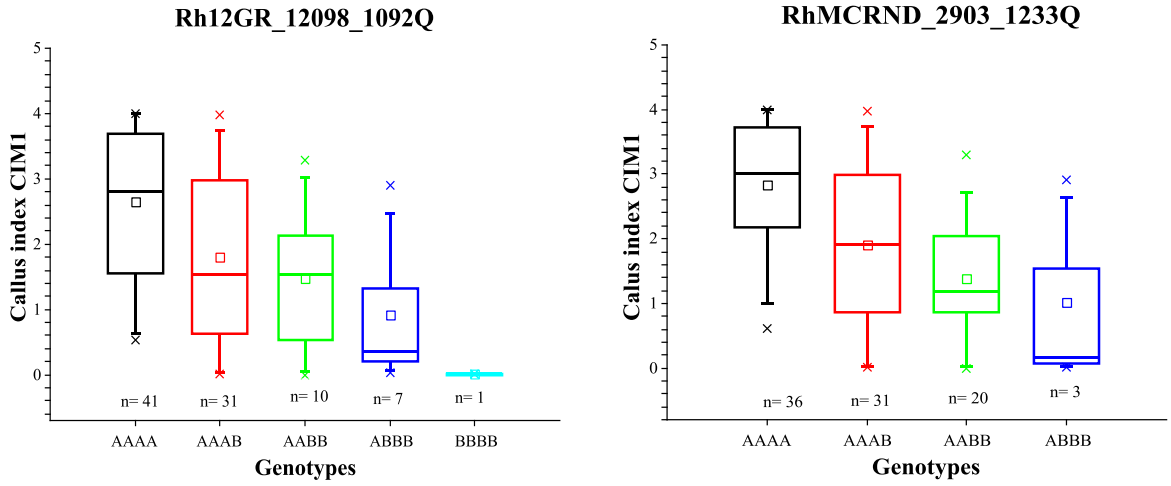

Fig. 9: Genotypic effects of SNP markers associated with the callus size on CIM1, Rh12GR_12098_1092Q (*Rosa chinensis* uncharacterized LOC112192505) and RhMCRND_2903_1233Q (*Rosa chinensis* pentatricopeptide repeat-containing protein At5g15010, mitochondrial-like (LOC112192673). Small square = mean; continuous line = median; asterisk = minimum, maximum; box = 1 stand3rd quartiles; and whisker = standard deviation)

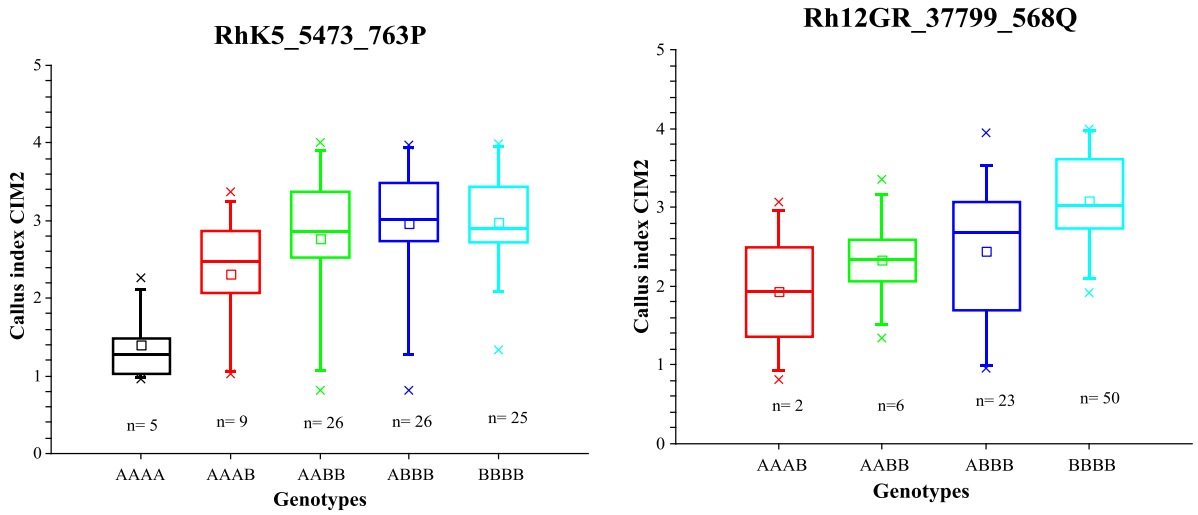

Fig. 10: Genotypic effects of SNP markers associated with the callus size on CIM2, RhK5_4734_773P (Rosa chinensis protein_transport_protein_Sec24-like_CEF (LOC112197354) and as Rh12GR_37799_568Q (NA). Small square = mean; continuous line = median; asterisk = minimum, maximum; box = 1 stand3rd quartiles; and whisker = standard deviation)

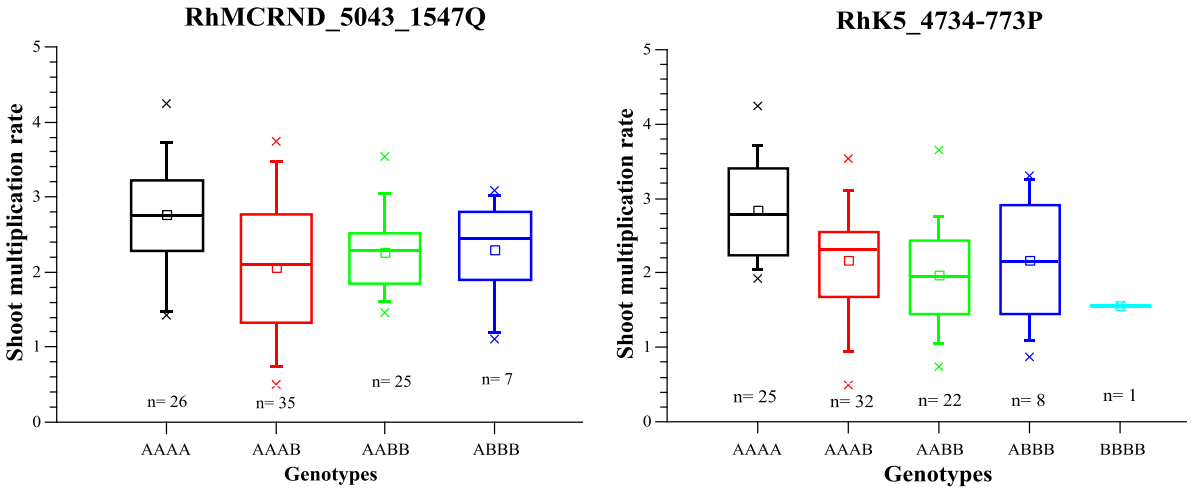

Fig. 11: Genotypic effects of SNPs associated with the shoot multiplication rate, RhMCRND_5043_1547Q (*Rosa chinensis* ATPase 10, plasma membrane-type (LOC112187313), transcript variant X2) and RhK5_4734_773P (*Rosa chinensis* cytochrome P450 71A1-like (LOC112187937). Small square = mean; continuous line = median; asterisk = minimum, maximum; box = 1 stand3rd quartiles; and whisker = standard deviation

Discussion

In this study, we presented the significant variation in callus formation and shoot proliferation of an association panel containing 96 rose genotypes and its correlation to other traits related to developmental processes. Furthermore, we identified the genomic regions and located a selection of candidate genes possessing known functions for callus and shoot proliferation traits.

Callus induction and shoot proliferation in a panel of 96 rose genotypes

Callus induction is the first step for plant regeneration via somatic embryogenesis for many plants, such as potato (Kumlay and Ercisli 2015), oil palm (Yusnista and Hapsoro 2011), (Jayanthi et al. 2015), bamboo (Yuan et al. 2013) and wolfberry (Osman et al. 2013). For roses, callus induction using leaf and stem explants was established first by Khosh-Khui and Sink (1982) with *Rosa manetti* Hort. and *R. hybrida* L. Tropicana. Different rose genotypes were used for callus induction by Kuusiene and Kandzezauskaite (2001) and different plant hormones were used for callus formation by Huang et al. (2018). Our comprehensive data set allows a detailed comparison of callus formation in two different media among 96 genotypes. Our data indicated that calluses induced on CIM2 formed more calluses on CIM1, but the group genotypes with small callus sizes were similar for both media. A high correlation of callus formation between the two media suggested that they were controlled at least in part by the same genetic factors.

In vitro shoot proliferation was applied to many plants for rapid multiplication, such as *Decalepis hamiltonii* or swallow root (Giridhar et al. 2005), *Ginkgo biloba* or Gymnosperm tree (Mantovani et al. 2013) and pear (Aygun and Dumanoglu 2015). Several rose cultivars were used for multiplication in different media by various studies (Davies 1980); (Ma et al. 1996); Pati et al. 2010). Rose shoot multiplication responded differently in media with larger differences in cytokinin concentrations, such as the Pau's Lemon Pillar, Plentiful, Parade, Garnet Yellow and Lili Marlene cultivars with rates of 2.8, 3.8 4.8, 2.9 and 5.8, respectively (Davies 1980) while the Frisco cultivar had a rate of 3.75 in a high concentration of BAP (10 mg/L) (Mahmood et al. 2016). Our experiment showed the variation of shoot multiplication in a panel of 96 rose genotypes in the same medium with a low concentration of BAP and gibberellic acid. The results demonstrated that *in vitro* shoot proliferation ability depended on genotype. Correlation between substantial shoot proliferation rate and callus size revealed they are most likely regulated by some similar genetic factors.

Marker-trait association analysis

Recently marker-trait associations have been analysed for callus induction in a number of plants, such as tomato (Phan et al. 2019), black cottonwood *Populus trichocarpa* (Tuskan et al. 2018a), rice (Zhang et al. 2018) and maize (Ma et al. 2018). In roses, previously marker-trait association mappings were performed in shoot organogenesis (Nguyen et al. 2017) as well as anthocyanin and carotenoids content of rose petals (Schulz et al. 2016).

Marker associations with callus formation

We detected 26 SNPs associated with the callus size after induction on CIM1 and 13 SNPs associated with callus size on CIM2. We found SNPs Rh12GR_59735_1764Q, in markers derived from a gene encoding a spliceosome-associated protein 130A, associated with the callus size on CIM1. This gene belong to alternative splicing factors which have roles in regulating gene expression during the development of multicellular organisms and are important for stress adaptation in plants (Staiger and Brown 2013). Moreover, spliceosome-associated protein 130A plays an indispensable role in the specific spatiotemporal events of reproduction (Aki et al. 2011). The SNPs Rh12GR_13539_496P and Rh12GR_13539_496Q are derived from genes encoding E3 ubiquitin-protein ligases *Arkadia,* which were found associated with the callus size inducted on CIM1. The SNP RhK5_12450_841P lies in a gene encoding a *Rosa chinensis* transmembrane E3 ubiquitin-protein ligase 1 and was associated with the callus size inducted on CIM2. The gene belong to the ubiquitination family and are involved in the regulation of cell cycle progression, transcriptional regulation, DNA repair, signal transduction and protein turnover. The E3 ubiquitin ligase for DNA-dependent protein kinase can promote DNA damage-

induced cell apoptosis (Ho et al. 2015; Pfeffer et al. 2015) and control organ size in a dosage-dependent manner in *Arabidopsis* (Disch et al. 2006). The gene underlying the Rh12RG_6077_815P encodes a putative lysophospholipase BODYGUARD and was associated with callus size on CIM1. This gene plays a critical role in plant survival during extreme drought conditions (Jakobson et al. 2016; Kurdyukov et al. 2006) and controls cuticle development and morphogenesis in *Arabidopsis* (Kurdyukov et al. 2006). The SNP RhMCRND_2903_1233Q (*Rosa chinensis* pentatricopeptide repeat-containing protein At5g15010, mitochondrial-like) associated with callus size on CIM1 was found on ChR 0. This gene plays a critical role in female gametophyte maturation and is important for central cell maturation and endosperm development, indicating the importance of mitochondria in female gametophyte maturation (Yagi et al. 2013). The gene *Rosa chinensis* protein C2-DOMAIN ABA-RELATED 5-like underlying RhMCRND_13074_681P was associated with the callus size on CIM1. This gene mediates the interaction of PYRABACTIN RESISTANCE1 (PYR1)/PYR1-LIKE /REGULATORY COMPONENTS OF ABA RECEPTORS abscisic acid receptors with plasma membranes and regulates abscisic acid sensitivity in *Arabidopsis* (Rodriguez et al. 2014).

A comparison with the position of candidate genes for callus induction were found in rose genomes such as *CYC, CDK* and *PASTICCINO,* some of which showed those positions near the peak regions of significant SNPs.

Marker associations with shoot multiplication rate

Among the six SNPs significantly associated with shoot multiplication rates, two SNPs had conspicuous effects between alleles. One of these was the RhMCRND_5043_1547Q which is derived from an EST that encodes the gene *Rosa chinensis* ATPase 10, plasma membrane-type (LOC112187313), transcript variant X2. This gene is an important ion pump for plant cell membranes, making it a prerequisite for growth (Falhof et al. 2016). This gene was also found to be regulating adult vegetative development and inflorescence architecture in *Arabidopsis* (George et al. 2008). We also found the RhK5_4734_773P from an EST that encodes the gene *Rosa chinensis* ChR P450 71A1-like (LOC112187937) with a clear effect between alleles. This gene belongs to the CYP79 family, produces phenylacetaldoxime and indole-3-acetaldoxime in heterologous systems and might contribute to auxin formation and plant defence (Irmisch et al. 2015). We also found the gene *Rosa chinensis* pentatricopeptide repeat-containing protein At3g62470, mitochondrial-like (PPR), which was encoded by an EST that harbours the marker RhK5_7015_457P at position 66.696.517 on ChR 7. This gene encodes a PPR protein and belongs to the huge PPR protein family that plays a central role in the post-transcriptional regulation of gene expression in plastids and mitochondria (Shikanai and Fujii 2013). This gene has also been revealed to have an essential role in plant embryogenesis (Cushing et al. 2005). The presence of this gene in shoot proliferation and callus induction analysis explains in part the correlation (0.54) between the traits. Finally, the SNP RhK5_4734_773P is derived from an EST that encodes *Rosa chinensis* protein_transport_protein_Sec24-like_CEF. This gene, in *Arabidopsis thaliana,* enhances the survival of yeast under oxidative stress (Belles-Boix et al. 2000). However, we did not find any known genes related to the shoot multiplication, such as *SHORT INTERNODES-like* and *PHOTOPERIOD RESPONSE1-like* genes at any position in the rose genome.

Conclusion

In this study, a large variation in callus formation and shoot proliferation among 96 rose genotypes was observed. GWAS for rose callus induction and shoot proliferation identified some significantly associated markers and some genomic regions where marker peaks were co-located to known candidate genes. These markers could provide tools for further attempts to identify genes influencing these traits.

Acknowledgements

This study was carried out with financial support from the Vietnamese Government and the department of Molecular Plant Breeding, Institute of Plant Genetics, Leibniz University Hannover, Germany.

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4.2 Analyses of correlations between these traits and potential overlap in the genetic pathways influencing these traits

Correlation of all the traits in this analysis was summarised in the table 3 and figure 5.

Table 3: Correlation of all the analysed traits

Pearson correlation	In vitro root length	In vivo root number	In vivo root length	In vivo root biomass	Callus CIM1	Callus CIM2	Regene-ration rate	Shoot ratio	Shoot proliferation
In vitro root number	0.7 1.85E-15	0.37 2.30E-03	0.43 1.58E-05	0.39 1.34E-03	0.37 1.83E-03	0.43 1.24E-05	0.04 6.80E-01	0.07 5.20E-01	0.59 1.45E-10
In vitro root length		0.38 1.60E-03	0.52 6.89E-08	0.43 2.59E-05	0.21 3.90E-02	0.27 7.10E-03	0.00 9.80E-01	0.02 8.20E-01	0.34 6.68E-03
In vivo root number			0.8 <2,2E-16	0.89 <2,2E-16	-0.1 0.34	-0.04 0.67	-0.03 0.53	-0.01 0.805	0 0.999
In vivo root length				0.89 <2,2E-16	-0.1 0.33	0.05 0.007	-0.07 0.53	-0.03 0.805	0.08 0.45
In vivo root biomass					-0.12 0.56	-0.01 0.54	-0.01 0.69	0.03 0.44	0 0.82
Callus CIM1						0.76 < 2,2E-16	0.29 0.0048	0.29 0.0038	0.54 1.50E-08
Callus CIM2							0.26 0.0098	0.28 0.0054	0.63 7.59E-12
Regeneration rate								0.98 <2,2E-16	0.06 0.56
Shoot ratio									0.08 0.41

Fig S1: Correlation of all analysed traits

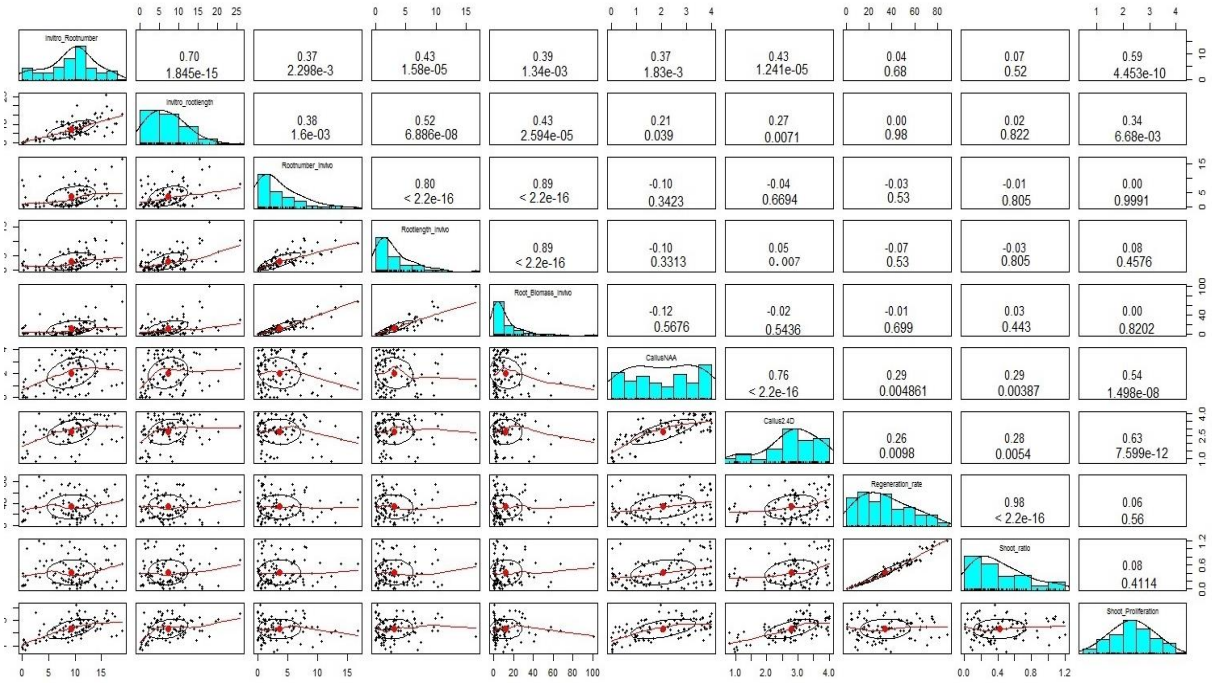

Table S4: Statistically significant SNPs associated with more than one of the phenotypic traits studied

Trait	Number of markers	Marker name	Gene prediction
In vivo root number/ Shoot ratio/ Shoot regeneration rate	1	RhK5_69_2438Q	gene probable phosphoinositide phosphatase SAC9 LOC101296222)
In vivo root length/ *In vivo* root number/ Shoot regeneration rate	1	RhK5_650_2680P	Protein_phosphatase_1_regulatory_ subunit_pprA_(probable)
Shoot ratio/ Shoot regeneration rate	19	RhMCRND_13148_267Q	gene uncharacterized RNA-binding protein C17H9.04c (LOC101291692)
		RhK5_13474_397Q	gene bifunctional protein FolD 1, mitochondrial-like (LOC101309186), transcript variant X2
		RhK5_570_626P	gene probable inactive serine/threonine-protein kinase scy1 (LOC101307983), transcript variant X2, mRNA
		Rh12GR_21174_1298Q	gene acidic leucine-rich nuclear phosphoprotein 32-related protein (LOC101303231),
		Rh12GR_28168_792Q	gene factor of DNA methylation 3-like (LOC101311119), transcript variant X2,
		RhK5_8293_614Q	gene probable receptor-like protein kinase At5g20050 (LOC101309575), mRNA
		RhK5_8844_469P	gene _IST1-like_protein_(probable)
		Rh12GR_21560_124Q	gene probable leucine-rich repeat receptor-like protein kinase
		RhK5_4154_515Q	gene probable CCR4-associated factor 1 homolog 7 (LOC101295595)
		RhK5_8_6985Q	gene dnaJ homolog subfamily C GRV2 (LOC101305987)
		Rh12GR_21282_4421P	gene BTB/POZ domain-containing protein
		Rh12GR_2555_1635P	gene _ Aristaless-related_homeobox_ protein_(ARX) _(similar_to)
		RhMCRND_6435_375P	gene probable receptor-like protein kinase At5g20050 (LOC101309575)
		RhK5_8_7501Q	Gene dnaJ homolog subfamily C GRV2 (LOC101305987)
		RhK5_3149_367Q	gene DELLA protein GAI-like (LOC101314119), mRNA
		Rh12GR_53908_964P	gene trihelix transcription factor GT-2-like (LOC101315082)
		RhK5_5078_253P	gene grpE protein homolog, mitochondrial -like (LOC101297042), transcript variant X4, mRNA
		Rh12GR_22138_343Q	gene nucleolar GTP-binding protein 2

			(LOC101314566)
		Rh12GR_26729_1408Q	gene transmembrane protein 19-like (LOC101291659), transcript variant X2,
In vivo root number/ Regeneration rate	2	RhK5_5772_666P	gene protein PAT1 homolog 1 (LOC101303919), mRNA
		RhK5_9050_472Q	gene ATP-dependent RNA helicase DHX36 (LOC101299095), mRNA
In vivo root length/ Regeneration rate	5	RhK5_41_5365P	gene dedicator of cytokinesis protein 6 (LOC101307146), mRNA
		RhK5_6730_852Q	gene_ribosomal_protein_L11_(similar_to)
		RhMCRND_21388_203P	Rosa multiflora breeding line 88/124-46 black spot resistance muRdr1 gene locus, complete sequence
		RhK5_6314_381Q	gene Putative_lipase_ROG1_(probable)
		RhMCRND_17848_232Q	gene ubiquitin-like-specific protease 1D (LOC101309441), transcript variant X2, mRNA
Callus CIM1/ Callus CIM2	2	Rh12GR_37799_568Q	Rosa chinensis psbP domain-containing protein 6, chloroplastic (LOC112191588), transcript variant X1, mRNA
		RhK5_4750_1179Q	Rosa chinensis spliceosome-associated protein 130 A
Callus CIM2/ *In vivo* root number	1	RhK5_107_2439P	*Rosa chinensis* chromatin modification -related protein EAF1 B-like (LOC112172241) , transcript variant X2, mRNA
In vivo root length/ *In vivo* root number	19	Rh88_10303_228Q	NA
		Rh12GR_70672_85P	gene Cell_differentiation_protein_ RCD1_homolog_(Rcd-1)_(similar_to)
		RhK5_7321_779Q	gene Histone_H4_transcription_factor _(HiNF-P)_(probable)
		Rh12GR_47780_467P	gene Cellulose_synthase-like_protein_G3 _(AtCslG3)_(probable)
		RhK5_15294_1220P	gene Nuclease_sbcCD_subunit_C _(probable)
		RhK5_2621_1523P	gene Phospholipase_C_4,_Precursor _(probable)
		RhK5_446_213P	gene Mitochondrial_Rho_GTPase_2_ (MIRO-2)_(probable)
		RhK5_1789_1730Q	gene RING_finger_protein_44_ (probable)
		RhK5_1017_1265P	gene Telomere-binding_protein_1 _(probable)
		RhK5_1049_2189P	gene Conserved_oligomeric_Golgi_ complex_subunit_3_ (COG_complex_subunit_3) _(probable)
		RhMCRND_23130_1044P	gene F-box_protein_At3g07870 _(probable)
		RhMCRND_3684_1281P	gene Dof_zinc_finger_protein_DOF3.3 _(AtDOF3.3)_(probable)

		RhK5_4056_658Q	gene Alcohol_dehydrogenase-like_1 _(probable)
		Rh12GR_14823_1243P	gene Copper-containing_nitrite_reductase, _Precursor_(probable)
		RhK5_16723_83Q	NA
		RhMCRND_13500_687Q	gene Anti-adapter_protein_iraM_(probable)
		RhK5_4957_957Q	gene Eukaryotic_translation_initiation _factor_3_subunit_J_(eIF3j)_(probable)
		RhK5_15295_125Q	gene E3_ubiquitin/ISG15_ligase _TRIM25_(probable)
		RhK5_7272_77Q	gene Period_circadian_protein_homolog_2_ (cPER1)_(probable)
In vivo root biomass/ *In vivo* root number	1	Rh12GR_49528_182P	NA

5. General discussion

The main goal of this study was to analyse genetic factors influencing the regeneration and micropropagation efficiency of rose cultivars. In this study, four chapters (representing four published manuscripts, one submitted manuscript and one manuscript ready for submission) are presented, each with a focus on different aspects: genetic dissection of adventitious shoot regeneration in roses by employing genome-wide association mapping (manuscript 1), markers development of shoot organogenesis in roses (manuscript 2), genetic analysis of AR formation *in vivo* and *in vitro* in a diversity panel of roses (manuscript 3) and genetic analysis of callus induction and shoot proliferation in roses by genome-wide association mapping (additional results). The main results were described and discussed in their respective manuscripts. More general aspects will be discussed in this chapter to describe the relationship among these findings and to provide an outlook for future objectives.

5.1 Regeneration and micropropagation traits' essential roles in roses

Regeneration and micropropagation of plants play essential roles in fundamental research and commercial applications, such as genetic engineering, clonal propagation and production of valuable metabolites. In roses, regeneration and micropropagation contribute to both research and commercial purposes. The development of genetic transformation protocols for plants in general (and roses in particular) requires a reliable and efficient plant regeneration system for the recovery of transgenic plants. In roses, few cultivars were used for genetic transformations (Dohm et al. 2001c; Lee et al. 2013; Li et al. 2002; Li et al. 2003; Uzunova 2000). Almost all transformation protocols used the SE of roses, but Uzunova (2000)) used organogenesis. Meanwhile, the micropropagation of roses has revolutionised the commercial nursery business: the benefit of micropropagation is its high multiplicative capacity to produce disease-free plants in a relatively short period of time, independent from seasonal factors and in a cost-effective manner. The plantlets that are developed through tissue culture are disease-free, will reduce input costs and increase effective management.

Regeneration via shoot organogenesis from various tissues and micropropagation has been reported for some rose cultivars. For organogenesis, some studies of rose cultivars have been published (Burger et al. 1990; Dubois et al. 2000; Lloyd et al. 1998; Pati et al. 2004a). Several publications involved the micropropagation of valuable rose cultivars, such as commercially-important species and genotypes of scented rose (*Rosa damascena* and *R. bourboniana*) (Pati et al. 2005) and those with medical value (*R. rugosa*) (Xing et al. 2010). In this study, a much larger and broader panel of 96 rose genotypes were used to investigate the traits that influence the *in vitro* regeneration and micropropagation of roses.

5.2 Roses are recalcitrant to particular manipulation *in vitro*

Recalcitrance is the inability of plant cells, tissues or organs to respond to the tissue culture. Recalcitrance can be a major limiting factor for *in vitro* manipulations of economically-important plant species, and it can also impair the wider application of *in vitro* conservation techniques. Roses are considered to be recalcitrant plants because of low regeneration, manipulation and transformation rates. Until now, no protocol of regeneration and manipulations has been applied for all rose varieties. In our study, however, the variation of shoot regeneration, callus induction, shoot proliferation and AR formation of 96 rose genotypes was demonstrated in one protocol.

In our investigation of *in vitro* regeneration and micropropagation, some genotypes, such as Raubritter, Rumba and Sterntaler, displayed the lowest regeneration rates and shoot ratios. However, they also demonstrated good responses for shoot proliferation and rooting performance. Rumba and Sterntaler also showed a high capacity for callus induction, with only Raubritter having a weaker response. In contrast, some genotypes with high regeneration capacity, such as Ausfather, Perenial Blush and Blue Pafume, had a low performance in callus induction, shoot proliferation and rooting. Other plant species are also recalcitrant to *in vitro* culture, such as cherry (Kaouther et al. 2017), peach (Park et al. 2017), black cotton (*Populus trichocarpa*) (Bao et al. 2009; Tuskan et al. 2018b), chili (*Capsicum* spp) (Haque and Ghosh 2018), black walnut (Stevens and Pijut 2018) and einkorn (*Triticum monococcum* L.) (Miroshnichenko et al. 2017). In these species, there are also pronounced genotypic, and therefore genetic, differences for *in vitro* competence, similar to roses.

5.3 Genetic differences between genotypes for all traits measured

In this study, we found differences between genotypes for shoot regeneration, callus induction, *in vitro* shoot proliferation and root formation. For direct regeneration traits, the organogenesis from petioles, shoot regeneration rate and shoot ratio were used as phenotypic descriptors for the regeneration capacity. Significant variation was found between the genotypes, ranging from a 0.88–88.33% regeneration rate and 0.008–1.2 in shoot ratios, which exceeded the rates reported by (Dubois et al. 2000) and (Pati et al. 2004). The results for callus formation from leaflet tissues on two kinds of media exhibited differences among genotypes. On medium CIM1, 95 of 96 genotypes showed callus formation, and only leaflets of the cultivar Jazz did not form callus. On the medium CIM2, leaflets of all genotypes formed calli, with callus size between 0.8–4.

The results of callus formation observed in all cultivars varied among genotypes between the two media. For shoot proliferation, some genotypes showed high multiplication rates, such as Bienenweide and Herzogin Friederike, with 3.74 and 4.24, respectively. In contrast, for some genotypes, multiplication was not possible and the dying off of some shoots even led to multiplication rates of lower than 1, for example, for Ausfather, Perennial Blush and Blue Perfume. Adventitious root

formation also showed variation among genotypes. For *in vitro* rooting experiments, the number of roots ranged from 0.12–18.7 and total root lengths ranged from 0.26–25.76 cm. For *in vivo* AR formation of rose genotypes, 90 of the 95 genotypes were able to form roots in the hydroponic system in the greenhouse. The average *in vivo* root number for 95 rose genotypes varied from 0–16.67, the average length of the roots ranged from 0–16.61 cm and the biomass of roots ranged from 0–55.23 mg. Therefore, our analyses reflect genotypic variation among the cultivars of the association panel that comprises partially non-overlapping genetic factors responsible for all the traits measured.

5.3.1 Potential for the improvement of research tools

An immediate application of the results generated in this thesis could be the selection of genotypes for research purposes displaying improved traits. For example, rooting capacity seems to be correlated to the success of induction of hairy roots via *Agrobacterium rhizogenes* (Debener, personal communication). Here, the selection of genotypes with high rooting capacity can improve experiments in functional genomics, in which genes are expressed and analysed in hairy roots. Furthermore, genotypes with improved callus formation and a higher capacity for direct regeneration from leaf petioles can be used in future research projects to improve current transformation methods. If markers associated with the traits investigated can be confirmed in independent populations, this information might even be used to identify the genes responsible for the genetic variation. This would be a crucial step in the functional analysis of the traits under study. As the rose genome has been recently sequenced (Hibrand Saint-Oyant et al. 2018b) regions around the associated markers can be screened for candidate genes for further studies.

5.3.2 Potential for practical application in rose production and breeding

Markers associated with some of the traits may be of immediate interest if their association can be verified in further experiments. For example, rooting capacity is an important trait for varieties propagated on their own roots, such as some landscaping or pot roses. Here, markers could be used to either preselect parents with improved allele composition and dosage or even progeny before other, more laborious tests for different traits (e.g. shelf life, disease resistance) are conducted during selection. Improved *in vitro* propagation might also be of immediate use for varieties kept in stock only under *in vitro* conditions or which are commercially propagated *in vitro*. Markers associated with axillary shoot proliferation might help to identify additional genotypes with improved proliferation capacity in order to avoid, or at least reduce, laborious *in vitro* experiments.

5.4 Correlation of the measured traits and cause of correlation

Studies on correlations between traits are critical to breeding programmes, as they may allow to perform indirect selection for a quantitative trait. They also provide information on how a trait might interfere with another (Machado et al. 2017). Some exemplary studies on the correlation between

traits were conducted with Spring oilseed Rape (Engqvist and Becker 1993) and Okra (*Abelmoschus esculents* [L.] Moechen) (Rashwan 2011).

In this study, correlations for all investigated traits were calculated, and the results are presented in Chapter 4. The strongest significant correlation was observed between regeneration rate and shoot ratio with a coefficient of 0.98. This is an obvious correlation because the regeneration of shoot organogenesis was apparent in the same explants (petioles) and culture conditions and the measures are not independent of each other. We also observed a strong significant correlation between *in vivo* root number and *in vivo* root length (coefficient 0.8), between *in vivo* root number and root biomass (coefficient 0.89) and between *in vivo* root length and *in vivo* root biomass (coefficient 0.89). These correlations are to be expected because the different measures were conducted on the same plants under the same *in vivo* rooting conditions. A high correlation was also observed between callus induction on CIM1 and CIM2, with a coefficient of 0.76. These correlations indicate common genetic mechanisms for callus induction, as in both cases, a callus is induced *in vitro* with the same treatment on the same explants, only using different PGRs. For *in vitro* rooting, we also found a correlation between *in vitro* root number and *in vitro* root length, with a coefficient of 0.7. The difference of growth and proliferation under *in vitro* conditions might have influenced the rooting response of the genotypes shoots since shoots of slightly different sizes were subjected to the analyses.

More correlations were observed between shoot proliferation and *in vitro* root number (coefficient 0.59), between callus CIM2 and shoot proliferation (coefficient 0.63), between shoot proliferation and callus CIM1 (coefficient 0.54) and between *in vitro* root length and *in vivo* root length (coefficient 0.52). These correlations indicate that there may be common developmental processes that partially overlap. However, only a few markers were found in common between these traits. This might be due to the absence of strong QTLs with large effects for each of the traits, indicating that each trait is influenced by many small effect QTLs. As these only partially overlap, many common factors may have remained undetected by our association study because of their small effect and the small population size, which only allowed the detection of major, large effect QTLs.

5.5 Outlook

The work described here outlined the first steps for the genetic analysis of developmental traits in roses. The small population studied and the limiting capacity for phenotyping led to a low genetic resolution and to only a comparatively small number of associated markers. In future experiments, this could be significantly improved by analysing more genotypes. As the costs for genotyping are expected to decrease, this will be a feasible endeavour. Markers with significant effects might be tested in additional populations by single marker analysis, such as the KASP technology described in Chapter 3. Most interesting, however, would be further analysis of the underlying genes for some of the traits. Here, markers with known functions related to the trait of interest (e.g. rooting traits) might

be used to isolate full-length genes, which then might be used in overexpression or knock out analysis, revealing the potential role of these genes in the developmental traits under study. An alternative to the time-consuming stable transformation of roses would be the induction of hairy roots for some of the traits (e.g. rooting, callus formation) or the use of heterologous systems, such as Arabidopsis or tobacco

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Acknowledgements

In the beginning, I would say thanks to my supervisors Prof. Dr. Thomas Debener and Prof. Dr. Traud Winkelmann to guide me well throughout the research work from title's selection to finding the results. Their immense knowledge, motivation and patience have given me more power and spirit to best in the research writing. Conducting the academic study regarding such a difficult topic couldn't be as simple as they made this for me. They are my mentors and better advisors for my doctorate study beyond the imagination.

Apart from my Supervisors, I won't forget to express the gratitude to Dr. Dietmar Schulz, Dr. Marcus Linde, Dr. Frank Schaarschmidt, Dr. Helgard Kaufmann, Dr. Juliane Geike and Dipl. Klaus Dreier who made my access simpler to the research facilities and laboratory. They all have played a major role in polishing my analysis experimental data skills. It wouldn't have been possible to conduct this research without their precious support. Their endless guidance is hard to forget throughout my life.

I would like to thank the Vietnamese Government for financial support and assistance in making my project come to life. This study might have never happen without the financial aid of this organization

I would like to thank to my colleges in the unit of Molecular Plant Breeding, Institute of Plant Genetics, Leibniz University Hannover, Germany for the assistance in my experiments, the courage to complete tasks before deadlines and for stimulating the discussions.

In the end, I am grateful to my parents, siblings, relatives and friends who remembered me in their prayers for the ultimate success. I consider myself nothing without them. They gave me enough moral support, encouragement and motivation to accomplish the personal goals.

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Thi Hong Nhung Nguyen**,** Dietmar Schulz, Traud Winkelmann, Thomas Debener. (2017). Genetic dissection of adventitious shoot regeneration in roses by employing genome-wide association studies. Plant Cell Reports September 2017, Volume 36, Issue 9, pp 1493–1505

Conference contribution

2017 “Development of molecular markers for shoot organogenesis in roses” VII International Symposium on Rose Research and Cultivation, Angers, France