Tetraose glycoalkaloids from potato can provide complete protection against fungi and insects

Pieter J. Wolters[1], Doret Wouters[1], Yury M. Tikunov[1], Shimlal Ayilalath[1], Linda Kodde[1], Miriam Strijker[1], Lotte Caarls[1], Richard G. F. Visser[1], Vivianne G. A. A. Vleeshouwers[1]

[1] Plant Breeding, Wageningen University and Research, P.O. Box 386, 6700, AJ, Wageningen, The Netherlands

Corresponding Author: Pieter J. Wolters, Plant Breeding, Wageningen University and Research, P.O. Box 386, 6700 AJ, Wageningen, The Netherlands, +31 317 482836, jaap.wolters@wur.nl

Keywords: *Alternaria solani*, Colorado potato beetle, Plant immunity, *Solanum commersonii*, *Solanum tuberosum*, steroidal glycoalkaloids

Abstract

Plants with innate disease and pest resistance can contribute to more sustainable agriculture. Resistance breeding typically relies on the use of immune receptors or impaired susceptibility genes, but these come with challenges in terms of strength, durability or pleotropic effects. Natural defence compounds from plants are not considered in breeding, because biosynthesis of these compounds is a complex trait and proof of their role in resistance is often indirect. Here, we identified a wild relative of potato, *Solanum commersonii*, that provides us with unique insight in the role of glycoalkaloids in plant immunity. We cloned two atypical resistance genes that can provide complete resistance to *Alternaria solani* and Colorado potato beetle through the production of tetraose steroidal glycoalkaloids. Moreover, we show that these compounds are active against a wide variety of fungi. This research highlights the potential of using natural defence compounds produced by plants to protect them against pests and diseases.

Introduction

Worldwide, up to 20-40% of agricultural crop production is lost due to plant diseases and pests (1). Many crops have become heavily dependent on the use of pesticides, but this is unsustainable as these can negatively affect the environment and their use can lead to development of pesticide resistance (2-7). The European Union's 'Farm to Fork Strategy' aims to half pesticide use and risk by 2030 (8), a massive challenge that illustrates the urgent need for alternative disease control methods.

Wild relatives of crop species are promising sources of natural disease resistance (9-12). Monogenic resistance caused by dominant resistance (*R*) genes, typically encoding immune receptors that belong to the class of nucleotide-binding leucine-rich repeat receptors (NLRs), are successfully employed by plant breeders to develop varieties with strong qualitative disease resistance. However, this type of resistance is usually restricted to a limited range of races and pathogens are often able to overcome resistance over time (13, 14).

More robust resistance can be obtained by combining NLRs with different recognition specificities (15-18), or by including pattern recognition receptors (PRRs), which recognize conserved (microbe- or pathogen-derived) molecular patterns (19). While there have been no reports yet that show that PRRs provide complete resistance, several studies indicate that they can provide a wider recognition spectrum and enhance disease resistance (20-22). Alternatively, susceptibility (*S*) genes provide recessive resistance that can be both broad-spectrum and durable (23-25). Unfortunately, their recessive nature complicates the use of *S* genes in conventional breeding of autopolyploids and many mutated *S* genes come with pleiotropic effects.

A wide range of secondary metabolites with antimicrobial or anti-insect properties has been identified in diverse plant species (26-28), suggesting that secondary metabolites can play a very direct role in plant immunity. However, there are only few studies to date in plants that demonstrate such a direct link between secondary metabolites and disease resistance (29-32). Avenacin A-1, a triterpenoid saponin from oat, is a well-known example (33, 34). Saponins are compounds with soap-like properties that consist of a triterpenoid or steroidal aglycone linked to a variable oligosaccharide chain (35). They

are widely distributed in plants from different families and their effect stems from the ability to interact with membrane sterols, disrupting the cell integrity from target organisms (35-38). Saponins from the Solanaceae and Liliaceae families are characterized by a steroidal alkaloid aglycone (39, 40). Different studies show that steroidal glycoalkaloids (SGAs) from tomato, potato and lily have antimicrobial and anti-insect activity (41-51).

Early blight is an important disease of tomato and potato that is caused by the necrotrophic fungal pathogen *Alternaria solani* (52-54). In a previous study, we found a wild potato species, *Solanum commersonii*, with strong resistance to *A. solani* (55). We showed that resistance is likely caused by a single dominant locus and that it can be introgressed in cultivated potato (55). Resistance to necrotrophs is usually considered to be a complex, polygenic trait, or recessively inherited according to the *inverse gene-for-gene* model (56-61). It therefore surprised us to find a qualitative dominant resistance against early blight in *S. commersonii* (55).

In this study, we explored different accessions of *S. commersonii* and *S. malmeanum* (previously *S. commersonii* subsp. *malmeanum* (62)) and developed a population that segregates for resistance to early blight. Using a Bulked Segregant RNA-Seq (BSR-Seq) approach (63), we mapped the resistance locus to the top of chromosome 12 of potato. We sequenced the genome of the resistant parent of the population and identified two glycosyltransferases that can provide resistance to susceptible *S. commersonii*. We show that resistance is based on the production of tetraose SGAs. Interestingly, these SGAs are active against a wide variety of pathogens. As a result, plants producing the compounds have a broad-spectrum disease and insect resistance.

Results

Early blight resistance maps to chromosome 12 of potato. To find suitable parents for a mapping study targeting early blight resistance, we performed a disease screen with *A. solani* isolate altNL03003 (64) on 13 different accessions encompassing 37 genotypes of *S. commersonii* and *S. malmeanum* (*SI Appendix,* Table S1). The screen showed clear differences in resistance phenotypes between and within accessions (Fig. 1a). Roughly half of the genotypes were completely resistant (lesion diameters < 3 mm

indicate that the lesions are not expanding beyond the size of the inoculation droplet) and the other half was susceptible (displaying expanding lesions), with only a few intermediate genotypes. CGN18024 is an example of an accession that segregates for resistance, with CGN18024_1 showing complete resistance and CGN18024_3 showing clear susceptibility (Fig. 1b). The fact that individual accessions can display such clear segregation for resistance suggests that resistance is caused by a single gene or locus. Because of its clear segregation, *S. commersonii* accession CGN18024 was selected for further studies.

To further study the genetics underlying resistance to early blight, we crossed resistant CGN18024_1 with susceptible CGN18024_3. Thirty progeny genotypes were sown out and tested with *A. solani*. We identified 14 susceptible genotypes and 16 fully resistant genotypes, with no intermediate phenotypes in the population (Fig. 1c). This segregation supports a 1:1 ratio (X^2 (1, N = 30) = 0.133, p=.72), which confirms that resistance to early blight is likely caused by a single dominant locus in *S. commersonii*.

To genetically localize the resistance, we isolated RNA from each progeny genotype and the parents of the population and proceeded with a BSR-Seq analysis (63). RNA from resistant and susceptible progeny genotypes were pooled in separate bulks and sequenced next to RNA from the parents on the Illumina sequencing platform (PE150). Reads were mapped to the DMv4.03 (65) and Solyntus potato genomes (66). To find putative SNPs linked to resistance, we filtered for SNPs that follow the same segregation as resistance (heterozygous in resistant parent CGN18024_1 and the resistant bulk, but absent or homozygous in susceptible parent and susceptible bulk). The resulting SNPs localize almost exclusively on chromosome 12 of the DM and Solyntus genomes, with most of them located at the top of the chromosome (*SI Appendix,* Dataset S1, Fig. 1d). We used a selection of SNPs distributed over chromosome 12 as high-resolution melt (HRM) markers to genotype the BSR-Seq population. This rough mapping proves that the locus for early blight resistance resides in a region of 3 Mb at the top of chromosome 12 (*SI Appendix,* Fig. S1).

Improved genome assembly of *S. commersonii*. A genome sequence of *S. commersonii* is already available (67), but we do not know if the sequenced genotype is resistant to *A. solani*. To help the

identification of additional markers and to explore the resistance locus of a genotype with confirmed resistance, we sequenced the genome of resistant parent CGN18024_1. High-molecular-weight (HMW) genomic DNA (gDNA) from CGN18024_1 was used for sequencing using Oxford Nanopore Technology (ONT) on a GridION X5 platform and for sequencing using DNA Nanoball (DNB) technology at the Beijing Genomics Institute (BGI) to a depth of approximately 30X. We used the ONT reads for the initial assembly and the shorter, more accurate, DNBseq reads to polish the final sequence. The resulting assembly has a size of 737 Mb, which is close to the size of the previously published genome of *S. commersonii* (730 Mb) (67). N50 scores and Benchmarking Universal Single-Copy Orthologs (BUSCO) score indicate a highly complete and contiguous genome assembly of *S. commersonii* (Table 1).

Identification of two glycosyltransferase resistance genes. To identify candidate genes that can explain the resistance of *S. commersonii*, it was necessary to further reduce the mapping interval. By aligning the ONT reads to the CGN18024_1 genome assembly, we could identify new polymorphisms that we converted to additional PCR markers (*SI Appendix,* Fig. S2-5). We performed a recombinant screen of approximately 3000 genotypes from the population to fine-map the resistance region to a window of 20 kb (*SI Appendix,* Fig. S6 and S7).

We inferred that the resistance locus is heterozygous in CGN18024_1 from the segregation in the mapping population. We used polymorphisms in the resistance region to separate and compare the ONT sequencing reads from the resistant and susceptible haplotype. This comparison showed a major difference between the two haplotypes. The susceptible haplotype contains a small insertion of 3.7 kb inside a larger region of 7.3 kb. The larger region is duplicated in the resistant haplotype (Fig. 2a). As a result, the resistance region of the resistant haplotype is 27 kb, 7 kb larger than the corresponding region of the susceptible haplotype (20 kb).

Two genes coding for putative glycosyltransferases (GTs) are located within the rearrangement of the resistant haplotype. The corresponding allele from the susceptible haplotype contains a frameshift mutation, leading to a truncated protein (*SI Appendix,* Fig. S8). Several other short ORFs with homology

to glycosyltransferases were predicted in the resistant haplotype, but *ScGTR1* (*S. commersonii* glycosyltransferase linked to resistance 1) and *ScGTR2* are the only full-length genes in the region. Reads from the BSR-Seq experiment show that both genes are expressed in bulks of resistant progeny and not in susceptible progeny (Fig. 2b), suggesting a putative role for these genes in causing resistance to *A. solani*. *ScGTR1* and *ScGTR2* are homologous sequences with a high similarity (97% amino acid identity). They show some similarity to characterized GTs with a role in zeatin biosynthesis (68-70) and to GAME17, an enzyme from tomato involved in biosynthesis of α-tomatine (71) (*SI Appendix,* Fig. S9, Table S2).

To test whether the identified candidate genes are indeed involved in resistance, we transiently expressed both alleles of the resistant haplotype (*ScGTR1* and *ScGTR2*) as well as the corresponding allele from the susceptible haplotype (*ScGTS*), using Agroinfiltration, in leaves of resistant CGN18024_1 and susceptible CGN18024_3 and *S. tuberosum* cultivar Atlantic. Following agroinfiltration, the infiltrated areas were drop-inoculated with a spore suspension of *A. solani*. Transient expression of *ScGTR1* as well as *ScGTR2* significantly reduced the size of the *A. solani* lesions in susceptible CGN18024_3, compared with *ScGTS* and the empty vector control. Resistant CGN18024_1 remained resistant, whereas susceptible Atlantic remained susceptible regardless of the treatment (Fig. 2c). We conclude that both *ScGTR1* and *ScGTR2* can affect resistance in *S. commersonii* CGN18024_3, but not in *S. tuberosum* cv. Atlantic.

Leaf compounds from resistant *S. commersonii* inhibit growth of diverse fungi, including pathogens of potato. Glycosyltransferases are ubiquitous enzymes that catalyse the transfer of saccharides to a range of different substrates. To test if resistance of *S. commersonii* to *A. solani* can be explained by a host-specific defence compound, we performed a growth inhibition assay using crude leaf extract from resistant and susceptible *S. commersonii*. Leaf material was added to PDA plates to equal 5% w/v and autoclaved (at 121 °C) or semi-sterilised at 60 °C. Interestingly, leaf material from resistant CGN18024_1 strongly inhibited growth of *A. solani*, while we did not see any growth inhibition on plates containing leaves from susceptible CGN18024_3 (Fig. 3a). Remarkably, on the plates containing

semi-sterilised leaves from susceptible *S. commersonii,* ample contamination with diverse fungi appeared after a few days, but not on plates with leaves from CGN18024_1 (Fig. 3a). Thus, leaves from CGN18024_1 contain compounds that can inhibit growth of a variety of fungi, not just *A. solani*.

To quantify the inhibitory effect of leaves from *S. commersonii* against different fungal pathogens of potato, we performed a growth inhibition assay with *A. solani* (altNL03003 (64)), *Botrytis cinerea* (B05.10 (73)) and *Fusarium solani* (1992 vr). As before, we added 5% (w/v) of leaf material from CGN18024_1 or CGN18024_3 to PDA plates and we placed the fungi at the centre of the plates. We measured colony diameters in the following days and compared it with the growth on PDA plates without leaf extract. Indeed, growth of all three potato pathogens was significantly reduced on medium containing leaf material from CGN18024_1 (Fig. 3b), compared to medium containing material from CGN18024_3 or on normal PDA plates. These results indicate that compounds from the leaves of resistant *S. commersonii* can have a protective effect against diverse fungal pathogens of potato.

Tetraose steroidal glycoalkaloids from *Solanum commersonii* provide resistance to *Alternaria solani* and Colorado potato beetle. Leaves from *Solanum* usually contain SGAs, which have a known activity against fungi and other plant pathogens (74). To test if SGAs can explain resistance of *S. commersonii*, we measured SGA content in leaves from Atlantic and susceptible/resistant *S. commersonii* by ultra high performance liquid chromatography (UPLC) coupled to mass spectrometry (MS). We found a remarkable difference in the SGA profile of resistant and susceptible genotypes (Fig 4a and *SI Appendix,* Table S3 and S4). Atlantic leaves contained only α-chaconine and α-solanine, which are the typical triose SGAs from cultivated potato. Susceptible *S. commersonii* CGN18024_3 also contained triose SGAs, but not the same ones as found in Atlantic. The SGAs from CGN18024_3 consist of a solanidine or demissidine (dihydrosolanidine) backbone conjugated to a trihexose sugar moiety. Remarkably, tetraose SGAs with an additional hexose or pentose, were additionally detected in resistant CGN18024_1. These tetraose SGAs from *S. commersonii* are not novel, but were characterized previously and identified as galactosyl-triglucosides (commersonine and dehydrocommersonine) and

galactosyl-diglucoside-xylosides (demissine and dehydrodemissine) (75-77). These results suggest that the triose SGAs present in susceptible CGN18024_3 are modified to produce the tetraose SGAs in resistant CGN18024_1, by addition of an extra glucose or xylose moiety.

To investigate a possible role for *ScGTR1* and *ScGTR2* in the production of tetraose SGAs from CGN18024_1 and their link to resistance, we generated stable transformants of *ScGTR1* and *ScGTR2* in CGN18024_3 (*SI Appendix,* Fig. S10). UPLC-MS analysis showed that both *ScGTR1* and *ScGTR2* transformants accumulate tetraose SGAs, while the amount of triose SGAs is markedly reduced (Fig 4a). Strikingly, ScGTR1 and ScGTR2 appear to have different specificities. Overexpression of *ScGTR1* resulted in the addition of a glucose to the triose SGAs from CGN18024_3 (yielding a commertetraose moiety), while overexpression of *ScGTR2* caused the addition of a xylose (resulting in a lycotetraose) (Figure 4a and d). This suggests that *ScGTR1* is a glucosyltransferase and that *ScGTR2* is a xylosyltransferase. However, we detect a slight overlap in activity. In addition to the lycotetraose products, we detect small amounts of commertetraose product in *ScGTR1* transformants and vice versa in the *ScGTR2* transformants. A multivariate Principal Components Analysis (PCA) on the full metabolic profile consisting of 1,041 mass peaks revealed that *ScGTR1* and *ScGTR2* are highly specific towards SGAs since 75% of the metabolic variation between the transformants and the wild types could be explained by the SGA modifications (*SI Appendix,* Fig. S11). Modifications catalysed by both enzymes can lead to resistance, as *ScGTR1* and *ScGTR2* transformants are both resistant to *A. solani* (Fig. 4b). We also analysed Atlantic *ScGTR1* and *ScGTR2* transformants, but did not detect differences in SGA profile, probably because they contain different triose SGA substrates than found in *S. commersonii* (*SI Appendix,* Table S4).

Leptine and dehydrocommersonine SGAs have previously been linked to resistance to insects such as Colorado potato beetle (CPB) (41, 44-48, 78). To see if the SGAs from *S. commersonii* can protect against insects, we performed a test with larvae of CPB on wildtype CGN18024_1/CGN18024_3 and on CGN18024_3 transformed with *ScGTR1* or *ScGTR2* (Fig. 4b). Wildtype CGN18024_3 is susceptible to CPB, but CGN18024_1 and CGN18024_3 transformed with *ScGTR1* or *ScGTR2* are completely resistant

to CPB, as illustrated by a very low larvae weight and survival (Fig. 4c). Thus, the transfer of a glucose or xylose moiety to triose SGAs from CGN18024_3 (demissidine-Gal-Glu-Glu and solanidine-Gal-Glu-Glu), catalysed by ScGTR1 and ScGTR2 from CGN18024_1 respectively, results in the production of tetraose SGAs that can offer protection against fungi and insects (Fig. 4d).

Discussion

In this study, we set out to characterise resistance of *S. commersonii* to *A. solani*. We showed that it is caused by a single dominant locus containing two GT candidate resistance genes. Both GTs are involved in the production of tetraose SGAs in *S. commersonii*, but they transfer distinct sugars. Both modifications cause resistance to *A. solani*. We demonstrate that the tetraose SGAs from *S. commersonii* can protect against other fungi besides *A. solani* and that plants producing the compounds are resistant to CPB. Collectively, our data establish a direct link between the tetraose SGAs from *S. commersonii* and resistance against different potato pathogens and insects.

It is known that specialized metabolites from plants have a role in plant defence and compounds with antimicrobial effects have been characterized in many different plant species (26-28). However, exact knowledge of how these compounds contribute to resistance and how they are produced is limited. As a result, saponins and other specialized defence metabolites are not considered in resistance breeding. Instead, the current focus is on using immune receptors or *S* genes. These different strategies each come with their own challenges in terms of durability, specificity, pleiotropic effects and strength of the resulting resistance. The tetraose SGAs from *S. commersonii* compare favourably in many of these aspects, as they provide a strong and broad-spectrum resistance without any noticeable negative effects on the plant.

Biosynthesis of SGAs in *Solanum* is a complex trait that is controlled by many genes. The discovery of *S. commersonii* genotypes with and without tetraose SGAs provides us with unique insight in the role of these compounds in plant immunity. To make a practical use of them, it is necessary to identify the genes upstream of *ScGTR1* and *ScGTR2*. The compounds that are found in resistant *S. commersonii* are an interesting combination of a solanidine or demissidine (dihydrosolanidine) aglycone and a

lycotetraose or commertetraose sugar moiety. Solanidine forms the aglycone backbone of α-solanine and α-chaconine from potato, while the lycotetraose decoration is found on α-tomatine from tomato (75, 79). The biosynthesis pathways leading to the production of these major SGAs from cultivated potato and tomato have largely been elucidated in recent years and it was found that the underlying genes occur in conserved clusters (71, 79). This knowledge and the similarities between SGAs from *S. commersonii* and potato/tomato will help to identify the missing genes from the pathway through comparative genomics.

The broad-spectrum activity of tetraose SGAs is attractive, but this non-specificity also presents a risk. The antifungal and anti-insect activity of SGAs from *S. commersonii* is not restricted to potato pathogens and pests, but could also affect beneficial or commensal micro-organisms or other animals that feed on plants (80, 81). In potato tubers, a total SGA content of less than 200 mg/kg is generally considered to be safe for human consumption (82-84), but little is known about the toxicity of individual SGAs. In tomato fruit, α-tomatine is converted to esculeoside A during fruit ripening in a natural detoxification process from the plant (85, 86) to facilitate dispersal of the seeds by foraging animals. Unintended toxic effects of SGAs should similarly be taken into account when used in resistance breeding.

Studies on α-tomatine and avenacin A-1 show that changes to the sugar moiety can affect toxicity of the compounds (43, 87-89). Tomato and oat pathogens produce enzymes that can detoxify these saponins through removal of one or more glycosyl groups (43, 49, 50, 90-92). The degradation products of saponins can also suppress plant defence responses (93, 94). Conversely, here we show that the resistance of *S. commersonii* is based on the addition of a glycosyl group to a triose saponin from *S. commersonii.* There is large variation in both the aglycone and the sugar moiety of SGAs from wild *Solanum*, with likely over 100 distinct SGAs produced in tubers (75) (95). This diversity suggests a pressure to evolve novel molecules, possibly to resist detoxification mechanisms, reminiscent of the molecular arms race that drives the evolution of plant immune receptors (14). Thus, wild *Solanum*

germplasm is not only a rich source of immune receptors, it also provides a promising source of natural defence molecules.

As crops are usually affected by multiple diseases and pests, significant reduction of pesticide use can only be achieved if plants are naturally protected against a range of pathogen species and pests. Different strategies towards this goal have been proposed and our study underlines the relatively unexplored potential of defence compounds that are naturally produced by plants. The fact that genes for specialized plant metabolites often occur in biosynthetic gene clusters (71, 96-98), means that introgression breeding can help to move these compounds from wild relatives to crop species. If the genes underlying the biosynthesis pathways are identified, it is also possible to employ them through metabolic engineering (28). Alternatively, the defence compounds could be produced in non-crop plants or other organisms and applied on crops as biological protectants. Studies on how natural defence compounds are produced in different plant tissues, how they are detoxified and how different modifications ultimately affect plant immunity, are essential to employ them in a safe and effective manner. Such studies at the interface of plant immunity and metabolism can help to design innovative solutions to complement existing resistance breeding strategies and improve sustainability of our food production.

Methods

An overview of all primers used in this study can be found in *SI Appendix,* Tables S5-7. More detailed information on methods can be found in the *SI Appendix* text.

Genome assembly and separation of haplotypes covering resistance region. ONT reads were filtered using Filtlong v0.2.0 (https://github.com/rrwick/Filtlong) with --min_length 1000 and --keep_percent 90. Adapter sequences were removed using Porechop (99). Fastq files were converted to Fasta using seqtk v1.3 (https://github.com/lh3/seqtk). Assembly was performed with smartdenovo (https://github.com/ruanjue/smartdenovo/) and a k-mer size of 17, with the option for generating a consensus sequence enabled. ONT reads were mapped back to the assembly using minimap2 v2.17 (100) and used for polishing with racon v1.4.3 (101) using default settings. DNBseq reads were mapped

to the resulting sequence using bwa mem v0.7.17 (102) and used for a second round of polishing with racon v1.4.3. This procedure to polish the assembly using DNBseq reads was repeated once. ONT reads were mapped back to the polished CGN18024_1 assembly using minimap2 v2.17 (100). The alignment was inspected using IGV v2.6.3 (103) to identify polymorphisms for new markers and marker information was used to identify ONT reads representative for both haplotypes spanning the resistance region of CGN18024_1. Bedtools v2.25.0 (104) was used extract the resistance region from the reads and to mask the corresponding region from the original CGN18024_1 assembly. The extracted resistance regions from both reads were appended to the assembly and the polishing procedure described above was repeated to prepare a polished genome assembly of CGN18024_1, containing a sequence of both haplotypes covering the resistance region. Quality of the genome was assessed using quast v5.0.2 with --eukaryote --large (105).

Transient disease assay. Agroinfiltration was performed as described previously using *Agrobacterium tumefaciens* strain AGL1 (72, 106). Agrobacterium suspensions were used at an OD_{600} of 0.3 to infiltrate fully expanded leaves of 3-week-old CGN18024_1, CGN18024_3 and *S. tuberosum* cv. Atlantic. *ScGTR1*, *ScGTR2*, *ScGTS* and pK7WG2-empty were combined as four separate spots on the same leaf and the infiltrated areas were encircled with permanent marker. The plants were transferred to a climate cell 48 h after agroinfiltration and each infiltrated area was inoculated with *A. solani* by pipetting a 10 µl droplet of spore suspension (1 x 10^5 conidia/mL) at the centre of each spot. Lesion diameters were measured 5 days post inoculation. Eight plants were tested of each genotype, using three leaves per plant.

Fungal growth inhibition assays. Mature leaf material from 5-week-old plants was extracted in phosphate-buffered saline (PBS) buffer using a T25 Ultra Turrax disperser (IKA) and supplemented to obtain a 5% w/v suspension in PDA and autoclaved (20 min at 121C), or added to PDA after autoclaving, followed by an incubation step for 15 min at 60C to semi-sterilise the medium. The medium was poured into Petri dishes. Small agar plugs containing mycelium from *A. solani* (CBS 143772) or *F. solani* (1992 vr) were placed at the centre of each plate and the plates were incubated at 25C in the dark.

Similarly, approximately 100 spores of *B. cinerea* B05.10 (73) were pipetted at the centre of PDA plates containing the different leaf extracts and the plates were incubated at room temperature in the dark. 3 plates per fungal isolate/leaf extract combination were prepared and colony diameters were measured daily using a digital calliper.

Data analysis. Data were analysed in RStudio (R version 4.02) (107, 108), using the tidyverse package (109). Most figures were generated using ggplot2 (110), but genomic data were visualised using Gviz and Bioconductor (111). PCA was performed using PAST3 software (https://past.en.lo4d.com/windows). *P* values for comparisons between means of different groups were calculated in R using Welch's Two Sample t-test.

Data and material availability.

RNAseq data from the BSR-Seq experiment was deposited in the NCBI Sequence Read Archive with BioProject ID PRJNA792513 (Sequencing Read Archive accession IDs SRR17334110, SRR17334111, SRR17334112 and SRR17334113). Raw reads used in the assembly of the CGN18024_1 genome were deposited with BioProject ID PPRJNA789120 (Sequencing Read Archive accession IDs SRR17348659 and SRR17348660). The assembled genome sequence of CGN18024_1 was archived in GenBank under accession number JAJTWQ000000000. Sequences of *ScGTR1* and *ScGTR2* were deposited in GenBank under accession numbers OM830430 and OM830431. *A. solani* isolate altNL03003 was deposited at the fungal collection at the Westerdijk Fungal Biodiversity Institute under number CBS 143772. All other data and materials used in this study are available from the corresponding author upon reasonable request.

Author contributions

P.J.W, R.G.F.V. and V.G.A.A.V designed the study. P.J.W, D.W., S.A., L.K. and M.S. performed experiments. P.J.W., Y.M.T., S.A. and L.C. analysed the data. P.J.W. and V.G.A.A.V wrote the manuscript with input from all authors. All authors approved the manuscript.

Competing interests

P.J.W, R.G.F.V. and V.G.A.A.V. are inventors on U.S. Patent Application No. 63/211,154 relating to *ScGTR1* and *ScGTR2* filed by the J.R. Simplot company. The other authors declare no competing interests.

Acknowledgements

This research was funded by the J.R. Simplot Company, we especially thank Craig Richael for his support and useful discussions. We thank Dirk Jan Huigen and Henk Meurs for taking care of the plants in the greenhouse and Jack Vossen for providing us with *F. solani* isolate 1992 vr from the collection of Biointeractions and Plant Health (Theo van der Lee, WUR). Jan van Kan and Yaohua You for insightful discussions and for providing us with *B. cinerea* isolate B05.10. Evert Jacobsen for his feedback on the manuscript. Martijn van Kaauwen and Richard Finkers for bioinformatics support. P.J.W. thanks Andrea Lorena Herrera for her support and helpful talks about secondary metabolites.

Table 1. Genome assembly metrics of *S. commersonii* cmm1t (67) and CGN18024_1

Genome	**CMM1t[a)]**	**CGN18024_1**
Total size (Mb)	730	737
Contig number	278460	637
Largest contig (Mb)	0,17	21,2
N50 (Mb)	0,007	4,02
Complete BUSCO (%)	81,9	95,7

[a)] Aversano et al (2015)

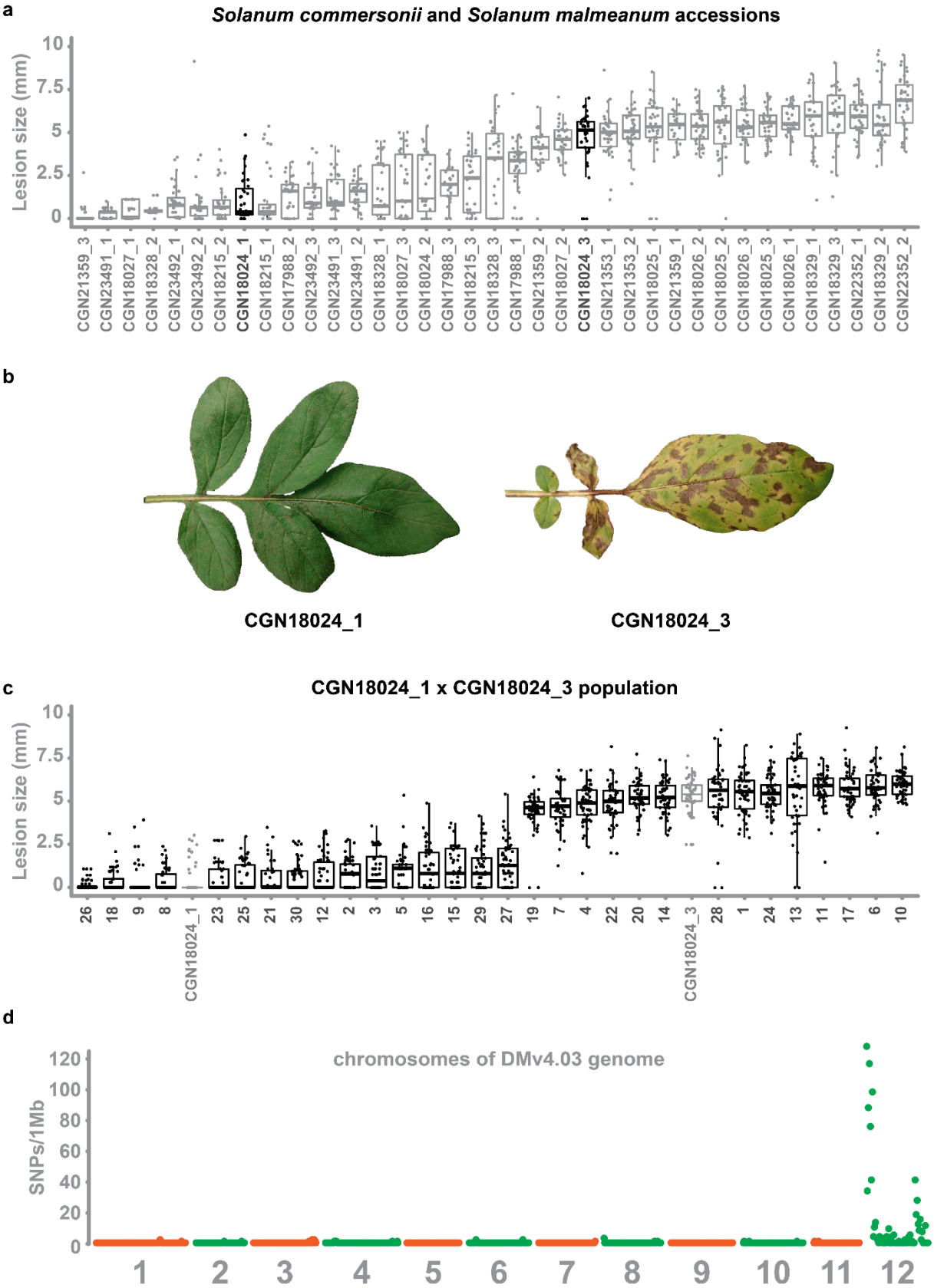

Fig. 1. Early blight resistance maps to chromosome 12 of potato. a. 2-3 genotypes of 13 different accessions of *S. commersonii* and *S. malmeanum* were inoculated with *A. solani* altNL03003. 3 plants of each genotype were tested and 3 leaves per plants were inoculated with 6 10 µl droplets with spore suspension. Lesion diameters were measured 5 days post inoculation and visualised using boxplots, with horizonal lines indicating median values and individual measurements plotted on top. Non-expanding lesions (<2-3 mm) indicate resistance and expanding lesions indicate susceptibility. Some accessions

segregate for resistance. **b.** Accession CGN18024 is an example of an accession that segregates for resistance to *A. solani*, with CGN18024_1 displaying complete resistance and CGN18024_3 displaying susceptibility at 5 days after spray-inoculation. **c.** Progeny from CGN18024_1 x CGN18024_3 was inoculated with *A. solani*. 3 plants of each genotype were tested and 3 leaves per plants were inoculated with 6 10 µl droplets with spore suspension each. Lesion diameters were measured 5 days post inoculation. 16 progeny genotypes are resistant (with lesion diameters < 2-3 mm) and 14 are susceptible (with expanding lesions). This corresponds to a 1:1 segregation ratio (X^2 (1, N = 30) = 0.133, ρ= 0.72). **d.** SNPs derived from a BSRseq analysis using bulks of susceptible and resistant progeny were plotted in 1 Mb windows over the 12 chromosomes of the potato DMv4.03 genome (65) They are almost exclusively located on chromosome 12.

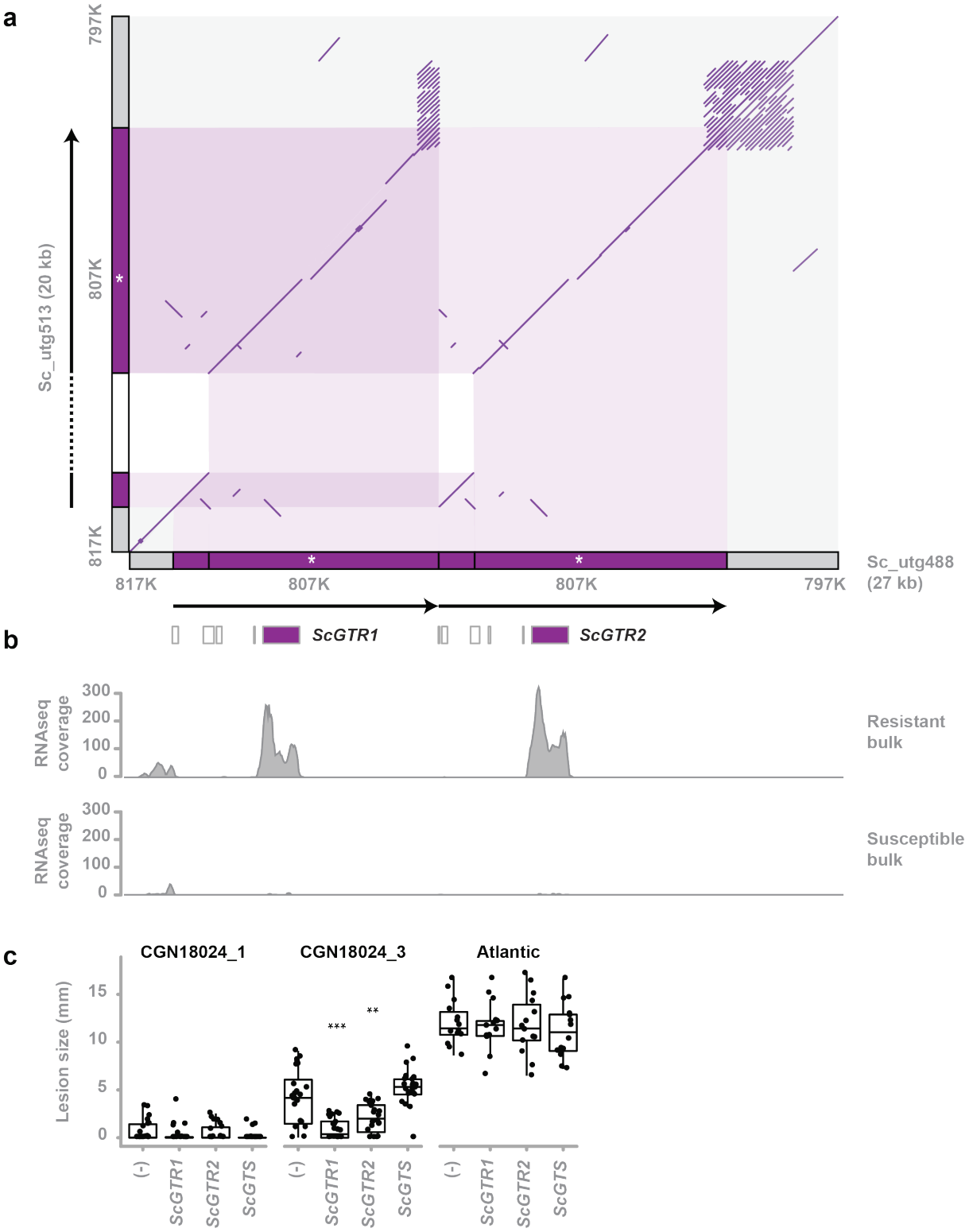

Fig. 2. Identification of two glycosyltransferase resistance genes. a. Comparison of the susceptible and resistant haplotype of the *Solanum commersonii* CGN18024_1 resistance region (delimited by markers 817K and 797K) in a comparative dot plot shows a rearrangement. Locations of markers used to map the resistance region are indicated in grey along the x- and y-axis. The duplicated region of the resistant haplotype contains marker 807K (white asterisk) and two predicted glycosyltransferases (*ScGTR1* and *ScGTR2*). Several short ORFs with homology to glycosyltransferases that were predicted in the resistance region are indicated by white boxes, but *ScGTR1* and *ScGTR2* are the only full-length genes. As a result of the rearrangement, the resistance region of the resistant haplotype (27 kb) is 7 kb larger than the corresponding region of the susceptible haplotype (20 kb). **b.** Alignment of RNAseq reads from the BSRSeq analysis shows that *ScGTR1* and *ScGTR2* are expressed in bulks of

resistant progeny, but not in bulks of susceptible progeny. **c.** *S. tuberosum* cv. 'Atlantic', *S. commersonii* CGN18024_1 and CGN18024_3 were agroinfiltrated with expression constructs for *ScGTR1* and *ScGTR2*, *ScGTS* and empty vector (-). *A. solani* is inoculated 2 days after agroinfiltration and lesion diameters are measured 5 days after inoculation. Lesion sizes were visualised with boxplots, with horizonal lines indicating median values and individual measurements plotted on top. Agroinfiltration with expression constructs for *ScGTR1* and *ScGTR2* results in a significant (Welch's Two Sample t-test, $^{**}P < 0.01$, $^{***}P < 0.001$) reduction of lesion sizes produced by *Alternaria solani* altNL03003 in *S. commersonii* CGN18024_3, but not in *S. tuberosum* cv. 'Atlantic'.

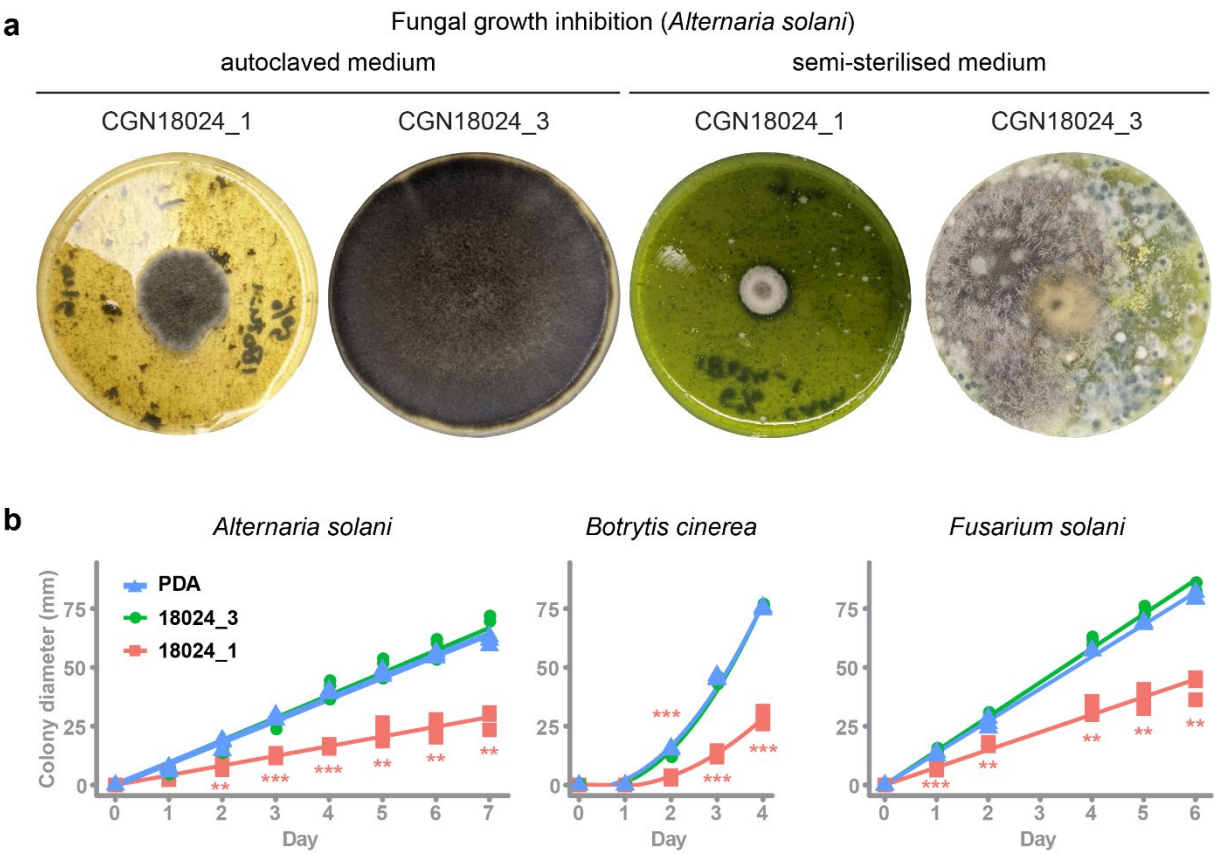

Fig. 3. Leaf compounds from resistant *S. commersonii* inhibit growth of diverse fungi, including pathogens of potato. a. Crude leaf extract from CGN18024_1/CGN18024_3 was added to PDA plates (5% w/v) and autoclaved (left) or semi-sterilised for 15 min at 60 °C (right)**.** Growth of *Alternaria solani* altNL03003 was strongly inhibited on PDA plates with autoclaved leaf extract from CGN18024_1 compared to plates with CGN18024_3, as shown on the left two pictures taken at 7 days after placing an agar plug with mycelium of *A. solani* at the centre of each plate. Abundant fungal contamination appeared after 4 days on plates containing semi-sterilized leaf from CGN18024_3, but not on plates containing material from CGN18024_1 (right two pictures). **b.** Growth of potato pathogenic fungi *A. solani*, *B. cinerea* (B05.10) and *F. solani* (1992 vr) was followed by measuring the colony diameter on PDA plates containing autoclaved leaf material from CGN18024_1/CGN18024_3. Growth of all three fungi was measured on PDA plates containing CGN18024_1 (red squares), CGN18024_3 (green circles) or plates with PDA and no leaf material (blue triangles). Significant differences in growth on PDA plates containing plant extract compared to PDA plates without leaf extract are indicated with asterisks (Welch's Two Sample t-test, $^{**}P < 0.01$, $^{***}P < 0.001$).

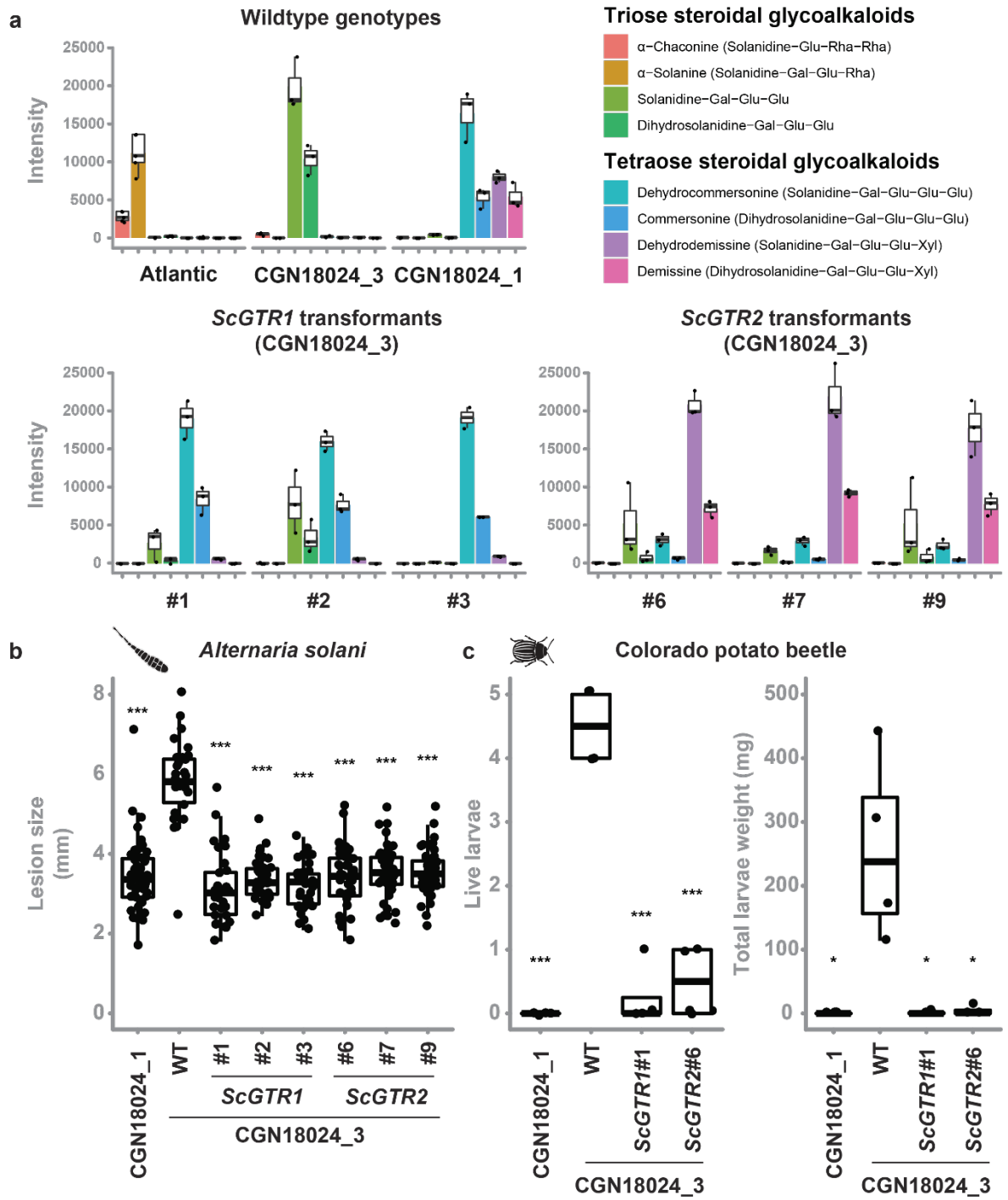
a
Wildtype genotypes
Intensity
Atlantic
CGN18024_3
CGN18024_1
Triose steroidal glycoalkaloids
α-Chaconine (Solanidine-Glu-Rha-Rha)
α-Solanine (Solanidine-Gal-Glu-Rha)
Solanidine-Gal-Glu-Glu
Dihydrosolanidine-Gal-Glu-Glu
Tetraose steroidal glycoalkaloids
Dehydrocommersonine (Solanidine-Gal-Glu-Glu-Glu)
Commersonine (Dihydrosolanidine-Gal-Glu-Glu-Glu)
Dehydrodemissine (Solanidine-Gal-Glu-Glu-Xyl)
Demissine (Dihydrosolanidine-Gal-Glu-Glu-Xyl)
ScGTR1 transformants (CGN18024_3)
ScGTR2 transformants (CGN18024_3)
#1
#2
#3
#6
#7
#9
b
Alternaria solani
Lesion size (mm)
CGN18024_1
WT
ScGTR1
ScGTR2
CGN18024_3
c
Colorado potato beetle
Live larvae
Total larvae weight (mg)
ScGTR1#1
ScGTR2#6
d
CGN18024_3
CGN18024_1
Demissidine-Gal-Glu-Glu
Solanidine-Gal-Glu-Glu
ScGTR1 (+ Glu)
ScGTR2 (+ Xyl)
Dehydrocommersonine
Commersonine
Dehydrodemissine
Demissine

Fig. 4. Tetraose steroidal glycoalkaloids from *Solanum commersonii* provide resistance to *Alternaria solani* and Colorado potato beetle. Data are visualised with boxplots, with horizonal lines indicating median values and individual measurements plotted on top. **a.** Tetraose steroidal glycoalkaloids (SGAs) were detected in resistant CGN18024_1 and in CGN18024_3 transformed with *ScGTR1*/*ScGTR2*. Susceptible *S. tuberosum* cv. 'Atlantic' and wildtype (WT) CGN18024_3 contain only triose SGAs. Overexpression of *ScGTR1* resulted in the addition of a hexose to the triose SGAs from CGN18024_3, resulting in a commertetraose (Gal-Glu-Glu-Glu), while overexpression of *ScGTR2* caused the addition of a pentose, resulting in a lycotetraose (Gal-Glu-Glu-Xyl). **b.** WT CGN18024_1/CGN18024_3 and CGN18024_3 transformants were inoculated with *Alternaria solani* altNL03003. 3 plants of each genotype were tested and 3 leaves per plants were inoculated with 6 10 µl droplets with spore suspension each. Lesions diameters were measured 5 days post inoculation. *ScGTR1* and *ScGTR2* can both complement resistance to *A. solani* in CGN18024_3, as the lesion sizes produced on CGN18024_3 transformants are comparable to resistant CGN18024_1. **c.** 3 plants per genotype were challenged with 5 Colorado potato beetle larvae each. The tetraose SGAs produced by *ScGTR1* and *ScGTR2* can provide resistance to Colorado potato beetle, as indicated by reduced larvae survival and total larvae weight. Significant differences with WT CGN18024_3 are indicated with asterisks (Welch's Two Sample t-test, $*P < 0.05$, $***P < 0.001$). d. CGN18024_3 produces triose SGAs and is susceptible to Colorado potato beetle and *A. solani*. ScGTR1 and ScGTR2 from CGN18024_1 convert these triose SGAs from susceptible *S. commersonii* to tetraose SGAs, through the addition of a glucose or xylose moiety respectively. Both sugar additions can provide resistance to Colorado potato beetle and *A. solani*.

References

1. S. Savary *et al.*, The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution* **3**, 430-439 (2019).
2. M. Calvo-Agudo *et al.*, Neonicotinoids in excretion product of phloem-feeding insects kill beneficial insects. *Proceedings of the National Academy of Sciences* **116**, 16817-16822 (2019).
3. C. A. Hallmann, R. P. B. Foppen, C. A. M. van Turnhout, H. de Kroon, E. Jongejans, Declines in insectivorous birds are associated with high neonicotinoid concentrations. *Nature* **511**, 341-343 (2014).
4. A. Mikaberidze, N. Paveley, S. Bonhoeffer, F. van den Bosch, Emergence of Resistance to Fungicides: The Role of Fungicide Dose. *Phytopathology®* **107**, 545-560 (2017).
5. J. A. Lucas, N. J. Hawkins, B. A. Fraaije, The evolution of fungicide resistance. *Adv Appl Microbiol* **90**, 29-92 (2015).
6. K. L. Fairchild, T. D. Miles, P. S. Wharton, Assessing fungicide resistance in populations of Alternaria in Idaho potato fields. *Crop Protection* **49**, 31-39 (2013).
7. S. Landschoot *et al.*, Boscalid-resistance in Alternaria alternata and Alternaria solani populations: An emerging problem in Europe. *Crop Protection* **92**, 49-59 (2017).
8. European Commission (2020) Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions: A Farm to Fork Strategy for a fair, healthy and environmentally-friendly food system.
9. J. Rodewald, B. Trognitz, Solanum resistance genes against Phytophthora infestans and their corresponding avirulence genes. *Molecular Plant Pathology* **14**, 740-757 (2013).
10. V. G. Vleeshouwers *et al.*, SolRgene: an online database to explore disease resistance genes in tuber-bearing Solanum species. *BMC Plant Biology* **11**, 116 (2011).
11. P. J. Wolters *et al.*, A rapid method to screen wild Solanum for resistance to early blight. *European Journal of Plant Pathology* **154**, 109-114 (2019).
12. S. Arora *et al.*, Resistance gene cloning from a wild crop relative by sequence capture and association genetics. *Nature Biotechnology* **37**, 139-143 (2019).
13. H. H. Flor, Current Status of the Gene-For-Gene Concept. *Annual Review of Phytopathology* **9**, 275-296 (1971).
14. J. D. Jones, J. L. Dangl, The plant immune system. *Nature* **444**, 323-329 (2006).
15. H. J. Kim *et al.*, Broad spectrum late blight resistance in potato differential set plants MaR8 and MaR9 is conferred by multiple stacked R genes. *Theor Appl Genet* **124**, 923-935 (2012).
16. S. Zhu, Y. Li, J. H. Vossen, R. G. Visser, E. Jacobsen, Functional stacking of three resistance genes against Phytophthora infestans in potato. *Transgenic research* **21**, 89-99 (2012).
17. V. G. Vleeshouwers *et al.*, Understanding and exploiting late blight resistance in the age of effectors. *Annual review of phytopathology* **49**, 507-531 (2011).
18. H. Rietman *et al.*, Qualitative and quantitative late blight resistance in the potato cultivar Sarpo Mira is determined by the perception of five distinct RXLR effectors. *Molecular plant-microbe interactions : MPMI* **25**, 910-919 (2012).
19. B. P. M. Ngou, H.-K. Ahn, P. Ding, J. D. G. Jones, Mutual potentiation of plant immunity by cell-surface and intracellular receptors. *Nature* **592**, 110-115 (2021).
20. J. Du *et al.*, Elicitin recognition confers enhanced resistance to Phytophthora infestans in potato. *Nature Plants* **1**, 15034 (2015).
21. I. Albert *et al.*, An RLP23–SOBIR1–BAK1 complex mediates NLP-triggered immunity. *Nature Plants* **1**, 15140 (2015).
22. D. Couto, C. Zipfel, Regulation of pattern recognition receptor signalling in plants. *Nature Reviews Immunology* **16**, 537-552 (2016).
23. C. C. van Schie, F. L. Takken, Susceptibility genes 101: how to be a good host. *Annual review of phytopathology* **52**, 551-581 (2014).
24. I. H. Jørgensen, Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. *Euphytica* **63**, 141-152 (1992).

25. K. Sun *et al.*, Silencing susceptibility genes in potato hinders primary infection with Phytophthora infestans at different stages. *Horticulture Research* **9** (2022).
26. R. A. Dixon, Natural products and plant disease resistance. *Nature* **411**, 843-847 (2001).
27. A. Piasecka, N. Jedrzejczak-Rey, P. Bednarek, Secondary metabolites in plant innate immunity: conserved function of divergent chemicals. *New Phytologist* **206**, 948-964 (2015).
28. G. Polturak, A. Osbourn, The emerging role of biosynthetic gene clusters in plant defense and plant interactions. *PLoS Pathogens* **17**, e1009698 (2021).
29. P. Bednarek *et al.*, A Glucosinolate Metabolism Pathway in Living Plant Cells Mediates Broad-Spectrum Antifungal Defense. *Science* **323**, 101-106 (2009).
30. K. Papadopoulou, R. Melton, M. Leggett, M. Daniels, A. Osbourn, Compromised disease resistance in saponin-deficient plants. *Proceedings of the National Academy of Sciences* **96**, 12923-12928 (1999).
31. B. P. Thomma, I. Nelissen, K. Eggermont, W. F. Broekaert, Deficiency in phytoalexin production causes enhanced susceptibility of Arabidopsis thaliana to the fungus Alternaria brassicicola. *The Plant Journal* **19**, 163-171 (1999).
32. R. Hain *et al.*, Disease resistance results from foreign phytoalexin expression in a novel plant. *Nature* **361**, 153-156 (1993).
33. E. Turner, The Nature of the Resistance of Oats to the Take-all Fungus: II. INHIBITION OF GROWTH AND RESPIRATION OF OPHIOBOLUS GRAMINIS SACC. AND OTHER FUNGI BY A CONSTITUENT OF OAT SAP. *Journal of Experimental Botany* **7**, 80-92 (1956).
34. K. Haralampidis *et al.*, A new class of oxidosqualene cyclases directs synthesis of antimicrobial phytoprotectants in monocots. *Proceedings of the National Academy of Sciences* **98**, 13431-13436 (2001).
35. A. Osbourn, Saponins and plant defence—a soap story. *Trends in plant science* **1**, 4-9 (1996).
36. E. A. Keukens *et al.*, Molecular basis of glycoalkaloid induced membrane disruption. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **1240**, 216-228 (1995).
37. C. Armah *et al.*, The membrane-permeabilizing effect of avenacin A-1 involves the reorganization of bilayer cholesterol. *Biophysical journal* **76**, 281-290 (1999).
38. G. R. Fenwick, K. R. Price, C. Tsukamoto, K. Okubo, "CHAPTER 12 - Saponins" in Toxic Substances in Crop Plants, J. P. F. D'Mello, C. M. Duffus, J. H. Duffus, Eds. (Woodhead Publishing, 1991), https://doi.org/10.1533/9781845698454.285, pp. 285-327.
39. H.-J. Li, Y. Jiang, P. Li, Chemistry, bioactivity and geographical diversity of steroidal alkaloids from the Liliaceae family. *Natural product reports* **23**, 735-752 (2006).
40. E. Heftmann, Biogenesis of steroids in solanaceae. *Phytochemistry* **22**, 1843-1860 (1983).
41. S. Chowański *et al.*, A Review of Bioinsecticidal Activity of Solanaceae Alkaloids. *Toxins (Basel)* **8**, 60 (2016).
42. J. P. Munafo Jr, T. J. Gianfagna, Antifungal activity and fungal metabolism of steroidal glycosides of Easter lily (Lilium longiflorum Thunb.) by the plant pathogenic fungus, Botrytis cinerea. *Journal of agricultural and food chemistry* **59**, 5945-5954 (2011).
43. Y. You, J. A. van Kan, Bitter and sweet make tomato hard to (b) eat. *New Phytologist* **230**, 90-100 (2021).
44. S. L. Sinden, L. L. Sanford, W. W. Cantelo, K. L. Deahl, Leptine glycoalkaloids and resistance to the Colorado potato beetle (Coleoptera: Chrysomelidae) in Solanum chacoense. *Environmental Entomology* **15**, 1057-1062 (1986).
45. S. L. Sinden, L. L. Sanford, S. F. Osman, Glycoalkaloids and resistance to the Colorado potato beetle in *Solanum chacoense* Bitter. *American Potato Journal* **57**, 331-343 (1980).
46. H. H. Tai *et al.*, Colorado potato beetle resistance in Solanum oplocense X Solanum tuberosum intercross hybrids and metabolite markers for selection. *American journal of potato research* **92**, 684-696 (2015).
47. H. H. Tai, K. Worrall, Y. Pelletier, D. De Koeyer, L. A. Calhoun, Comparative Metabolite Profiling of Solanum tuberosum against Six Wild Solanum Species with Colorado Potato Beetle Resistance. *Journal of Agricultural and Food Chemistry* **62**, 9043-9055 (2014).
48. J. R. Paudel *et al.*, Genetic mapping of steroidal glycoalkaloids using selective genotyping in potato. *American Journal of Potato Research* **96**, 505-516 (2019).
49. O. Kaup, I. Gräfen, E.-M. Zellermann, R. Eichenlaub, K.-H. Gartemann, Identification of a tomatinase in the tomato-pathogenic actinomycete Clavibacter michiganensis subsp. michiganensis NCPPB382. *Molecular plant-microbe interactions* **18**, 1090-1098 (2005).
50. R. F. Seipke, R. Loria, Streptomyces scabies 87-22 possesses a functional tomatinase. *Journal of bacteriology* **190**, 7684-7692 (2008).
51. J. R. Paudel *et al.*, Pathogen and pest responses are altered due to RNAi-mediated knockdown of GLYCOALKALOID METABOLISM 4 in Solanum tuberosum. *Molecular Plant-Microbe Interactions* **30**, 876-885 (2017).
52. B. Christ, S. Maczuga, The effect of fungicide schedules and inoculum levels on early blight severity and yield of potato. *Plant disease* **73**, 695-698 (1989).
53. J. Rotem, *The genus Alternaria: biology, epidemiology, and pathogenicity* (American Phytopathological Society, 1994).
54. D. Shtienberg, S. Bergeron, A. Nicholson, W. Fry, E. Ewing, Development and evaluation of a general model for yield loss assessment in potatoes. *Phytopathology* **80**, 466-472 (1990).
55. P. J. Wolters *et al.*, Qualitative and Quantitative Resistance against Early Blight Introgressed in Potato. *Biology* **10**, 892 (2021).
56. J. Glazebrook, Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* **43**, 205-227 (2005).

57. V. G. Vleeshouwers, R. P. Oliver, Effectors as tools in disease resistance breeding against biotrophic, hemibiotrophic, and necrotrophic plant pathogens. *Molecular plant-microbe interactions* **27**, 196-206 (2014).
58. J. M. Lorang, T. A. Sweat, T. J. Wolpert, Plant disease susceptibility conferred by a "resistance" gene. *Proceedings of the National Academy of Sciences* **104**, 14861-14866 (2007).
59. E. D. Nagy, J. L. Bennetzen, Pathogen corruption and site-directed recombination at a plant disease resistance gene cluster. *Genome Research* **18**, 1918-1923 (2008).
60. J. D. Faris *et al.*, A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proceedings of the National Academy of Sciences* **107**, 13544-13549 (2010).
61. G. Shi *et al.*, The hijacking of a receptor kinase–driven pathway by a wheat fungal pathogen leads to disease. *Science advances* **2**, e1600822 (2016).
62. D. M. Spooner, M. Ghislain, R. Simon, S. H. Jansky, T. Gavrilenko, Systematics, diversity, genetics, and evolution of wild and cultivated potatoes. *The Botanical Review* **80**, 283-383 (2014).
63. Y. Torres Ascurra, X. Lin, P. J. Wolters, V. G. Vleeshouwers, "Identification of Solanum Immune Receptors by Bulked Segregant RNA-Seq and High-Throughput Recombinant Screening" in Solanum tuberosum. (Springer, 2021), pp. 315-330.
64. P. J. Wolters *et al.*, Gapless Genome Assembly of the Potato and Tomato Early Blight Pathogen Alternaria solani. *Molecular Plant Microbe Interactions* **31**, 692-694 (2018).
65. X. Xu *et al.*, Genome sequence and analysis of the tuber crop potato. *Nature* **475**, 189-195 (2011).
66. N. van Lieshout *et al.*, Solyntus, the New Highly Contiguous Reference Genome for Potato (Solanum tuberosum). *G3 Genes|Genomes|Genetics* **10**, 3489-3495 (2020).
67. R. Aversano *et al.*, The Solanum commersonii Genome Sequence Provides Insights into Adaptation to Stress Conditions and Genome Evolution of Wild Potato Relatives. *The Plant Cell* **27**, 954 (2015).
68. R. C. Martin, M. C. Mok, D. W. Mok, Isolation of a cytokinin gene, ZOG1, encoding zeatin O-glucosyltransferase from Phaseolus lunatus. *Proceedings of the National Academy of Sciences* **96**, 284-289 (1999).
69. R. C. Martin, M. C. Mok, D. W. Mok, A gene encoding the cytokinin enzyme zeatin O-xylosyltransferase of Phaseolus vulgaris. *Plant Physiology* **120**, 553-558 (1999).
70. M. C. Mok *et al.*, Topolins and hydroxylated thidiazuron derivatives are substrates of cytokinin O-glucosyltransferase with position specificity related to receptor recognition. *Plant Physiology* **137**, 1057-1066 (2005).
71. M. Itkin *et al.*, Biosynthesis of antinutritional alkaloids in solanaceous crops is mediated by clustered genes. *Science* **341**, 175-179 (2013).
72. G. R. Lazo, P. A. Stein, R. A. Ludwig, A DNA Transformation–Competent Arabidopsis Genomic Library in Agrobacterium. *Bio/Technology* **9**, 963-967 (1991).
73. J. Amselem *et al.*, Genomic Analysis of the Necrotrophic Fungal Pathogens Sclerotinia sclerotiorum and Botrytis cinerea. *PLOS Genetics* **7**, e1002230 (2011).
74. M. Friedman, Potato Glycoalkaloids and Metabolites: Roles in the Plant and in the Diet. *Journal of Agricultural and Food Chemistry* **54**, 8655-8681 (2006).
75. M. Distl, M. Wink, Identification and quantification of steroidal alkaloids from wild tuber-bearing Solanum species by HPLC and LC-ESI-MS. *Potato Research* **52**, 79-104 (2009).
76. A. Vázquez, G. González, F. Ferreira, P. Moyna, L. Kenne, Glycoalkaloids of Solanum commersonii Dun. ex Poir. *Euphytica* **95**, 195-201 (1997).
77. I. Ginzberg, J. G. Tokuhisa, R. E. Veilleux, Potato steroidal glycoalkaloids: biosynthesis and genetic manipulation. *Potato Research* **52**, 1-15 (2009).
78. B. Sagredo, N. Balbyshev, A. Lafta, H. Casper, J. Lorenzen, A QTL that confers resistance to Colorado potato beetle (Leptinotarsa decemlineata [Say]) in tetraploid potato populations segregating for leptine. *Theoretical and applied genetics* **119**, 1171-1181 (2009).
79. P. D. Cárdenas *et al.*, The bitter side of the nightshades: Genomics drives discovery in Solanaceae steroidal alkaloid metabolism. *Phytochemistry* **113**, 24-32 (2015).
80. E. Eich, *Solanaceae and Convolvulaceae: Secondary metabolites: Biosynthesis, chemotaxonomy, biological and economic significance (a handbook)* (Springer Science & Business Media, 2008).
81. J. G. Roddick, "Steroidal Glycoalkaloids: Nature and Consequences of Bioactivity" in Saponins Used in Traditional and Modern Medicine, G. R. Waller, K. Yamasaki, Eds. (Springer US, Boston, MA, 1996), 10.1007/978-1-4899-1367-8_25, pp. 277-295.
82. J. P. Valkonen, M. Keskitalo, T. Vasara, L. Pietilä, K. Raman, Potato glycoalkaloids: a burden or a blessing? *Critical reviews in plant sciences* **15**, 1-20 (1996).
83. E. P. o. C. i. t. F. Chain *et al.*, Risk assessment of glycoalkaloids in feed and food, in particular in potatoes and potato-derived products. *EFSA Journal* **18**, e06222 (2020).
84. L. C. Dolan, R. A. Matulka, G. A. Burdock, Naturally Occurring Food Toxins. *Toxins (Basel)* **2**, 2289-2332 (2010).
85. M. Nakayasu *et al.*, Identification of α-tomatine 23-hydroxylase involved in the detoxification of a bitter glycoalkaloid. *Plant and Cell Physiology* **61**, 21-28 (2020).
86. J. Szymański *et al.*, Analysis of wild tomato introgression lines elucidates the genetic basis of transcriptome and metabolome variation underlying fruit traits and pathogen response. *Nature Genetics* **52**, 1111-1121 (2020).
87. J. Roddick, The steroidal glycoalkaloid α-tomatine. *Phytochemistry* **13**, 9-25 (1974).
88. B. C. Campbell, S. S. Duffey, Tomatine and parasitic wasps: potential incompatibility of plant antibiosis with biological control. *Science* **205**, 700-702 (1979).
89. R. W. Sandrock, H. D. VanEtten, Fungal sensitivity to and enzymatic degradation of the phytoanticipin α-tomatine. *Phytopathology* **88**, 137-143 (1998).

90. B. Ökmen *et al.*, Detoxification of α-tomatine by C ladosporium fulvum is required for full virulence on tomato. *New Phytologist* **198**, 1203-1214 (2013).
91. A. Osbourn, P. Bowyer, P. Lunness, B. Clarke, M. Daniels, Fungal pathogens of oat roots and tomato leaves employ closely related enzymes to detoxify different host plant saponins. *MPMI-Molecular Plant Microbe Interactions* **8**, 971-978 (1995).
92. P. Bowyer, B. R. Clarke, P. Lunness, M. J. Daniels, A. E. Osbourn, Host range of a plant pathogenic fungus determined by a saponin detoxifying enzyme. *Science* **267**, 371-374 (1995).
93. S.-i. Ito *et al.*, Tomatidine and lycotetraose, hydrolysis products of α-tomatine by Fusarium oxysporum tomatinase, suppress induced defense responses in tomato cells. *FEBS Letters* **571**, 31-34 (2004).
94. K. Bouarab, R. Melton, J. Peart, D. Baulcombe, A. Osbourn, A saponin-detoxifying enzyme mediates suppression of plant defences. *Nature* **418**, 889-892 (2002).
95. R. Shakya, D. A. Navarre, LC-MS Analysis of Solanidane Glycoalkaloid Diversity among Tubers of Four Wild Potato Species and Three Cultivars (Solanum tuberosum). *Journal of Agricultural and Food Chemistry* **56**, 6949-6958 (2008).
96. X. Qi *et al.*, A gene cluster for secondary metabolism in oat: implications for the evolution of metabolic diversity in plants. *Proceedings of the National Academy of Sciences* **101**, 8233-8238 (2004).
97. H.-W. Nützmann, A. Osbourn, Gene clustering in plant specialized metabolism. *Current opinion in biotechnology* **26**, 91-99 (2014).
98. H. W. Nützmann, A. Huang, A. Osbourn, Plant metabolic clusters–from genetics to genomics. *New phytologist* **211**, 771-789 (2016).
99. R. R. Wick, L. M. Judd, C. L. Gorrie, K. E. Holt, Completing bacterial genome assemblies with multiplex MinION sequencing. *Microbial genomics* **3** (2017).
100. H. Li, Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* **34**, 3094-3100 (2018).
101. R. Vaser, I. Sović, N. Nagarajan, M. Šikić, Fast and accurate de novo genome assembly from long uncorrected reads. *Genome Res* **27**, 737-746 (2017).
102. H. Li, Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv preprint arXiv:1303.3997* (2013).
103. J. T. Robinson *et al.*, Integrative genomics viewer. *Nature Biotechnology* **29**, 24-26 (2011).
104. A. R. Quinlan, I. M. Hall, BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841-842 (2010).
105. A. Gurevich, V. Saveliev, N. Vyahhi, G. Tesler, QUAST: quality assessment tool for genome assemblies. *Bioinformatics* **29**, 1072-1075 (2013).
106. E. Domazakis *et al.*, Effectoromics-Based Identification of Cell Surface Receptors in Potato. *Methods in Molecular Biology* **1578**, 337-353 (2017).
107. RStudio Team (2020) RStudio: Integrated Development for R. RStudio, PBC, Boston. (Boston, MA).
108. R Core Team (2020) R: A Language and Environment for Statistical Computing. (Vienna, Austria).
109. H. Wickham *et al.*, Welcome to the Tidyverse. *Journal of open source software* **4**, 1686 (2019).
110. H. Wickham, *ggplot2: Elegant Graphics for Data Analysis* (Springer-Verlag New York, 2016).
111. F. Hahne, R. Ivanek, "Visualizing Genomic Data Using Gviz and Bioconductor" in Statistical Genomics: Methods and Protocols, E. Mathé, S. Davis, Eds. (Springer New York, New York, NY, 2016), 10.1007/978-1-4939-3578-9_16, pp. 335-351.