# The molecular basis of the plant-pathogen interaction of potato and *Rhizoctonia solani*

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von
Franziska Genzel

Präsidentin der Humboldt-Universität zu Berlin
Prof. Dr.-Ing. Dr. Sabine Kunst
Dekan der Lebenswissenschaftlichen Fakultät
Prof. Dr. Bernhard Grimm

Gutachter/innen: 1. Prof. Dr. Philipp Franken
2. Prof. Dr. Carmen Büttner
3. Prof. Dr. Frederik Börnke

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## ZUSAMMENFASSUNG

Die Kartoffel ist eines der wichtigsten Nahrungsmittel, Futtermittel sowie Industrierohstoffe der Welt. Wie bei zahlreichen Kulturpflanzen wird auch die Produktion der Kartoffel von verschiedenen Schaderregern beeinträchtigt. Zu diesen zählt der bodenbürtige pilzliche Erreger *Rhizoctonia solani* Kühn, der in nahezu allen Kartoffelanbaugebieten auftritt. Die an Kartoffeln häufigste Anastomosegruppe (AG) 3 verursacht Auflaufschäden und Nekrosen an den Stängeln und Stolonen. Das Auftreten von deformierten Knollen und ein starker Befall der Knollen mit Sklerotien vermindern Qualität und Ertrag, da diese Knollen nicht handelsfähig sind. Die bodenbürtige Herkunft und die Bildung von melanisierten Sklerotien begrenzen die Wirksamkeit chemischer Pflanzenschutzmittel. Deshalb sind alternative Bekämpfungsmaßnahmen dringend notwendig. Der Anbau resistenter Sorten stellt eine effektive Bekämpfungsmaßnahme dar. Solche Sorten stehen jedoch nicht zur Verfügung. Unterschiede im Grad der Anfälligkeit von Sorten gegenüber *R. solani* AG3PT wurden dagegen häufig beschrieben. Ziel dieser Arbeit war es daher, Merkmale aufzufinden, die mit einer erhöhten Feldresistenz der Kartoffelsorte gegenüber diesem Pathogen korrelieren und in Zukunft als Marker in der Züchtung zur Einschätzung des Resistenzgrads von Sorten genutzt werden können.

Auf der Grundlage von in Zusammenarbeit mit Züchtern durchgeführten Feldversuchen wurden zwei Kartoffelgenotypen mit einem unterschiedlichen Grad der Feldresistenz gegenüber *R. solani* AG3PT für vergleichende molekularbiologische und biochemische Analysen ausgewählt. Die Bewertung der Anfälligkeit beruht auf der Bonitur des prozentualen Besatzes der Ernteknollen mit Sklerotien von *R. solani* AG3PT. Darauf basierend wurden die Hypothesen aufgestellt, dass der unterschiedliche Grad in der Anfälligkeit im Feld mit der Ausbreitung von *R. solani* AG3PT in der Pflanze korreliert, also mit deren Resistenz, und dass diese mit der pflanzlichen Abwehrreaktion gegenüber dem Pilz korreliert. Daher wurden sieben in der Literatur beschriebene Gene, die mit der generellen Abwehrreaktion der Pflanze gegenüber pilzlichen Pathogenen verbunden sind, ausgewählt und deren Expressionsniveau im Pflanzengewebe der Kartoffel in Reaktion auf *R. solani* AG3PT auf Transkript- und zum Teil auch auf Proteinebene untersucht. Die Expression der ausgewählten Gene [Pathogenese-Proteine (*PR-1*, *PR-2*, *PR-3*, *PR-10*), Glutathion-S-Transferase (*GST*), Phenylalanin-Ammoniak-Lyase (*PAL*), Proteinase-Inhibitor 2 (*PI2*)] wird über die Phytohormone Salizylsäure (SA) bzw. Jasmonatsäure/Ethylen (JA/ET) reguliert. Dabei wurde die dritte Hypothese der Arbeit überprüft, dass eine Infektion mit einem nekrotrophen Erreger wie *R. solani* AG3PT insbesondere mit dem JA/ET-Regulationsweg assoziiert ist. Zunächst sollte aber geklärt werden, ob die unterschiedliche Anfälligkeit der ausgewählten Sorten mit der Besiedlung durch *R. solani* AG3PT einhergeht.

Deshalb wurde eine quantitative PCR zur Bestimmung der Erregerdichte im Gewebe der Kartoffel etabliert. In einer vergleichenden Analyse wurde des Weiteren die Frage beantwortet, ob das Expressionsniveau der ausgewählten Abwehrgene bei Vergleich der Sorten mit unterschiedlichem Besiedlungsgrad von Spross und Wurzel mit *R. solani* AG3PT korreliert. Aufgrund ihrer Bedeutung für die Pflanzenabwehr wurde zusätzlich die Analyse des Gehaltes an ausgewählten pflanzlichen Sekundärmetaboliten (Carotinoide, Glykoalkaloide, Phenylpropanoide) in Reaktion auf einen Befall mit *R. solani* AG3PT in die vergleichenden Untersuchungen der beiden Sorten einbezogen.

Die Ergebnisse der Untersuchungen zeigen, dass nicht nur die Keime sondern auch die Wurzeln nach Inokulation der primären Keime mit *R. solani* AG3PT sehr schnell von dem Erreger besiedelt werden, dieser sich jedoch in der wachsenden Wurzel nicht weiter extensiv ausbreitet. Der Vergleich der Erregerdichte in den pflanzlichen Geweben der beiden Sorten ergab, dass die weniger empfindliche Sorte in geringerer Dichte mit *R. solani* AG3PT besiedelt wird. Diese zeigte also eine höhere Resistenz. Eine verstärkte Expression der ausgewählten Abwehrgene wurde in Reaktion auf eine Infektion mit *R. solani* AG3PT in Spross und Wurzel beobachtet. Die Untersuchungen ergaben dabei, dass die Verteidigungsreaktion der Pflanze auf die Infektion mit *R. solani* AG3PT sowohl über den JA/ET- als auch über den SA-Weg reguliert wird. Bei dem Vergleich des Expressionsniveaus der Sorten mit unterschiedlichem Anfälligkeitsgrad bzw. unterschiedlicher Resistenz gegenüber *R. solani* AG3PT zeigte die resistentere Sorte eine höhere konstitutive Expression abwehrassoziierter Gene im Vergleich zur anfälligeren Sorte. Im Gegensatz zur empfindlicheren Sorte führte die Infektion mit *R. solani* AG3PT in der resistenteren Sorte zu keiner signifikanten Erhöhung des Expressionsniveaus der untersuchten Abwehrgene (*PR-1*, *PR-3*, *PR-10*, *GST*, *PAL*, *PI2*). Interessanterweise wurden in der resistenteren Sorte höhere Gehalte an den Glykoalkaloiden α-Chaconin und α-Solanin sowie dem Flavonol Nicotiflorin im Spross- und Wurzelgewebe nachgewiesen. *In vitro* Untersuchungen zeigten, dass die genannten Sekundärmetabolite das Myzelwachstum von *R. solani* AG3PT hemmen. Es ist daher davon auszugehen, dass die Glykoalkaloide α-Chaconin und α-Solanin sowie das Flavonol Nicotiflorin ebenfalls von Bedeutung für die Pathogenabwehr sind.

Es kann daher geschlussfolgert werden, dass die geringere Empfindlichkeit mit einer quantitativ höheren Resistenz der Kartoffel gegenüber *R. solani* AG3PT und diese mit einer höheren, präformierten pflanzlichen Immunabwehr korreliert ist. Die erhöhte Expression bestimmter pflanzlicher Abwehrgene und die erhöhte konstitutive Synthese pflanzlicher Abwehrmetabolite resultiert in einer geringeren Besiedlung der Pflanze durch das Pathogen und trägt somit vermutlich auch zu einer geringeren Symptomausprägung im Feld bei. Die zur präformierten Abwehr zusätzlich induzierte Verteidigungsreaktion der Pflanze wird sowohl über den JA/ET- als auch über den SA-Weg reguliert.

## SUMMARY

Potato is one of the most important food crops, feeds, and industrial raw materials worldwide. Similar to many other crops, potato production is impaired by various pests and pathogens. Among these is *Rhizoctonia solani* Kühn, a soil-borne fungal pathogen which occurs in almost all potato production areas. The main potato-infecting anastomosis group (AG) 3 delays plant emergence and causes necrotic lesions on stems and stolons. Deformation of tubers and severe infestation of tubers with sclerotia considerably reduce quality and yield because these tubers are not marketable. The control of this pathogen is hampered due to the soil-borne origin, the formation of melanised sclerotia, and the limited efficacy of chemical treatments which can lead to an accumulation of *R. solani* AG3PT in the soil over the long term. Therefore, alternative control strategies are urgently necessary. The use of resistant cultivars represents an effective and environmentally friendly alternative. However, resistant cultivars are not available. Nevertheless, differences in the degree of resistance of potato cultivars to *R. solani* AG3PT have been repeatedly observed in the field. Thus, it was aimed to reveal traits correlating with an increased field resistance of potato to *R. solani* AG3PT which can be used as markers in future breeding programs to evaluate the degree of a cultivar's resistance to this pathogen.

Based on field experiments carried out in cooperation with potato breeders, two potato genotypes showing different levels of field resistance against *R. solani* AG3PT were selected for comparative molecular and biochemical analyses. The evaluation of the degree of resistance was based on the assessment of the percentage of tuber skin covered with sclerotia (black scurf severity). Differences between the selected cultivars regarding the level of resistance to *R. solani* AG3PT were verified in additional field experiments. On this basis, two hypotheses of the thesis are that differences in the degree of resistance in the field are correlated with the colonisation of the plant by the pathogen, in other words with the plant's resistance, and that this resistance correlates with the defence response of the plant to the fungus. Therefore, seven genes known to be involved in defence responses of potato to fungal pathogens were selected and their expression was analysed, at transcript and partly at protein level, in potato tissue following infection with *R. solani* AG3PT. The expression of the selected genes [pathogenesis-related proteins (*PR-1*, *PR-2*, *PR-3*, *PR-10*), glutathione S-transferase (*GST*), phenylalanine ammonia-lyase (*PAL*), proteinase inhibitor 2 (*PI2*)] is regulated by the phytohormones salicylic acid (SA) or ethylene/jasmonic acid (ET/JA). In this context the third hypothesis which states that an infection with a necrotrophic pathogen particularly induces the JA/ET-associated pathway was tested. At first, it was aimed to investigate whether differences in the degree of resistance of the selected cultivars are accompanied by colonisation with *R. solani* AG3PT. Thus, a quantitative real-time PCR

(qPCR) was established to quantify the fungal density in plant tissue. In comparative analyses it was investigated whether the two potato genotypes differ regarding the expression level of the selected defence-related genes and whether this expression level is correlated with the pathogen density determined in roots and sprouts. Since plant secondary metabolites are important key players in plant defence, comparative analyses regarding the contents of selected metabolites (carotenoids, glycoalkaloids, phenylpropanoids) in both cultivars in response to an infection with *R. solani* AG3PT were included.

Results show that, in addition to sprouts, roots are quickly colonised by *R. solani* AG3PT following inoculation of the primary sprout. However, the fungus did not further spread within the growing root. Moreover, quantification of pathogen DNA in plant tissue revealed a significantly lower pathogen density in the more resistant cultivar. Thus, this cultivar showed a higher resistance to *R. solani* AG3PT. An increased expression of the selected defence genes following infection with the pathogen was determined in sprouts as well as in roots. Analyses revealed that defence responses of the plant against *R. solani* AG3PT are regulated by the JA/ET- as well as SA-pathway. Comparing the two cultivars with different degrees in field resistance to *R. solani* AG3PT revealed a significantly higher constitutive expression of the analysed defence-related genes in the more resistant cultivar compared to the less resistant cultivar. In contrast to the less resistant cultivar, infection with the pathogen did not result in a significant up-regulation of defence-related genes (*PR-1*, *PR-3*, *PR-10*, *GST*, *PAL*, *PI2*) in the more resistant cultivar. Interestingly, higher contents of the glycoalkaloids α-chaconine and α-solanine and of the flavonol nicotiflorin were determined in roots and shoots of this cultivar. *In vitro* culture studies show that these secondary plant metabolites reduce mycelial growth of *R. solani* AG3PT which implicates their involvement in pathogen defence in potato.

This leads to the conclusion that a lower *Rhizoctonia* disease severity is correlated to a higher quantitative resistance of potato to *R. solani* AG3PT which is accompanied by a higher preformed expression of defence. The increased expression of certain defence-related genes and the increased constitutive biosynthesis of defensive plant metabolites result in a reduced colonisation of the potato by the pathogen and may thus presumably contribute to a decreased disease severity in the field. The defence reaction of the plant, which was additionally induced upon infection with the pathogen, is regulated by the JA/ET- as well as the SA-pathway.

## ABSTRACT

Potato is the fourth most important food crop worldwide. However, it is also target of many pests and microbial pathogens including the fungus *Rhizoctonia solani* Kühn. The infection of potato with this pathogen leads to considerable economic losses, primarily caused by reduction of tuber quality. The soil-borne nature, the formation of melanised sclerotia, and the limited efficacy of fungicides impair the control of this pathogen and strengthen the necessity for alternative control measures. A very effective alternative is the use of resistant cultivars. Quantitative differences in the degree of resistance of potato to *R. solani* AG3PT have been repeatedly observed in the field. However, until now there is no information available regarding the underlying mechanisms contributing to the resistance level. This thesis aimed at revealing mechanisms in potato which contribute to the manifestation of a certain degree of field resistance to *R. solani* AG3PT. Based on the screening of various potato genotypes in field trials performed in cooperation with potato breeders, two potato cultivars distinctly differing in the level of resistance to *R. solani* AG3PT were selected for further molecular and biochemical analyses. The cultivar with a higher degree of resistance showed higher constitutive expression of defence-related genes. In contrast to the less resistant cultivar, no distinct increase of the defence-gene expression was detectable upon pathogen infection in this cultivar. Moreover, contents of the glycoalkaloids α-chaconine and α-solanine and of the flavonol nicotiflorin were higher compared to the less resistant cultivar. Using *in vitro* culture tests, a growth-reducing effect of these compounds on *R. solani* AG3PT was confirmed. Concluding, a higher resistance of potato cultivars to *R. solani* AG3PT seems to be related to a higher expression level of defence-related genes and to a higher content of plant secondary metabolites. This enhanced constitutive defence level resulted in a lower pathogen colonisation of the plant, thus contributing to a reduced disease severity in the field.

CONTENTS

## FIGURES

## TABLES

## ABBREVIATIONS

| | |
|---|---|
| 18S | 18S ribosomal RNA |
| ABA | abscisic acid |
| ABAR | ABA receptor |
| ACT | actin |
| AG | anastomosis group |
| AG3PT | anastomosis group 3 potato |
| ANOVA | analysis of variance |
| APCI | atmospheric-pressure chemical ionization |
| APRT | adenine phosphoribosyltransferase |
| *Avr* | avirulence gene |
| Ben3 | *Rhizoctonia solani* AG3PT isolate used in these studies |
| bp | base pair |
| BSA | bovine serum albumin |
| cDNA | complementary DNA |
| $C_q$ | quantification cycle (synonym to threshold cycle) |
| $C_t$ | threshold cycle |
| CTAB | hexadecyl-trimethyl-ammonium bromide |
| CV | coefficient of variation on the normalised relative quantities |
| CYC | cyclophilin |
| DNA | desoxyribonucleic acid |
| dNTP | deoxynucleotide |
| dpi | days post inoculation |
| DTT | dithiothreitol |
| DW | dry weight |
| E | efficiency |
| EC | electrical conductivity |
| EDTA | ethylenediaminetetraacetic acid |
| *TEF1* | name of gene encoding elongation factor 1-α |
| ET | ethylene |
| ETI | effector-triggered immunity |
| FA | formic acid |
| FM | fresh mass |
| for | forward |

| | |
|---|---|
| gDNA | genomic DNA |
| GST | glutathione S-transferase |
| hoi | hours of incubation |
| HPLC | high-performance liquid chromatography |
| HR | hypersensitive response |
| HST | host-specific toxin |
| IGZ | Leibniz-Institute of Vegetable and Ornamental Crops |
| ISR | induced systemic resistance |
| ITS | internal transcribed spacer |
| JA | jasmonic acid |
| kb | kilobase |
| L2 | cytoplasmatic ribosomal protein L2 |
| M value | measure of the gene stability |
| MAPK | mitogen-activated protein kinase |
| MS | mass spectrometry |
| m/z | mass-to-charge ratio |
| NB-LRR | nucleotide-binding leucine rich repeat |
| NCBI | National Center for Biotechnology Information |
| NCED | 9-*cis*-epoxy-carotenoid dioxygenase |
| OD | optical density |
| PAL | phenylalanine ammonia-lyase |
| PAMP | pathogen-associated molecular pattern |
| PCR | polymerase chain reaction |
| PDA | potato dextrose agar |
| PI2 | proteinase-inhibitor II |
| PR | pathogenesis-related |
| PSY | phytoene synthase |
| PTFE | polytetrafluoroethylene |
| PTI | PAMP-triggered immunity |
| PVDF | polyvinylidene fluoride |
| qPCR | quantitative real-time polymerase chain reaction |
| qRT-PCR | quantitative real-time reverse-transcription polymerase chain reaction |
| *R* gene | resistance gene |
| rev | reverse |

| | |
|---|---|
| RNA | ribonucleic acid |
| ROS | reactive oxygen species |
| RQ | relative quantity |
| RT | reverse transcriptase |
| RT | retention time |
| SA | salicylic acid |
| SAR | systemic acquired resistance |
| SDS | sodium dodecyl sulphate |
| SDS–PAGE | SDS polyacrylamide gel electrophoresis |
| Seq | sequencing |
| SGT3 | β-solanine/ β-chaconine rhamnosyl-transferase |
| SQS | squalene synthase |
| *Taq* polymerase | *Thermphilus aquaticus* polymerase |
| TBE | Tris/Borate/EDTA |
| TBS | Tris buffered saline |
| TEMED | Tetramethylethylenediamine |
| ToF-MS | time-of-flight mass spectrometry |
| Tris | Tris(hydroxymethyl)aminomethane |
| TUB-B | β-tubulin |
| UHPLC-DAD | Ultrahigh-performance liquid chromatograph diode array detection |
| UV | ultra violet |
| wpi | weeks post inoculation |

# 1. INTRODUCTION

## 1.1 Potato crop

*Origin*

Potato (*Solanum tuberosum* L.) belongs to the family of *Solanaceae*, genus *Solanum* L. and was already domesticated over 10 000 years ago in the Andes in South America (Ames and Spooner, 2008). The potato was introduced into Spain in the 16$^{th}$ century from where it spread over other European countries (Ruiz de Galarreta et al., 2006; Massard, 2009). The question concerning the exact origin of European potatoes has been controversially discussed for a long time. Several researchers proposed that the European potato originated from Chilean landraces while others claimed the Andes to be the place of origin (Ames and Spooner, 2008). Investigations of Ames and Spooner (2008) on plastid 241-bp deletion markers from historical herbarium specimens encourage the hypothesis of an Andean origin. The landraces of the Andes can be distinguished from landraces of Chile by means of morphology, cytoplasmic sterility aspects, microsatellite markers, 241-bp deletion in the intergenic region of a plastid DNA molecule, and by tuberisation behaviour. Chilean landraces tuberise under long-day conditions, while Andean landraces tuberise under short-day conditions (Ames and Spooner, 2008). Today, commercially cultivated potatoes are combined under the name *Solanum tuberosum*. The taxonomy of *Solanum* species is still controversially discussed. According to Hawkes (1990) a total of seven cultivated species exists, including *Solanum tuberosum*, with additional 219 wild tuber-bearing species. However, Spooner et al. (2005) suggested that the domestication of a group from the wild species complex of *Solanum brevicaule* resulted in diploid *Solanum stenotomum* (*S. tuberosum*) from which cultivars like diploid *Solanum phureja*, tetraploid *S. tuberosum* subsp. *Andigena,* and tetraploid *S. tuberosum* subsp. *tuberosum* originated. Several cytotypes (di-, tri-, tetra- and pentaploid) of cultivated potato species have been described (Huamán and Spooner, 2002). The modern, high yielding cultivars grown worldwide are tetraploid (Huamán and Spooner, 2002). Still, wild species of potato show a much higher biodiversity than commercially grown cultivars (Camire et al., 2009) and are thus a valuable source for breeding programs.

*The potato plant characteristics*

The life cycle of a potato consists of various developmental stages, from sprouting of tubers and emergence of plants, over stolon formation and induction of tuberisation, until tuber development and dormancy (Fig. 1.1, BBCH scale). These processes entail large changes in physiology and metabolism, which are regulated by numerous interacting genes and hormones (Bachem et al., 2000). During growth of the tubers, starch and storage proteins

are accumulated while the general metabolic activity decreases (Lehesranta et al., 2006). With the break of tuber dormancy, reserves are mobilised and subsequently photosynthesis is activated (Liu et al., 2015). The tuberisation processes are controlled by environmental and genetic factors. Within the genus *Solanum*, a great variability exists concerning the tuberisation behaviour in reaction to specific environmental conditions (van den Berg et al., 1996). Consequently, cultivars can be organised in maturity classes, according to the number of days needed from planting until tuber maturity (Camire et al., 2009). The tubers reach their full maturity when the aboveground plant is already in the stage of senescence (Fig. 1, BBCH stage 9).

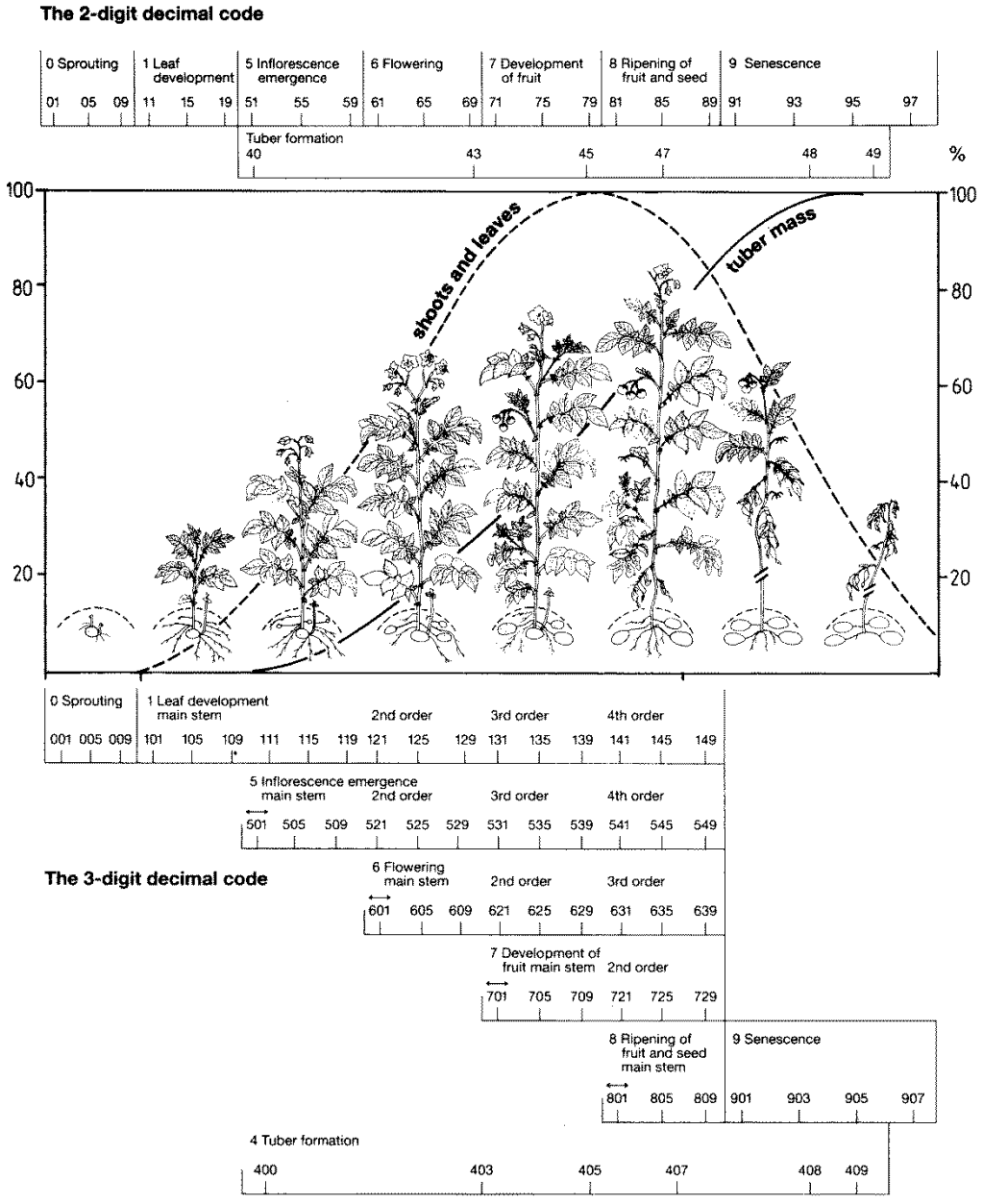


**Figure 1:** BBCH scale for *Solanum tuberosum* L. (Hack et al., 1993).

*Value and consumption*

Although the potato was already introduced into Europe in the 16th century, it was still occasionally rejected by the population in some European countries in the 18th century (Ames and Spooner, 2008). Today, potato is the fourth most important food crop worldwide (Fiers et al., 2012) and has an increasing importance, in China, for instance (Thompson et al., 2009). Considering world food security, the potato's role in the fight against hunger in developing countries increases considerably and replaces grain-based food sources in cases of unavailability or unaffordability of rice, corn or wheat (Camire et al., 2009). Over the last 16 years a slight decrease of potato growing areas (ha) in European countries has been observed, while there was an increase recorded for South America, Africa, and Asia (AMI Marktbilanz Kartoffeln 2014/15; Camire et al., 2009). In summary, potato production increases in developing countries and in contrast, decreases in developed countries (Tab. 1).

**Table 1:** World potato production in the years 1991-2007 (http://.fao.org/potato-2008/en/world/index/.html).

| | **1991** | **1993** | **1995** | **1997** | **1999** | **2001** | **2003** | **2005** | **2007** |
|---|---|---|---|---|---|---|---|---|---|
| **Countries** | | | | | **million tonnes** | | | | |
| **Developed** | 183.13 | 199.31 | 177.47 | 174.63 | 165.93 | 166.93 | 160.97 | 159.97 | 159.89 |
| **Developing** | 84.86 | 101.95 | 108.50 | 128.72 | 135.15 | 145.92 | 152.11 | 160.01 | 165.41 |
| **World** | 267.99 | 301.26 | 285.97 | 303.35 | 301.08 | 312.85 | 313.08 | 319.98 | 325.30 |

**Source: FAOSTAT**

The increasing popularity is not only based on the enormous yield per unit growing area in comparison to other crops. Furthermore, the potato has a high nutritional value. It is a remarkable source for carbohydrates, shows a higher content of proteins compared to cereals, and contains important vitamins like ascorbic acid (vitamin C), dehydroascorbic acid (vitamin C), folic acid (vitamin B), and pyridoxine (vitamin B6). The xanthophylls β-carotene and lutein are also present in potato tubers. These derivatives of carotenoids serve as an important vitamin A source (Camire et al., 2009). Carotenoids also act as antioxidants (Boba et al., 2011). Other antioxidants present in potatoes are flavone aglycones, quercetin, and chlorogenic acids. Phenolic antioxidants like flavonoids are known for their role in reducing the risk of cardiovascular diseases in humans (Niggeweg et al., 2004). Moreover, potatoes possess valuable minerals including potassium, phosphorus, and calcium, but also zinc and iron.

Potatoes show a high genetic diversity (Navarre et al., 2011) and there is a great variety of cultivars. These cultivars differ considering amount and composition of vitamins, pigments, and minerals (Buckenhüskes, 2005; Camire et al., 2009) as well as other traits strongly demanded by consumer preferences. These include, for instance, tuber shape, colour of tuber flesh and skin, storage properties and cooking traits (Camire et al., 2009).

*Diseases of potato*

Approximately 40 diseases are known to occur on potato worldwide. Causal agents include bacteria, fungi, viruses, insects, and nematodes (Fiers et al., 2012). In spite of crop protection strategies, losses in potato production amount approximately 39 % (Oerke and Dehne, 2004). Viral diseases mainly cause damage on foliage of potato, although there are some tuber damaging viruses like tobacco rattle virus (TRV) and potato mop-top virus (PMTV) (Fiers et al., 2012). An increasing occurrence of the potato virus Y (PVY), a highly variable virus, has been reported in South Africa (Visser and Bellstedt, 2009). Moreover, various bacteria infect potato plants leading to wilt and rot on plants and tubers (Christ, 1998). Several of these bacteria, like the soft rot causing bacteria *Pectobacterium atrosepticum*, *Pectobacterium carotovorum,* and *Dickeya dadantii,* lead to economically important losses worldwide (Ngadze et al., 2012). However, the most prevalent pathogens on potato are fungi and oomycetes (Muzhinji, 2016). Worldwide occurring fungal potato pathogens are *inter alia Colletotrichum coccodes* (black dot), *Fusarium* spp. (dry rots), *Helminthosporium solani* (silver scurf), *Rhizoctonia solani* (black scurf), *Sclerotinia sclerotinium* (white mold), *Spongospora subterreanea* (powdery scab), and *Verticillium dahliae* (wilt) (Fiers et al., 2012). The oomycete *Phytophthora infestans* is still one of the major disease-causing agents on potato. Moreover, an increasing aggressiveness of this pathogen has been observed in the last 15 years (Landwirtschaftskammer Nordrhein-Westfalen, 2011). Soil-borne diseases can result in damages on tubers or other plant parts, like in the case of *Verticillium* wilt, chloroses and wilting of leafy plant parts (Fiers et al., 2012). Nematodes like the potato cyst nematode represent another important causal agent of soil-borne diseases on potato (Oerke and Dehne, 2004).

Diseases of potato tubers mainly affect quality as they appear as blemishes, rots or galls also leading to tuber deformations (Fiers et al., 2012), but demands regarding the quality of agronomical products increased within the last years. The majority of potatoes offered for sale had been washed, so that quality deficiencies like blemishes or black scurf become more obvious to the consumer. To fulfill consumer demands, potato growers have to reduce the risk of these quality-reducing diseases caused by pathogens. The worldwide occurring natural soil inhabiting fungus *R. solani* can lead to considerable qualitative losses due to

infection of underground organs of potato, which makes it one of the most important soil-borne pathogens on potato (Banville, 1989; Campion et al., 2003, Tsror, 2010).

## 1.2 The genus *Rhizoctonia*

### 1.2.1 Taxonomy

Since the first mentioning of the genus *Rhizoctonia* in 1815 by De Candolle, a multitude of species has been ascribed to this group, mainly based on vegetative characteristics (Garcia et al., 2006). The genus *Rhizoctonia* is a heterogeneous group of filamentous fungi which do not produce asexual spores and are mostly associated with roots of living plants (Garcia et al., 2006). The group is very divers, includes pathogens, saprophytes, and mycorrhizal fungi on orchidiaceous plants and occurs worldwide in cultivated as well as non-cultivated soil (Ogoshi, 1987; Garcia et al., 2006). Initially, classification of *Rhizoctonia* species was based on the texture of sclerotia and the association of mycelium with roots of living plants (Parmeter, 1970). The specificity of classification features has been extended by Ogoshi (1987) to the characteristics: branching near distal septum of young vegetative hyphal cells, formation of septum near the point of origin in the branch, construction of the branch, dolipore septum, absence of clamp connections, absence of conidia, sclerotia without differentiation into rind and medulla, and absence of rhizomorph. Pathogenicity, morphology, and colouration have been additionally used to classify different *Rhizoctonia* species (Garcia et al., 2006). According to Ogoshi (1987), *Rhizoctonia* isolates can be divided into the following three different groups. One group consists of multinucleate fungi like *Rhizoctonia oryzae* and *Rhizoctonia zeae*, teleomorph *Waitea* Warcup and Talbot. Binucleate *Rhizoctonia*, which belong to the teleomorph form *Ceratobasidium* Rogers, build the second group. *Rhizoctonia solani* is member of the third group whose species belong to the teleomorph form *Thanatephorus* Donk.

### 1.2.2 *Rhizoctonia solani* Kühn

*Rhizoctonia solani* Kühn (teleomorph, *Thanatephorus cucumeris*) is a multinucleate fungal pathogen of the phylum *Basidiomycota* with a broad host range. This necrotrophic fungus infects many important crops worldwide (Ogoshi, 1987; Woodhall et al., 2007). It represents a large species complex with many intraspecific groups which can differ considerably regarding pathogenicity, sclerotial morphology, cultural appearance on media, and physiology (Ogoshi, 1987). In earlier studies, these groups were either organised based on the characteristics pathogenicity, sclerotial morphology, cultural appearance, and physiology or on hyphal anastomosis reactions on cultural media. There are four possible reaction types of hyphal anastomosis (C0-C3). In stages C0 and C1, there is no interaction or hardly any

hyphal fusion detectable between the isolates which presumably belong to different anastomosis groups (AG). C2 and C3 however, describe reactions of isolates belonging to the same AG. Somatic incompatibility of genetically distinct isolates within an AG leads to killing reaction, also known as C2. In contrast, C3 describes the case of perfect fusion of isolates which are genetically identical or closely related (Cubeta and Vilgalys, 1997). Since hyphal interactions are affected by various factors like laboratory and nutritional conditions as well as genetic instability, interpretation of anastomosis is hampered and not always reproducible (Cubeta and Vilgalys, 1997). The use of molecular tools to distinguish AGs and sub-groups within an AG however, is less laborious and bears several advantages over the previously described methods (Okubara et al., 2008; Woodhall et al., 2008). The internal transcribed spacer region (ITS) has been suggested as a standard barcode for the discrimination of species, especially in the phylum of *Basidiomycota* (Schoch et al., 2012). In fungi, the ITS region constitutes the spacer regions ITS1 and ITS2, which are situated between the 18S and 25S genes of the eukaryotic ribosomal ribonucleic acid (rRNA) cistron (Bellemain et al., 2010; Schoch et al., 2012). The two ITS regions are separated by the 5.8S gene (Fig. 2).

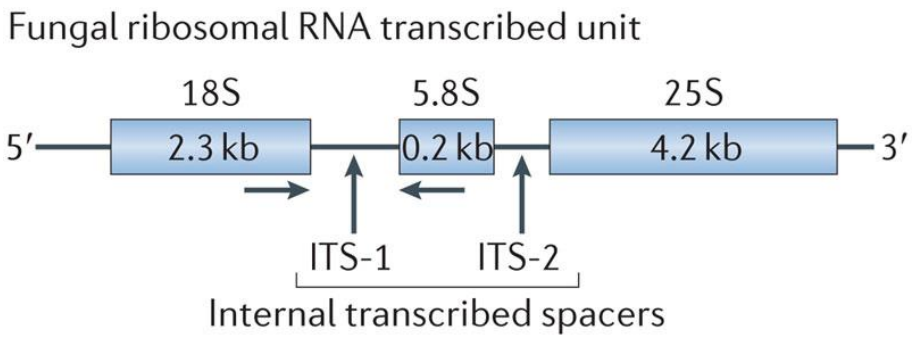


**Figure 2:** Internal transcribed spacer region (Underhill and Iliev, 2014).

The intra-specific diversity of ITS regions and of restriction sites of nuclear 5.8S ribosomal DNA is suitable for the identification of sub-groups, also called intra-specific groups, in *R. solani* (Kuninaga et al., 2000). Thus, the two phylogenetic groups AG3 potato (PT) and AG3 tobacco (TB) within *R. solani* AG3 were identified (Ceresini et al., 2002). Additionally, molecular tools like real-time PCR provide the possibility of rapid quantification of fungal DNA over a broad range of concentrations at species and AG level (Okubara et al., 2008).

### 1.2.3 *Rhizoctonia* diseases on potato

*Rhizoctonia* diseases on potato occur in all potato growing regions worldwide. An infection of potato with *R. solani* can lead to severe disease symptoms on various underground plant parts (Ceresini et al., 2002, Lehtonen et al., 2009). Shortly after planting, necrosis on emerging sprouts (Fig. 3), also referred to as stem canker, appears and this results in delayed plant emergence as well as reduced number of stems and disturbed nutrient flow (Djébali and Belhassen, 2010). Additionally, the fungus colonises stolons which can compromise the plant's growth considerably (Banville, 1989). The most obvious symptom of *Rhizoctonia* disease appears later in the season as black scurf on tubers (Fig. 4A) when maturing potato tubers are covered with sclerotia (Banville et al., 1996; Woodhall et al., 2007; Atkinson et al., 2011). Other possible quality defects on progeny potatoes are malformations (Fig. 4B) or tubers of heterogeneous size (Hide and Horrocks, 1994). Infection can originate from tuber- and soil-borne inoculum. Carling et al. (1989) observed a significant reduction of tuber yield, delay of plant emergence, and a high incidence of stem canker caused by tuber-borne inoculum. Furthermore, tuber-borne inoculum represents a source for the dispersal of the pathogen over long distances into new growth areas via seed tubers (Ceresini et al., 2002; Tsror and Peretz-Alon, 2005). Yield reduction and occurrence of misshapen tubers are also caused by soil-borne inoculum (Scholte, 1989). Moreover, Tsror and Peretz-Alon (2005) described that the combination of tuber- and soil-borne inoculum increases the severity of disease symptoms. Reduction of marketable tuber yield due to *Rhizoctonia* infection can amount 21–34 % (Banville, 1989). Most of the studied pathogenic isolates have been ascribed to *Rhizoctonia solani*, however, other *Rhizoctonia* species have also been described to be pathogenic (Garcia et al., 2006). For instance, binucleate *Rhizoctonia* isolates (BNR) AG A and AG R caused severe disease symptoms on potato (Muzhinji et al., 2015). Several AGs (2, 3, 4, 5, 7, 8, 9) of *R. solani* are able to colonise and infect potato, although great differences in mode of infection and severity have been observed (Woodhall et al., 2007; Ritchie et al., 2013). Carling et al. (1998) for instance, found an isolate of AG7 originating from Mexico which infected stems, stolons, and tubers of potato. Additionally, AG5 was isolated from stem canker in China (Yang and Wu, 2015). In Australia, isolates of AG4 were found on potato roots and underground stems, where they also produced sclerotia (Balali et al., 1995). Yet, several studies confirm that AG3 potato (PT) is the main *R. solani* sub-group infecting potato worldwide and being responsible for stem canker and black scurf symptoms (Campion et al. 2003; Woodhall et al. 2007; Lehtonen et al., 2008). *R. solani* AG3PT bears the highest ability to form sclerotia (Bains et al., 2002; Campion et al. 2003; Justesen et al. 2003; Woodhall et al., 2007; Lehtonen et al., 2009) and seems to be very specific to potato.

**Figure 3:** Stem canker on emerging potato sprouts caused by *Rhizoctonia solani* AG3PT.

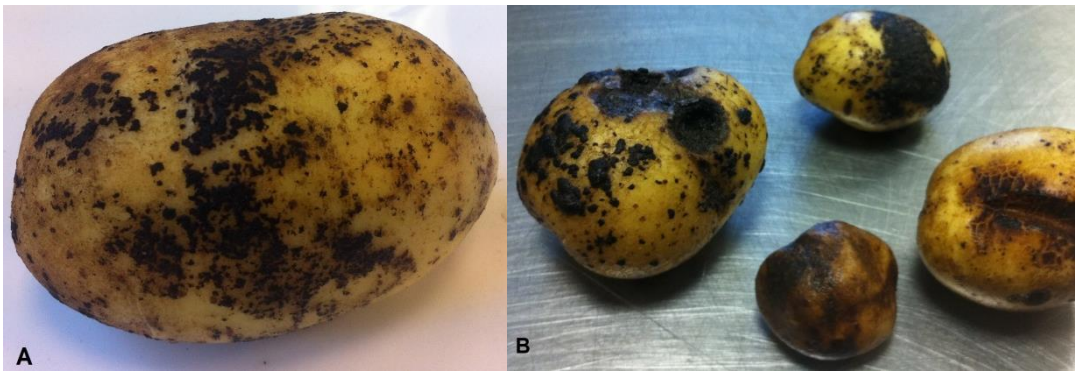


**Figure 4:** Typical black scurf symptom on progeny tubers (A) and deformed progeny tubers (B) caused by *Rhizoctonia solani* AG3PT.

### 1.3 Potato crop protection

In general, the occurrence of diseases is affected by many factors including abiotic and biotic factors as well as cultural practices (Larkin and Honeycutt, 2006; Fiers et al., 2012). Due to a constantly increasing world population, agricultural production has to be raised considerably. Crop protection is a key aspect of an enhanced productivity.

#### 1.3.1 General crop protection strategies

The application of chemicals constitutes a large part in the disease management of potatoes. However, due to environmental concerns and increasing appearance of resistances among the pathogens, the use of chemicals is supposed to be reduced. Other conventional control

strategies include cultural practices like adapted rotation systems, suitable irrigation, fertilisation, and optimised storage conditions (Fiers et al., 2012). An alternative approach of pest management is the use of biological control agents. It is known that potato roots, stolons, and tubers are hosts of an essential microbial community which presents a valuable source for beneficial microorganisms (Diallo et al., 2011). Effects arising from these microorganisms can result *inter alia* from nutrient and spatial competition, mycoparasitism or induced systemic resistance (ISR). For instance, non-pathogenic *Streptomyces* spp., which were isolated from scab lesions of potato tubers, reduced probability of severe disease caused by pathogenic *Streptomyces turgidiscabies* under greenhouse conditions (Hiltunen et al., 2009). Furthermore, *in vitro*-cultured plants colonised by the endophytic bacteria *Pseudomonas* sp. and *Methylobacterium* sp. showed a reduced foliar disease development after challenging them with *P. atrosepticum* than non-colonised plants (Pavlo et al., 2011). *Talaromyces flavus*, a soil-borne fungus with antagonistic activity, reduced potato wilting caused by *Verticillium albo-atrum* in the field (Naraghi et al., 2010). The most efficient alternative control strategy is still the use of resistant cultivars. Potato cultivars show a wide range of resistance against various types of pathogens and pests. However, most of the commercially cultivated potatoes outside South America show a limited genetic base and high susceptibility to numerous pests (The Potato Genome Sequencing Consortium, 2011). In contrast, wild potato species possess resistance genes against a high number of important pathogens (Jansky, 2003). For instance, species *Solanum chacoense* Bitt., *Solanum commersonii* Dunal ex Poir., and *Solanum yungasense* Hawkes are sources conferring resistance to *Streptomyces scabies* (Thaxt.), known as common scab on potato (Sedláková, 2013). Thus, wild species of *Solanum* serve as a valuable source of disease resistance genes (Fiers et al., 2012). *Solanum* hybrids of crosses from *S. tuberosum* Gp. tuberosum with wild species showed higher resistance to *Erwinia carotovora subsp. atroseptica* than the commonly used cultivars ‘Atlantic’, ‘Russet Norkotah’, and ‘Russet Burbank’ which also possess moderate levels of resistance (Jansky, 2003).

### 1.3.2 Specific crop protection strategies against *Rhizoctonia* diseases

Plant protection measures of *Rhizoctonia* diseases include cultural practices and chemical treatment (Gallou, 2011; Zhang et al., 2014). However, the primary control method used against this pathogen is the application of fungicides (Campion et al., 2003). A broad range of chemicals like tolclofos-methyl, flutolanil, azoxystrobin, metam sodium or pencycuron is available (Ratgeber Landesbetrieb Landwirtschaft Hessen, Fachinformation-Pflanzenproduktion 1/2015). Yet, there is a high variation in sensitivity within *Rhizoctonia* species and within the species complex of *R. solani* to fungicides, so that efficacy of the treatment is highly variable (Campion et al., 2003).

Alternative control measures include for instance the application of biological antagonists. Several biological antagonists have already been investigated regarding their efficacy in suppressing *R. solani* diseases. During *in vitro* culture experiments, *Bacillus* strains inhibited growth of AG2, AG4, AG5, and AG7, while there was no specific antagonism of the tested *Bacillus* strains detectable against AG3 (Virgen-Calleros et al., 2000). In the field however, Virgen-Calleros et al. (2000) observed no disease reducing effect of any of the tested *Bacillus* strains. In contrast, *Bacillus amyloliquefaciens* FZB42, a constituent of the commercially available biological soil conditioner RhizoVital® 42, significantly reduced bottom rot on lettuce caused by *R. solani* AG1-IB (Chowdhury et al., 2013). The application of *Pythium oligandrum* on seed tubers also significantly reduced disease symptoms on stolons caused by *R. solani* AG3PT in the field (Ikeda et al., 2012). Mycoparasitism, visible as *Pythium oligandrum* hyphae coiling around *R. solani* hyphae, was observed as well. In addition, *Clonostachys rosea* and *Trichoderma* spp. have shown mycoparasitic behaviour in interaction with *R. solani* (Xue, 2003; Elad et al., 1983; Lahlali and Hijri, 2010). Furthermore, isolates of *Trichoderma virens*, *Trichoderma atroviride,* and *Trichoderma barbatum* promoted growth of potato plants and simultaneously reduced disease symptoms on potato stems and stolons in fields inoculated with *R. solani* (Hicks et al., 2014). Several studies reported an influence of *Trichoderma* spp. on defence-associated mechanisms, e.g. the up-regulation of pathogenesis-related proteins, leading to priming of the plants and thus reduction of disease (Yedidia et al., 2000; Gallou et al., 2009; Singh et al., 2011). Wilson et al. (2008) described a decreased incidence of black scurf due to a combined application of flutolanil and *Trichoderma harzianum* in the field. However, the efficacy varied between different experiments and the total marketable tuber yield was still lower compared to the non-inoculated control.

Pathogen management also includes crop rotation with non-host plants. For instance, green manure from *Brassica* crops used in crop rotation was connected with a reduction of soil-borne diseases like *R. solani* (Larkin et al., 2007). Supposedly, the pathogen-reducing effect of *Brassica* manure is associated with volatile sulphur compounds (glucosinolates and isothiocyanates) and changes in the composition of the soil microbiome (Larkin et al., 2007). Furthermore, Bains et al. (2002) reported that *Beta vulgaris*, *Brassica campestris*, *Hordeum vulgare,* and *Pisum sativum* were not infected by five tested isolates of *R. solani*, and thus suitable for crop rotation with potato. Since *R. solani* AG3 was isolated from roots of various plants (Bains et al., 2002), choice of catch crops and weed control strategies require particular attention.

Despite this variety of control measures, an increasing occurrence of *Rhizoctonia* diseases has been reported by potato growers. *R. solani*, and particularly AG3PT, is among others one causal agent of quality deficiencies which have been increasingly criticised by potato

industry and traders in the last years (Keiser, 2007). Currently available control strategies are not able to efficiently control *Rhizoctonia* diseases on potato. Another important aspect is the formation of completely melanised sclerotia, which persist various environmental conditions over long periods of time, promoting an accumulation of the soil-borne inoculum (Ritchie et al., 2013). The increasing occurrence of this disease and the lack of efficient control strategies strengthen the necessity of new control mechanisms.

The most favourable, environmentally friendly strategy to control *R. solani* is the use of resistant potato genotypes. Cultivars with different degrees of resistance to *R. solani* AG3PT have often been observed, yet, none of these cultivars showed complete resistance (Bains et al., 2002; Daami-Remadi et al., 2008; Djébali and Belhassen, 2010; Thangavel et al., 2014). So far, little is known about the molecular background and the physiological mechanisms underlying quantitative differences in susceptibility against *R. solani* AG3PT. Moreover, there is no method available which allowed rapid evaluation of potato cultivars regarding their degree of disease resistance against this pathogen. Qualitative resistance in potato against the necrotrophic pathogen *R. solani* AG3PT has not been described until now. Therefore, it is assumed that differences in resistance are based on quantitative resistance involving multiple mechanisms of plant defence from expression of pathogenesis-related (PR) proteins to biosynthesis of secondary plant metabolites which are known for their antimicrobial properties. Finding key players crucial for different degrees of resistance would serve the breeding of cultivars with higher resistance to *R. solani* AG3PT. Presumably, cultivars with a higher degree of resistance to *R. solani* AG3PT possess higher levels of defence-related proteins and plant secondary metabolites.

### 1.4 Mechanisms of plant defence

Since plants are sessile, they have to cope with surrounding environmental conditions, including adaptation to abiotic and biotic stresses. In order to defend themselves against microbial pathogens like bacteria, fungi, viruses as well as insect herbivores, they developed a complex defence system (Thomma et al., 1998; Glazebrook, 2005). Defence mechanisms can either be preformed or induced. Preformed defence mechanisms include physical barriers like the cuticle or the cell wall which prevent microbial invasion of plants (Fu and Dong, 2013). A biochemical barrier is built by constitutively produced peptides, proteins, and secondary plant metabolites (phytoanticipines) which have a repulsive effect on potential pathogens (Heath, 2000). On the one hand, this constitutive defence represents an important component of non-host resistance. On the other hand, it can result in considerable fitness costs due to reallocation of energy sources from growth to defence in the absence of stress (Huot et al., 2014). Due to pathogen attack an array of defence responses is activated in

plants. These include physical changes like callose deposition and cell wall thickening as well as biochemical changes like the formation of reactive oxygen species (ROS), changes in level of signalling hormones, and the *de novo* synthesis of secondary plant metabolites, also known as phytoalexins (Bolton, 2009). Plant receptor kinases, known as pattern recognition receptors (PRRs), enable the plant to recognise the invading pathogen, whereupon defence cascades are activated (Aubel et al., 2013). These PRRs bind pathogen-associated molecular patterns (PAMPs), which are microbial-derived compounds like chitin, leading to PAMP-triggered immunity (PTI), the first phase of active defence (Huot et al., 2014). These PAMPs are often compounds which are typical for a whole class of microorganisms, e. g. chitin is a cell wall component present in nearly all fungi (Gomez-Gomez and Boller, 2002; Adams, 2004). PTI involves *inter alia* mitogen-activated protein kinase (MAPK) signalling, the formation of ROS, the induction of pathogen-responsive defence genes, and the deposition of callose at the infection site and aims to limit the spreading of the pathogen (Chisholm et al., 2006). Successful pathogens however, are able to inhibit the PTI by emitting effectors, also known as products of avirulence (*Avr*) genes, which prevent pathogen recognition at the membrane site or interfere with the plant immune system (Chisholm et al., 2006; Bolton, 2009). These effectors might for instance inhibit MAPK signalling, promote nutrient leakage or act as plant hormones and thus affect different levels of PTI leading to effector-triggered susceptibility (ETS) (Jones and Dangl, 2006). In turn, plants evolved resistance (*R*) genes to recognise these effectors or their activity. After successful recognition of effectors, a stronger immune response called effector-triggered immunity (ETI) is activated (Bolton, 2009; Huot et al., 2014). It is interesting to note that this interaction of pathogen and plant molecules has already been predicted in the 1940s by Harold Henry Flor in various articles leading to the concept of the Gene-for-Gene hypothesis (Flor, 1942, 1955, 1971). In case of successful recognition of the pathogen effector, plant defence responses are activated leading to host resistance. In this interaction, characterised as incompatible, the pathogen is unable to cause disease (Glazebrook, 2005). Conversely, a lack of recognition, due to either absence of the compatible *R* gene or *Avr* gene, results in a susceptible host and thus disease is induced by the pathogen. This interaction is called compatible. In recent years many *R* and *Avr* genes have been identified. *R* genes are ubiquitous in plant genomes and have for the most part similar structures within the plant kingdom (Stukenbrock and McDonald, 2009). Based on their conserved domains, *R* genes can be divided into five different groups (Wanderley-Nogueira et al., 2012). The largest group is represented by the nucleotide-binding leucine rich repeat (NB-LRR) gene family containing highly conserved NB and LRR regions which are associated with recognition and specificity (Chisholm et al., 2006). There is a lack of knowledge considering effectors from eukaryotic phytopathogens. However, it is known that fungal and oomycete effectors can act extracellular or inside the plant cell (Jones and Dangl,

2006). ETI often leads to hypersensitive response (HR), the programmed cell death of the pathogen-infected tissue. In addition, ETI can initiate systemic acquired resistance (SAR) through the induction of the salicylic acid (SA) synthesis. SAR terms the expression of immune responses in distal uninfected tissue in order to protect the plant from secondary infections. The activation of SAR entails an enormous transcriptional reprogramming and is associated with the induction of PR proteins (Fu and Dong, 2013). The resistance is long-lasting and can continue for weeks or months and sometimes for the whole lifetime of a plant (Durrant and Dong, 2004). However, the above described concept of *R* gene-*Avr* gene interaction has primarily been shown for bio- or hemibiotrophic pathogens. For necrotrophs like *R solani*, different mechanisms have been suggested (Glazebrook, 2005). Biotrophic pathogens rely on living host cells, whereas necrotrophic pathogens kill their host and feed on dead tissue (Glazebrook, 2005). Since necrotrophic pathogens utilise dead tissue, it was assumed that the SA-mediated HR induction supports their growth (Govrin and Levine, 2000).

Plant pathogens are divided into biotrophs, which possess *Avr* genes, and necrotrophs, which can possess host-specific toxin (*HST*) genes. Toxins can be grouped into non-specific, affecting a broad range of plants, and host-specific, affecting only a particular genotype. Similar to the products of *Avr* genes, HSTs have a virulence function, are recognised by specific plant *R* genes, undergo evolution and thus might be termed effectors (Oliver and Solomon, 2010, Wang et al., 2014). Recently, several effectors of necrotrophic fungi have been identified. They include polyketide, peptide, and proteinaceous effectors (Oliver and Solomon, 2010). Zheng et al. (2013) revealed three effectors (glycosyltransferase GT family 2, cytochrome C oxidase assembly protein CtaG/cox11 and peptidase inhibitor I9) of the sheath blight pathogen *R. solani* AG1 IA. It has been shown that an activation of R protein-mediated ETI through HSTs leads to HR and an effector-triggered susceptibility (ETS) (Lorang et al., 2004; Wang et al., 2014). So in contrast to the concept where the recognition of a compatible *Avr* protein by the *R* protein leads to resistance of the plant, the recognition of the HST by the compatible plant protein leads to induction of necrosis and thus susceptibility (Fig. 5). Differences in the outcome of these interactions might be based on the different lifestyles of biotrophic and necrotrophic pathogens (Stukenbrock and McDonald, 2009). In addition, resistance against necrotrophic fungal pathogens has been primarily attached to the signalling molecules jasmonic acid (JA) and ethylene (ET) but not to SA which has a crucial role in plant resistance against biotrophic/hemibiotrophic pathogens (Glazebrook, 2005, Tsuda et al., 2013). It has been shown that acidic *PR* genes are induced by SA, while the expression of basic *PR* genes is mediated by ET or JA (Kitajima and Sato, 1997; Kasprzewska, 2003). Recent research indicated an unexpectedly important role of SA in the resistance of Arabidopsis against the necrotrophic pathogen *Botrytis cinerea*

(Glazebrook, 2005). According to Wang et al. (2014) plant immune responses to necrotrophs can be similar or different from those to biotrophs, depending on the specific pathogen. Although the interaction of a plant with a necrotroph can be non-host-specific as well as host-specific, gene-for-gene resistance is rarely found during these interactions. It has been noticed that plant immune systems combating necrotrophic pathogens are very complex (Wang et al., 2014). Until now, host-specific resistance has not been described within the pathosystem potato/*R. solani* AG3PT. Therefore, a general response of potato, involving various basic defence mechanisms, is more likely.

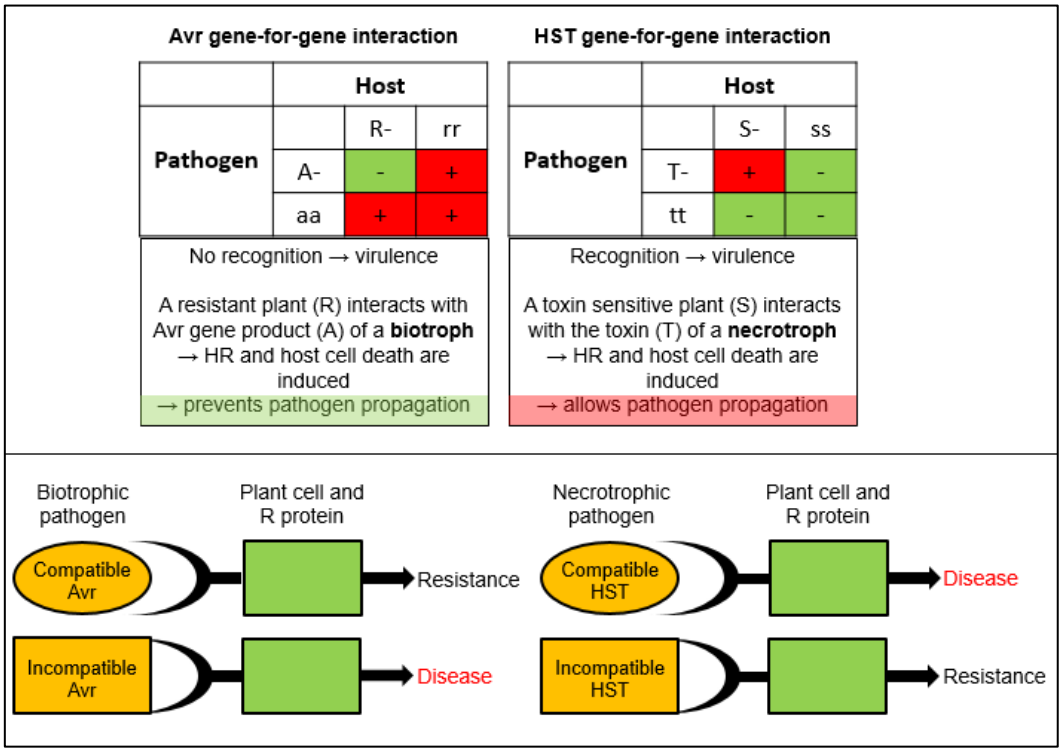


**Figure 5:** Comparison of gene-for-gene-interactions of plants with biotrophic and nectrophic pathogens (suggested by Stukenbrock and McDonald, 2009). Avr = avirulence, HST = host specific toxin, R = resistant, S = sensitive, A = avirulence gene product, T = toxin.

### 1.4.1 Induction of specific defence-related genes

Plants respond to different kinds of stresses with the induction of a variety of proteins. Many of these proteins are assigned to the group of pathogenesis-related (PR) proteins. These PR proteins form a group of at least 17 structurally and functionally different protein families which are ubiquitous in plants (Liu and Ekramoddoullah, 2006). They play an important role in constitutive and induced defence following pathogen attack, drought, cold, UV light or wounding for instance (van Loon et al., 2006, Jashni et al., 2015). In addition, PR proteins have been shown to be regulated dependent on the plant developmental stage (Develey-Rivière and Galiana, 2007). Many PR proteins, like the SA-marker PR-1, possess

antimicrobial properties. Due to the sterol binding capacity of PR-1, it is assumed that its antimicrobial effect is caused by the sequestration of sterols of the pathogen (Gamir et al., 2017). Beta-1,3-glucanases (PR-2) and chitinases (PR-3) for example degrade the fungal cell wall components glucan and chitin, respectively (Jashni et al., 2015). Various successful attempts to increase resistance of plants against pathogens have been done by inserting a gene encoding a chitinase (Nishizawa et al., 1999; Tabaeizadeh et al., 1999; Datta et al., 2001). Furthermore, microarray analysis revealed a systemic transcriptional induction of *PR-3* and *PR-2* in potato sprouts in response to a *R. solani* AG3PT infection (Lehtonen et al., 2008). The expression of PR-10 proteins, encoded by a multi-gene family, is regulated by developmental processes and is induced by biotic and abiotic stresses (Agarwal and Agarwal, 2014). Their induction can be dependent on ABA, JA, ET, and/or SA (Wang et al., 1999; McGee et al., 2001; Hashimoto et al., 2004). Furthermore, antifungal activity has been observed *in vitro* (Agarwal et al., 2013). In order to protect themselves from plant cell wall degrading hydrolases secreted by pathogens, plants synthesise proteinase inhibitors (PI). Gvozdeva et al. (2006) revealed that potato plants synthesise various proteinases which are able to suppress trypsin-like extracellular proteinases of *R. solani in vitro*. In addition to PR proteins, a variety of proteins can be induced following pathogen attack. Out of this pool, phenylalanine ammonia-lyase (PAL) and glutathione S-transferase (GST) were selected for further analysis in these studies. PAL is a key enzyme in the phenylpropanoid metabolism. Phenylpropanoids are precursors of a broad range of secondary plant metabolites like flavonoids, anthocyanins, hormones, and lignins which are involved in plant defence against abiotic and biotic stresses (Dixon and Paiva, 1995). PAL has a crucial role during plant defence and it has been shown to be associated with different plant hormones. In pepper, the *PAL1* gene regulates the SA-dependent defence signalling (Kim and Hwang, 2014). Furthermore, *PAL* was induced by JA and ET but suppressed by abscisic acid (ABA) (Gundlach et al., 1992; Kumar and Knowles, 2003; Asselbergh et al., 2008). The main role of GSTs is cellular detoxification (Dixon et al., 2002). However, recent studies revealed an important role in plant defence, more precisely, in SA-dependent defence signalling (Ghanta et al., 2011). Different levels of resistance are presumably related to expression levels of these above-mentioned defence-related genes. It has been suggested that the constitutive expression of e.g. *PR* genes is associated with non-specific resistance of *Solanum* species to *P. infestans* (Vleeshouwers et al., 2000). Similar mechanisms may be expected in the pathosystem potato/*R. solani* AG3PT.

### 1.4.2 Secondary plant metabolites involved in plant defence

It is supposed that most of the known secondary plant metabolites are involved in plant defence, so that a higher content of these compounds is associated with a higher resistance of the plant (Mazid et al., 2011). In general, plants produce a high diversity of secondary metabolites for protection against abiotic and biotic stresses. Secondary plant metabolites are often specific to one plant species, but can be specific to related plant species as well (Mazid et al., 2011). They can be divided into the three classes phenolics, terpenes, and sulphur- or nitrogen-containing compounds such as alkaloids (Bartwal et al., 2013; Pusztahelyi et al., 2015), which will be described further in the following sections.

*Phenolics*

Phenolic compounds are derived from the shikimate and phenylpropanoid pathway in plants (Pusztahelyi et al., 2015). They are involved in plant resistance due to antimicrobial and signalling properties but can also act as physical barriers like lignin for instance (Kröner et al., 2012). Thus, an important role in quantitative resistance against pathogens has been attached to these compounds (Niggeweg et al., 2004).

*Terpenoids*

The terpenoids are the largest group of secondary plant metabolites occurring in nearly all plants (Bartwal et al., 2013). They possess various functions in plants including the regulation of growth, defence or attraction, and of interactions with neighbouring plants. Terpenoids are derived from the mevalonate pathway and the non-mevalonate pathway, specifically 2-methyl-erythritol 4-phosphate/1-deoxy-xylulose 5-phosphate (MEP/DOXP) pathway, and, based on the number of isoprene units, can be divided into subclasses: monoterpenes, sesquiterpenes, diterpenes, triterpenes, tetraterpenes, and polyterpenes (Baldwin, 2010; Mazid et al., 2011; Bartwal et al., 2013). Representatives are e. g. carotenoids (tetraterpenes), abscisic acid (ABA, sesquiterpenes), and gibberellins (GA3, diterpenes) (Mazid et al., 2011; Pusztahelyi et al., 2015). Phytoalexins of potato also belong to the class of sesquiterpenes. It is known that ABA is induced due to abiotic stress, but the role in response to pathogens is contradictory (Asselbergh et al., 2008). According to Asselbergh et al. (2008), ABA can be a positive or negative regulator of disease resistance. The relevance of carotenoids during plant-pathogen interactions has been rarely investigated until now. Since carotenoids protect phospholipids of the plant membrane from peroxidation by quenching free radicals and toxins, which are produced under abiotic and biotic stress reactions, they might be important for plant resistance against pathogens (Boba et al., 2011). Carotenoids also provide substrates for the biosynthesis of ABA (Tanaka et al., 2008).

Moreover, an influence of arbuscular mycorrhizal fungi on carotenoid biosynthesis in roots has already been described (Fester et al., 2005).

*Nitrogen containing compounds – alkaloids and glycoalkaloids*

Glycoalkaloids are representatives of a large group of nitrogen (N) containing secondary plant metabolites (Mazid et al., 2011). Based on their toxicity, they are important compounds of plant resistance against pathogens and herbivores (Ginzberg et al., 2009). However, they are also toxic for mammals, because they interfere with functions of the nervous system (Mazid et al., 2011). Glycoalkaloids are derived from the mevalonate pathway (Kozukue et al., 2001). In potato, they are well studied. All plant parts of potato contain glycoalkaloids, but the highest levels were determined in sprouts or floral organs (Oda et al., 2002; Ginzberg et al., 2009). Until now, more than 80 different steroidal glycoalkaloids have been identified from potato. Nevertheless, α-chaconine and α-solanine are the two major glycoalkaloids in commonly grown potatoes (Ginzberg et al., 2009).

## 2. OBJECTIVES

The rejection of production batches of potato due to quality deficiencies caused by *R. solani* AG3PT leads to economic losses in potato production worldwide. A lack of efficient control strategies strengthens the necessity of alternative methods like the use of resistant cultivars. Although there are no cultivars with complete resistance against *R. solani* AG3PT, differences in the degree of susceptibility have been frequently observed under field conditions. However, there is currently no available method for a rapid evaluation of potato cultivars regarding their degree of resistance against *Rhizoctonia* diseases. It is presumed that the degree of quantitative resistance to *R. solani* AG3PT is related to the plant immunity level which affects interaction processes between plant and pathogen. Currently, there is only little information on the molecular background and on the relationship between degree of resistance and quantity of distinct secondary metabolites involved in plant defence available. Therefore, the goal of this thesis was to get insight into the molecular background of the interaction between potato and *R. solani* AG3PT and into the role of secondary metabolites for plant defence.

Molecular and biochemical analyses of cultivars with different degrees of field resistance to *R. solani* AG3PT enable the detection of key compounds which are crucial for the manifestation of different resistance levels. Thus, the first objective was the selection of potato cultivars with different degrees of field resistance to *R. solani* AG3PT based on the percentage infestation of harvested potato tubers with sclerotia of *R. solani* AG3PT. These cultivars are prerequisite for comparative analysis of the plant's response during interaction with the pathogen and for analysis of the importance of secondary plant metabolites within this interaction.

Furthermore, it was assumed that the pathogen density varied dependent on cultivar resistance resulting in a lower density in the less susceptible potato cultivar. We hypothesise that a higher expression level of defence-related genes in the cultivar less susceptible to *R. solani* AG3PT reduces pathogen colonisation, leading to a lower pathogen density in plant tissue in this cultivar compared to the more susceptible cultivar. For this purpose, a method for specific and rapid quantification of the pathogen in plant tissue was established.

Since the molecular background of different degrees of resistance awaits clarification, the third objective was to elucidate defence responses of potato which are induced upon infection with *R. solani* AG3PT. We assume that a higher plant innate immunity level is related to a higher expression level of defence-related genes. Hence, a higher expression level of these genes would be possessed by the more resistant cultivar compared to a less resistant cultivar.

Quantitative differences in resistance are related to the amount of defence-related proteins and secondary plant metabolites, and were thus studied in two selected cultivars with different degrees of resistance to *R. solani* AG3PT. The detection of a *R. solani* AG3PT-induced up-regulation of defence-related genes in a highly susceptible cultivar was followed by comparative analyses of a highly and a less susceptible cultivar considering their expression levels of defence-related genes as well as their contents of secondary plant metabolites.

# 3. MATERIAL AND METHODS

## 3.1 Field experiments

*Cultivation and experimental design*

Field experiments were performed in cooperation with potato breeders with the aim to select potato cultivars with different degrees in field resistance to black scurf disease. These cultivars should be used to analyse the molecular background of quantitative differences in resistance to *Rhizoctonia* diseases. Therefore, an assortment of different potato cultivars, selected based on the experience of the potato breeders, was investigated in field experiments carried out in 2013, 2014, and 2015 (see appendix Tab. 17, p. 145, for more details). Conventional seed tubers were provided by different potato breeders in Germany [Norika GmbH, Sanitz, Germany; Böhm-Nordkartoffel Agrarproduktion GmbH & Co. OHG, Hohenmocker, Germany; Saatzucht Firlbeck GmbH & Co.KG, Atting, Germany; Zuchtstation Niehoff, Bütow, Germany; Solana GmbH & Co. KG, Hamburg, Germany; SaKa Pflanzenzucht GmbH & Co. KG (belongs to Solana GmbH & Co. KG), Hamburg, Germany].

Additionally, the cultivars ‘Arkula’ and ‘Granola’, which had shown different degrees in resistance to *Rhizoctonia* diseases in the field experiments performed in cooperation with potato breeders, were tested again in field experiments at the research stations of the IGZ in 2015 and 2016. In 2015, seed tubers were planted in a loamy soil (Alluvial loam, soil value 76) at the external research station of the IGZ in Golzow, located in Brandenburg (52° 34’ N, 14° 30’ O). In 2016, the experiment was carried out at the field station in Großbeeren (Dilluvial sand, soil value 26), located in Brandenburg (52° 33’ N, 13° 22’ O) as well. Experimental set-up and cultural practices like fertilisation measures, irrigation as well as phytosanitary measures were comparable for both experiments. An amount of 160 kg/ha nitrogen and 300 kg/ha potassium (calcium ammonium nitrate and Kali 40, Kausek GmbH & Co. KG, Mittenwalde, Germany) was applied prior to planting. A second mineral fertilisation was given four weeks after planting by application of 80 kg/ha nitrogen and 150 kg/ha potassium (calcium ammonium nitrate and Kali 40, Kausek GmbH & Co. KG). The treatments were arranged in a randomised block design with six replicates per treatment (each cultivar with and without *R. solani* AG3PT inoculation, respectively), containing ten tubers per replicate (Fig. 6). The tubers were planted with an intra-row distance of 0.3 m and an inter-row distance of 0.65 m. Irrigation was performed as required. Phytosanitary measures during vegetation period included removal of weeds by hand and the application of fungicides against *Phytophthora infestans* and application of insecticides against *Leptinotarsa decemlineata* (see appendix Tab. 18, p. 146). Harvest of progeny tubers was carried out four weeks after haulm death for both cultivars, respectively.

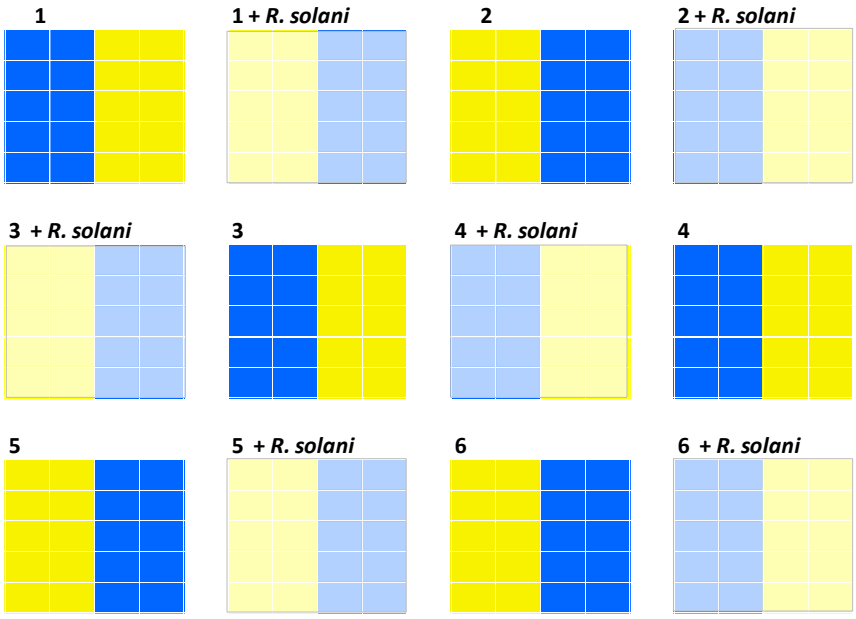


**Figure 6:** Experimental design of field experiment included 12 plots. Each treatment was performed without and with *Rhizoctonia solani* AG3PT (isolate Ben3) inoculation. Each plot contained both tested potato cultivars 'Arkula' and 'Granola' (indicated by different colours), planted in rows.

After harvest, tubers were graded according to square dimensions into the following size categories ≤ 25 mm, ≤ 28 mm, ≤ 30 mm, ≤ 35 mm, ≤ 40 mm, ≤ 45 mm, ≤ 50 mm, ≤ 55 mm, ≤ 60 mm, > 60 mm. Thus, the total amount of tubers could be divided into marketable (35 – 55 mm) and non-marketable (< 35 mm, > 55 mm) yield based on tuber size. Afterwards, fresh mass [g] and number of tubers per size category were determined.

For assessment of black scurf disease severity, harvested tubers were washed and following size classification, a sample of 30 tubers of the categories 35–55 mm was taken from each replicate. In addition, the incidence of tuber deformation and drycore symptoms was evaluated. Black scurf disease severity was assessed using a 9-point scale determining the percentage of the tuber surface covered with sclerotia (Tab. 2). Simultaneously, the percentage of deformed tubers and of tubers with drycore symptoms was recorded.

**Table 2:** Rating scale for evaluation of black scurf severity on progeny tubers in the field.

| **Rating scale** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** |
|---|---|---|---|---|---|---|---|---|---|
| **Surface covered with sclerotia (%)** | 0 | 1 | 1-5 | 5-10 | 10-15 | 15-25 | 25-35 | 35-45 | >45 |

### 3.1.1 Preparation of fungal inocula

In all experiments the pathogenic strain *R. solani* AG3PT isolate Ben3 (kindly provided by Marianne Benker, North Rhine-Westphalia – Plant Protection Service, Germany), which originated from sclerotia on potato tubers, was used for plant inoculation.

For preparation of inocula, barley kernels (Davert, Ascheberg, Germany) infested with the isolate Ben3 were used. The inocula were generated as follows: barley kernels were autoclaved twice and then inoculated with three plugs from a Petri dish colonised with *R. solani* AG3PT isolate Ben3, which was grown on potato dextrose agar (PDA, Merck, Darmstadt, Germany) at 20-22 °C for five days. Then, kernels were incubated for three weeks at 25 °C. At planting, inoculation was done by placing five pathogen-infested grain kernels (Fig. 7) below and above the seed tuber, respectively.

**Figure 7:** Barley kernels infested with *Rhizoctonia solani* AG3PT were used for inoculation in field experiments and for inoculation of *in vitro* plantlets in the greenhouse and growth chamber.

### 3.2 Pot experiments using potato mini tubers

First investigations regarding plant-pathogen interactions between *R. solani* AG3PT and potato were carried out using the cultivar 'Arkula' (Norika GmbH). In order to ensure working with pathogen-free plant material, potato mini tubers were produced from a culture of *in vitro* plantlets (kindly provided by Norika GmbH), because conventionally available seed tubers can be latently infested with *R. solani* and other pathogens.

Before planting, mini tubers were pre-sprouted in the dark applying a cycle of changing temperatures (8 °C for one week, 4 °C for three weeks, 8 °C for three days, 20-22 °C until sprouting). Additionally, the tubers were treated with 10 mg/L gibberellic acid (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) for 20 min to induce emergence of potato sprouts.

When the sprouts had reached a length of approximately 3-4 cm, the mini tubers were potted (pot size: 12 × 12 × 20 cm) in a quartz sand/grit mixture (Euroquarz, Dorsten, Germany) and further cultivated in a growth chamber (York, Mannheim, Germany) under following conditions: 18/15 °C day/night temperature, with 16 h/8 h day/night cycle, 400 µmol $m^{-2}$ $s^{-1}$ light and a relative humidity of 80 %. The tubers were poured with B'cuzz Hydro A+B nutrient solution (Atami B.V., Rosmalen, The Netherlands) twice a week and if required osmotic water was added. The nutrient solution had been adjusted to an EC of

2.1 dS $m^{-1}$ and a pH of 5.8 by adding 4 M $HNO_3$. The experimental set-up was built as a completely randomised block containing three replicates per treatment (potato plants without and with *R. solani* AG3PT inoculation). At each sampling date (3, 6 and 13 dpi), entire roots and all sprouts of five individual plants per replicate were sampled and pooled respectively.

For inoculum production, the isolate Ben3 was grown on potato dextrose agar (PDA, Merck) at 20-22 °C. Fourteen days after the potato plants had been transplanted into pots, they were inoculated with the *R. solani* isolate Ben3. For this purpose, a 5-day-old agar plug (Ø 10 mm) of a *R. solani* AG3PT culture was taken from the margin of a Petri dish (Fig. 8) and placed on the tuber next to the first emerging sprout when it had reached a length of 4 cm. In order to maintain humidity and promote fungal growth, every pot was sprayed with osmotic water. After inoculation, tubers were covered with quartz sand.

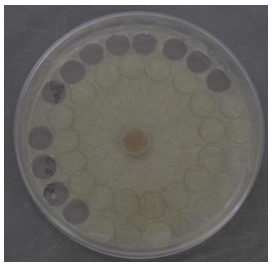

**Figure 8:** 5-day-old *in vitro* culture of *Rhizoctonia solani* AG3PT which was used for inoculation of potato mini tubers.

During cultivation, emerging sprouts were examined for necrotic lesions and growth anomalies. At harvest, in addition to sprouts, roots and tubers were investigated. Thus, disease symptoms like necrotic lesions and the occurrence of sclerotia were visually assessed.

The number of sprouts per tuber varies, so that all formed sprouts were collected. Sprouts and roots were separately shock frozen in liquid nitrogen and stored at -80 °C. Every tissue sample was ground using a mixer mill (2 min, 30/s; MM400, Retsch, Haan, Germany) with two grinding balls (7 mm, 3 mm; Askubal, Korntal-Münchingen, Germany) under constant cooling in liquid nitrogen. All items (grinding balls, tweezer, etc.) used during tissue sampling and proceeding were cleaned with RNase Zap (Thermo Fisher Scientific, Waltham Massachusetts, USA) to avoid degradation of RNA. The experiment was run twice.

### 3.3 Pot experiments using potato plantlets

The two cultivars 'Arkula' and 'Granola' were used for analysis of the plant's response to *R. solani* AG3PT (gene expression analysis and determination of secondary metabolites) and for assessment of pathogen density in plant tissue during cultivation time. These two cultivars had shown different degrees of susceptibility to *R. solani* AG3PT in previous field experiments. To assure working with pathogen-free plant material, *in vitro*-cultured plantlets ('Arkula' provided by Norika GmbH; 'Granola' provided by SaKa Pflanzenzucht GmbH & Co. KG) were used.

*Cultivation of potato plants for assessment of pathogen density*

For assessment of *R. solani* AG3PT density in potato roots and shoots, plantlets of 'Arkula' and 'Granola' were cultivated in the greenhouse after acclimatisation. The plantlets were also fertilised with B'cuzz Hydro A+B nutrient solution (Atami B.V.) and if required watered. In this study, the set-up consisted of four replicates per treatment with four plants per replicate. At each sampling date [3, 5 and 7 weeks post inoculation (wpi)], roots and underground sprouts of four plants per replicate were sampled and pooled respectively. Subsequently, samples of the bottom part of shoots (2 cm) were stored at -20 °C for DNA extraction. After determining fresh mass of roots, they were vertically separated into three sections (upper, centre, lower section) and stored at -20 °C as well. For DNA-extraction, plant material of all types of tissue was lyophilised and ground using a mixer mill (2 min, 30/s; Retsch MM400) with two grinding balls (7 mm, 3 mm; Askubal) per sample.

*Cultivation of potato plants for assessment of the plant's response to a Rhizoctonia solani AG3PT infection*

Plantlets were transferred into pots (13 cm diameter x 11 cm height) filled with a quartz sand/grit mixture (Euroquarz) and acclimatised for four to five days to autotrophic conditions in mini-greenhouses. For analysis of the expression of defence-related genes and the content of secondary metabolites (terpenoids and phenylpropanoids), plantlets of 'Arkula' and 'Granola' were further cultivated in the growth chamber (York) under following conditions: 18/15 °C day/night temperature, with 16 h/8 h day/night cycle, 400 µmol $m^{-2}$ $s^{-1}$ light and a relative humidity of 80 %. They were fertilised with B'cuzz Hydro A+B nutrient solution (Atami B.V.) and watered if required. In this case the experiment contained six replicates with four plants per replicate. Roots and shoots of four plants per replicate were sampled and pooled respectively at 10 days post inoculation (dpi). Afterwards, samples were separately shock frozen in liquid nitrogen and stored at -80 °C. For the extraction of RNA, proteins, and for the non-targeted metabolome analysis, an aliquot of frozen plant tissue was ground using a mixer mill (2 min, 30/s; Retsch MM400) with two grinding balls (7 mm, 3 mm; Askubal) under

constant cooling in liquid nitrogen. All items (grinding balls, tweezer, etc.) used during tissue sampling and proceeding were cleaned with RNase Zap (Thermo Fisher Scientific) to avoid degradation of RNA. A second aliquot of each sample was lyophilised (Beta 1-16, Christ, Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and ground using a mixer mill (2 min, 30/s; Retsch MM400) with two grinding balls (7 mm, 3 mm; Askubal) for the extraction of alkaloids, carotenoids, and phenylpropanoids.

In both experiments the treatments were arranged as completely randomised blocks. In each experimental setup the nutrient solution had been adjusted to an EC of 2.1 dS $m^{-1}$ and a pH of 5.8 by adding 4 M $HNO_3$. For inoculation of plants, barley kernels infested with the isolate Ben3 were used. The inoculum was produced as described in 2.1.1. Plantlets were inoculated two weeks after planting with five barley kernels, colonised with the pathogen, which were placed 2 cm apart from the plant and 2 cm deep into the quartz sand/grit mixture.

During cultivation and at harvest disease symptoms like lesions on shoots and the formation of sclerotia were visually assessed. Furthermore, fresh mass (FM) of roots, shoots, leaves, and progeny tubers was recorded at each sampling date.

### 3.4 Microscopic observation

To investigate the colonisation of roots by *R. solani* AG3PT, hyphae were stained using a solution of ink (Pelikan Vertriebsgesellschaft mbH & Co.KG, Hannover, Germany) and household vinegar (25 %) according to Vierheilig et al. (1998). Shortly after sampling, roots were cut into 1-2 cm long fragments, and cleared in 10 % KOH (Carl Roth GmbH & Co. KG) for 10 min at 100 °C. After removing KOH, the roots were rinsed twice with distilled water and subsequently stained for 5 min in a 10 % ink-vinegar solution at 100 °C. After removal of the staining solution, roots were rinsed twice with distilled water and bleached in 90 % lactic acid (Carl Roth GmbH & Co. KG) for at least two days. Bleached root segments were transferred onto glass slides and examined by light microscopy (EVOS® XL Core Imaging System for Transmitted Light Applications, Waltham Massachusetts, USA).

### 3.5 Molecular analyses

The response of potato to an infection with *R. solani* AG3PT was studied using common defence-related genes associated with the salicylic acid (SA)-pathway and the jasmonic acid/ethylene (JA/ET)-pathway. Expression of the selected genes [Tab. 3; pathogenesis-related (*PR*) genes *PR-1*, *PR-2*, *PR-3*, *PR-10*, glutathione S-transferase (*GST*), phenylalanine ammonia-lyase (*PAL*), proteinase-inhibitor II (*PI2)*] was investigated in roots and sprouts of ‘Arkula’ over a time course of 13 days after inoculation with the pathogen. To

test whether a relation between the expression level of the selected genes and the degree of resistance to black scurf disease exists, their expression was also determined in ‘Arkula’ and ‘Granola’ at 10 days post inoculation (dpi).

In addition, the expression of genes encoding key enzymes of the secondary plant metabolism [Tab. 3; abscisic acid receptor (*ABAR*), 9-*cis*-epoxy-carotenoid dioxygenase (*NCED*), phytoene synthase (*PSY*), β-solanine/β-chaconine rhamnosyltransferase (*SGT3*), squalene synthase (*SQS*)] was analysed in both cultivars.

### 3.5.1 Primer design and validation

All primers used, including accession numbers, annealing temperature and amplicon length, are listed in the tables 3 and 4 For the detection and quantification of *R. solani* AG3PT, primers specific to the internal transcribed spacer (ITS) regions of *R. solani* AG3PT were used (provided by Daniel Rivard, McGill University, Canada). The specificity of the ITS primers was verified by sequencing their PCR product (Eurofins Genomics GmbH, Ebersberg, Germany). The generated sequence was examined by comparison with GenBank data. All other primers are specific to *Solanum tuberosum* L. and were either designed based on potato sequences deposited in Genbank NCBI (NCBI, Bethesda, Maryland, USA) or chosen from the literature. Primer design was done using Primer-BLAST (NCBI) and DNAStar SeqBuilder software 12 (DNASTAR, Inc. Madison, Wisconsin, US). In the process, it was considered to design primers with a length of 20 to 25 base pairs (bp) to ensure a good sequence specifity and an adequate reaction efficiency (Dieffenbach et al., 1993). All primers used were generated by Eurofins MWG Operon (Eurofins Genomics).

**Table 3:** Primers used for analysis of potato gene expression by quantitative RT-PCR. All primers for analysis of defence genes and genes encoding enzymes of the secondary plant metabolism were generated from target genes of potato. Primers for specific quantification of *Rhizoctonia solani* AG3PT in potato tissue were designed from Internal Transcribed Spacer region (ITS).

| **Function** | **Abbre-viation of gene** | **Target gene coding for** | **Forward primer (5'-3')**<br>**Reverse primer (3'-5')** | **Annealing temperature (°C)** | **Amplicon length (bp)** | **Primer efficiency (%)** | **GenBank accession number** | **Reference for primer sequences** |
|---|---|---|---|---|---|---|---|---|
| Specific for *R. solani* AG3PT | *ITS* | Internal transcribed spacer region (ITS) 1 and 2 of AG3PT | For: GTTATTTTTTGTAATAAAATGATAATAAGTC<br>Rev: ATCAAACTAATTGAGTTAACAAAAAGAT | 57 | 273 | 89.0 | KJ170331 | Dr. Daniel Rivard (McGill University, Canada) |
| Reference-gene | *ACT* | Actin | For: GCTTCCCGATGGTCAAGTCA<br>Rev: GGATTCCAGCTGCTTCCATTC | 61 | 101 | 107.0 | X55749 | Nicot et al. (2005) |
| Reference-gene | *TEF1* | Elongation factor 1-α | For: ATTGGAAACGGATATGCTCCA<br>Rev: TCCTTACCTGAACGCCTGTCA | 61 | 101 | 103.0 | AB061263 | Nicot et al. (2005) |
| Defence-related | *PR-1* | Pathogenesis-related protein 1 | For: GGTGCAGGAGAGAACCTT<br>Rev: GGTACCATAGTTGTAGTTTGGCT | 61 | 82 | 99.2 | AJ250136 | Own study |
| Defence-related | *PR-2* | Basic glucan endo-1,3-betaglucanase | For: CACATTGCTTCTGGGATGGA<br>Rev: TTTAACATCTGGCCAGAAATCTTTAA | 61 | 75 | 95.5 | BQ118564 | Lehtonen et al., 2008 |
| Defence-related | *PR-3* | Acidic class II chitinase ChtA2 | For: ATGGCTGCCTTTTTCGGTCA<br>Rev: TACCTTGTCCAGCTCGTTCG | 57 | 169 | 104.2 | U49969.1 | Own study |
| Defence-related | *PR-10* | Pathogenesis-related protein 10 | For: TGATGTTAAGAGCATTGAGGTTGT<br>Rev: ATTGGACCACCTTCAACAAAGTT | 61 | 84 | 99.0 | XM_006340827 | Own study |
| Defence-related | *GST* | Glutathione S-transferase | For: AGTCGTGGCAGAGAACGAAG<br>Rev: AGGCCTAGCATCGAACAAGC | 57 | 175 | 93.2 | XM_006355737 | Own study |

**Table 3 (continued):** Primers used for analysis of potato gene expression by quantitative RT-PCR. All primers for analysis of defence genes and genes encoding enzymes of the secondary plant metabolism were generated from target genes of potato. Primers for specific quantification of *Rhizoctonia solani* AG3PT in potato tissue were designed from Internal Transcribed Spacer region (ITS).

| **Type of primer** | **Abbre-viation** | **Target gene** | **Forward primer (5'-3')**<br>**Reverse primer (3'-5')** | **Annealing temperature (°C)** | **Amplicon length (bp)** | **Primer efficiency (%)** | **GenBank accession number** | **Reference for primer sequences** |
|---|---|---|---|---|---|---|---|---|
| Defence-related | *PAL* | Phenylalanine ammonia-lyase | For: TCGAGGACGAATTGAAGGCAA<br>Rev: GCACATTGCTGTGAACACCTT | 57 | 215 | 107.1 | NM_001318638<br>XM_015311818 | Own study |
| Defence-related | *PI2* | Proteinase-inhibitor II (PR-6) | For: TGCCCACGTTCAGAAGGAAG<br>Rev: TGGGTCAGATTCTCCTTCGC | 61 | 120 | 94.9 | KJ475532 | Own study |
| ABA receptor | *ABAR* | Abscisic acid receptor PYL4-like | For: TCCTTGATGAGGAACGCCAC<br>Rev: ATCGGAGTGGAGGGTGGTAA | 59.2 | 95 | 96.0 | XM_006359495.2 | Own study |
| ABA bio-synthesis | *NCED* | 9-*cis*-epoxy-carotenoid dioxygenase | For: TCGGGCTTGTGGATCATAGC<br>Rev: TGAAGATCGCCAGAAGGCAA | 59.2 | 133 | 97.8 | 102577733 | Own study |
| Carotenoid bio-synthesis | *PSY* | Phytoene synthase 2, chloroplastic-like | For: GGGCTCTCCGATGAAGACAT<br>Rev: TTTTTCCTCTCCTCGGCCAT | 59.2 | 157 | 93.8 | XM_006339930.2 | Own study |
| Formation of α-chaconine and α-solanine | *SGT3* | β-solanine/ β-chaconine rhamnosyl-transferase | For: TTCCTGGTTTGCCTCTCGAC<br>Rev: TGGCTACGAACCTCAGCTTG | 60.0 | 118 | 92.7 | ABB84472.1 | Own study |
| Steroid bio-synthesis | *SQS* | Squalene synthase | For: TATGTAGCTGGGCTTGTTGG<br>Rev: GCCATGACCTGTGGAATGGC | 61.0 | 368 | 89.1 | XM_006361945 | Yoshioka et al., 1999 |

**Table 4:** Primers of reference genes which had to be excluded from normalisation of quantitative real-time PCR.

| **Type of primer** | **Abbre-viation** | **Target gene** | **Forward primer (5'-3')**<br>**Reverse primer (3'-5')** | **Amplicon length (bp)** | **GenBank accession number** | **Reference for primer sequences** |
|---|---|---|---|---|---|---|
| Reference gene | *APRT* | Adenine phosphoribosyl-transferase | For: GAACCGGAGCAGGTGAAGAA<br>Rev: GAAGCAATCCCAGCGATACG | 121 | CK270447 | Nicot et al. (2005), Lopez-Pardo et al. (2013) |
| Reference gene | *CYC* | Cyclophilin | For: CTCTTCGCCGATACCACTCC<br>Rev: TCACACGGTGGAAGGTTGAG | 121 | AF126551 | Nicot et al. (2005) |
| Reference gene | *L2* | Cytoplasmatic ribosomal protein L2 | For: GGCGAAATGGGTCGTGTTAT<br>Rev: CATTTCTCTCGCCGAAATCG | 121 | 39816659 | Nicot et al. (2005) |
| Reference gene | *TUB-B* | β-tubulin | For: ATGTTCAGGCGCAAGGCTT<br>Rev: TCTGCAACCGGGTCATTCAT | 101 | 609267 | Nicot et al. (2005) |
| Reference gene | *18S* | 18S ribosomal RNA | For: GGGCATTCGTATTTCATAGTCAGAG<br>Rev: CGGTTCTTGATTAATGAAAACATCCT | 101 | X67238 | Nicot et al. (2005) |

### 3.5.2 Nucleic acid extraction

Purity and integrity of the extracted nucleic acid affect gene expression results. A contamination of nucleic acids with proteins and other compounds can be determined spectrophotometrically by measuring the optical density (OD) at different wavelengths. Nucleic acids have an absorption maxima at 260 nm, whereas proteins have an absorption maximum of 280 nm. Thus, the absorbance ratio 260/280 should be above 1.8 to exclude impairment of amplification reactions due to proteins (Kirchner et al., 2014). Contamination with carbohydrates, phenol, and guanidine is indicated by an absorbance ratio 260/230 < 2.0. Thus, only nucleic acid with absorbance ratios above 1.8 (260/280) and 2.0 (260/230) was used for further analyses.

All centrifugation steps were run in the 5415R centrifuge (Eppendorf, Wesseling-Berzdorf, Germany).

*DNA extraction*

DNA was extracted from approximately 50 mg (balance Acculab max 620 g, d = 0.001 g Sartorius AG, Goettingen, Germany) of lyophilised ground plant tissue according to the method of Tinker et al. (1993) using CTAB (hexadecyl-trimethyl-ammonium bromide) in extraction buffer. For this purpose, ground plant tissue was merged with 500 µl of CTAB extraction buffer [100 mM Tris-HCl (Tris(hydroxymethyl)aminomethane-HCl, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) pH 8.0, 1.4 M NaCl (Serva Electrophoresis GmbH, Heidelberg, Germany), 20 mM ethylenediaminetetraacetic acid (EDTA) (Amresco, Ohio, USA), 2 % hexadecyl-trimethyl-ammonium bromide (CTAB) (Merck), 0.2 % 2-Mercaptoethanol (Carl Roth GmbH & Co. KG)] and incubated for 60 min at 65 °C. After centrifugation for 10 min at 14000 g, the supernatant was transferred into a new tube and inverted with 200 µl chloroform:isoamyl alcohol (24:1) (AppliChem GmbH, Darmstadt, Germany). Following centrifugation, the supernatant was inverted with 0.6 volumes of chilled isopropanol (AppliChem GmbH) and centrifuged. After precipitation (70 % ethanol (Carl Roth GmbH & Co. KG) for 30 min), the DNA pellet was air-dried and dissolved in 200 µl Tris (10 mM)-EDTA (1 mM)-buffer. For purification (Gebhardt et al., 1989), DNA was inverted with 15 µl RNase (10 µg/mL) (Serva Electrophoresis GmbH), incubated for 30 min at 37 °C and mixed with 200 µl phenol (AppliChem GmbH). After centrifugation, the upper phase was inverted with 400 µl chloroform:isoamyl alcohol (24:1). The upper phase was transferred into a new tube and DNA was precipitated with 2x volumes of absolute ethanol (Carl Roth GmbH & Co. KG) and 0.05 volumes of 4 M ammonium acetate (AppliChem GmbH) at -80 °C for 15 min. Following centrifugation for 30 min (14000 g) the pellet was washed with 70 % ethanol, air-dried and dissolved in 50 µl Tris (10 mM)-EDTA (1 mM)-buffer. The

concentration of extracted DNA was equalised (50 ng/µl) in order to confirm uniform DNA templates for the PCR assays. Quantity and purity of extracted DNA was determined with the NanoDrop ND-1000 spectral photometer (Thermo Scientific, Wilmington, USA).

*RNA extraction*

For gene expression analyses, total RNA was extracted from 70-90 mg (balance Acculab max 620 g, d = 0.001 g Sartorius AG) of ground sprout or root material using the RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. At first, buffer of the kit was added together with 5 µl β-mercaptoethanol (Carl Roth GmbH & Co. KG) to the samples, mixed and transferred into spin columns. After washing lysate with ethanol, a DNase treatment (Rnase Free Dnase Set, QIAGEN) followed. Pure RNA was eluted in RNase free water (Sigma-Aldrich Chemie GmbH) and stored at -80 °C until further use.

Quantity and purity of extracted RNA was determined with the NanoDrop ND-1000 spectral photometer (Thermo Scientific). Purity and integrity of the RNA affect gene expression results. The most important hampering factors are: first, contamination with proteins, carbohydrates or phenol; second, degradation of RNA due to nucleases; and third, contamination with genomic DNA (gDNA). A contamination of RNA with proteins and other compounds was determined spectrophotometrically by measuring the optical density (OD) at different wavelengths as mentioned above. In order to ensure the integrity of RNA, a modified TBE-based (Bioline GmbH, Luckenwalde, Germany) 1.0 %-agarose gel electrophoresis was performed (Aranda et al., 2012). Therefore, 1 % of a 12 % sodium hypochlorite (NaOCl) solution was added to the agarose (Carl Roth GmbH & Co. KG) gel, mixed and heated. After cooling down to room temperature, 0.5 % of ethidium bromide was added. Subsequently, 3 µl RNA was mixed with 1 µl RNase free water (Sigma-Aldrich Chemie GmbH) and 1 µl loading buffer and applied on the gel with a 1 kilobasepair (kb) ladder. The electrophoresis was carried out on a gel electrophoresis apparatus (Biometra GmbH, Göttingen, Germany) at 100 V for approximately 120 min. Sodium hypochlorite was added, because bleach destroys RNases present in the gel and prevents RNA denaturation, thus allowing the investigation of RNA quality (Aranda et al., 2012). Intact RNA is indicated by two bands, which represent the 18 S (2 kb) and 28 S (4 kb) ribosomal RNA.

Staining was done with ethidium bromide because staining with GelRed nucleic acid stain (Biotium, Fremont, California, USA) resulted in inferior quality of bands. Contamination with gDNA was checked performing qPCRs with negative reverse transcriptase controls. Therefore, during cDNA synthesis reverse transcriptase was replaced with nuclease free water.

*Gel extraction of PCR amplicon generated by primers ITS L396 and ITS U114*

The PCR amplicon of the ITS primers ITS L396 and ITS U114 was applied on agarose gel electrophoresis (Biometra GmbH) at 250 V for 20 min. After staining in ethidium bromide solution (0.0001 %), the respective band was excised and DNA fragments were extracted with the MinElute Gel Extraction Kit (QIAGEN). For this purpose, the excised gel band (approximately 200 mg) was incubated with 600 µl of QG buffer, included in the kit, at 50 °C for 10 min until the gel was dissolved. After adding 100 µl isopropanol (AppliChem GmbH), the solution was inversed and then transferred into a MinElute column. Before washing with 750 µl PE buffer, included in the kit, a centrifugation step (13000 rpm for 1 min) was included. Following renewed centrifugation, the DNA was eluted with 30 µl EB buffer, included in the kit, and incubated for 1 min at room temperature before centrifugation.

### 3.5.3 cDNA synthesis

The extracted RNA was reverse transcribed into cDNA using iScript™ cDNA Synthesis Kit (Bio-Rad, München, Germany) following the manufacturer's instructions. For this purpose, 1 µg RNA was added to 4 µl iScript reaction mix, 1 µl iScript reverse transcriptase and filled up to a volume of 25 µl with RNase free water (Sigma-Aldrich Chemie GmbH). The cDNA was synthesised in a *T Gradient* thermal cycler (Biometra GmbH) using the following program: 25 °C for 5 min, 42 °C for 30 min, 85 °C for 5 min. Thereupon, cDNA was diluted 10-fold with RNase free water and stored at -20 °C until further use.

### 3.5.4 Polymerase chain reaction

Polymerase chain reactions (PCRs) were performed in a *T Gradient* thermal cycler (Biometra GmbH).

For the verification of the fragment sizes of PCR products of the respective primers (Tab. 3 and 4), PCRs were run in a 25 µl reaction containing 16.875 µl PCR water (Sigma-Aldrich Chemie GmbH), 2.5 µl 10x buffer (Applied Biosystems, Darmstadt, Germany), 2.0 µl dNTP (deoxynucleotide) mix (VWR International GmbH, Darmstadt, Germany), 1.25 µl of each primer (10 pmol/µl), 0.125 µl *Taq* (*Thermus aquaticus*) polymerase (AmpliTaq Gold® DNA Polymerase, Applied Biosystems) and 1 µl DNA. The PCR temperature program used for amplification is shown in table 5.

**Table 5:** PCR temperature program used for 30 repeating cycles.

| Step | | Temperature [°C] | Time |
|---|---|---|---|
| **Initial denaturation** | | 94 | 5 min |
| **Denaturation** | x30 | 94 | 30 sec |
| **Annealing** | x30 | depending on primer pair (57-61) | 30 sec |
| **Elongation** | x30 | 72 | 1 min |
| **Final elongation** | | 72 | 5 min |

### 3.5.5 Agarose gel electrophoresis

The PCR products (15 µl) of each primer pair were mixed with 2.5 µl loading dye [according to Pietrek (2000) with 0.25 M EDTA (Amresco), 40 % sucrose (Carl Roth GmbH & Co. KG), 0.25 % bromphenol blue (Amresco)] and applied on 1.5 %-agarose (Carl Roth GmbH & Co. KG) gel, together with 4 µl of a 100 bp plus DNA (VWR International GmbH) ladder. Gel electrophoresis was performed on a gel electrophoresis apparatus (Biometra GmbH) at 250 V for 20 min. Gels were either stained by incubating them for 30 min in a 0.0001 % ethidium bromide (Carl Roth GmbH & Co. KG) solution or by adding 0.01 % of GelRed (Biotium) to the gel. The bands were visualised under UV-light (Transilluminator BioStep, Jahnsdorf, Germany).

### 3.5.6 Real-time polymerase chain reaction

All real-time PCRs were performed on the Thermal Cycler CFX96 C1000 Touch (Bio-Rad).

*Optimisation and validation of primer suitability and reaction conditions*

Following the verification of product fragment sizes of respective primer pairs, their suitability for qPCR was checked. Via melt curve data analysis it was confirmed that only one amplicon of the expected melt temperature was produced per primer pair (e.g. Fig. 9). Primers which did not fulfil this criterion were excluded (Tab. 4).

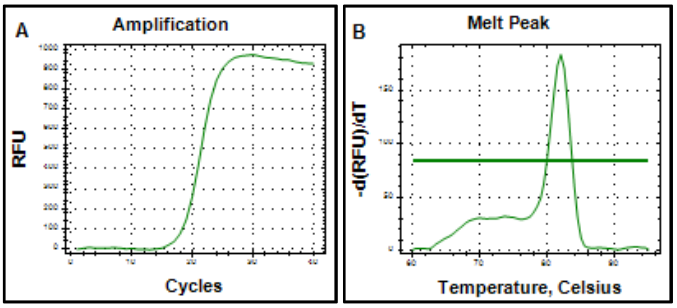


**Figure 9:** Amplification (A) and melt curve (B) of the reference gene *TEF1* (coding for elongation factor 1-α). The melting temperature is 82 °C.

The optimal annealing temperature was determined for each primer pair via temperature gradient PCR. Subsequently, amplification efficiency was estimated for each primer pair via dilution calibration curve of cDNA. Therefore, the slope of the log linear portion of the calibration curve is used to calculate the efficiency:

$$\text{PCR efficiency} = 10^{-1/\text{slope}} - 1$$

Furthermore, it is crucial to choose reference genes which are stably expressed and not influenced by tissue type, developmental stage or applied treatments (Udvardi et al., 2008). Therefore, various potential reference genes were tested in pre-experiments. Reference gene stabilities were checked with the qbase (Biogazelle, Gent, Belgium) software and the CFX Manager software 3.0 (Bio-Rad). Both calculate a measure (M) of the stability of the reference gene and the coefficient of variation on the normalised relative quantities (CV). The lower these values, the more stably the genes are expressed (Hellemanns et al., 2007). Generally, the M value ranges between 0.5 in homogeneous samples and 1.0 in heterogeneous samples and the CV between 0.25 and 0.5. Based on M and CV values in pre-experiments, a preselection of three reference genes was made for normalisation. In addition, the M and CV values were calculated and evaluated for each individual experiment.

#### 3.5.6.1 Quantitative real-time PCR for quantification of *R. solani* AG3PT

For quantification of *R. solani* AG3PT DNA in plant tissue, 2 µl of gDNA, which had been diluted to obtain a concentration of 50 ng/µl, were used as a template in the reaction mix (Tab. 6) under the temperature program listed in table 7.

**Table 6:** Composition of 20 µl reaction mix for quantification of *Rhizoctonia solani* AG3PT DNA via qPCR.

| | Volume [µl] | Concentration |
|---|---|---|
| **SsoAdvanced Universal SYBRGreen supermix (Bio-Rad)** | 10.0 | 1x |
| **Primer forward (10 pmol/µl)** | 1.0 | 500 Nm |
| **Primer reverse (10 pmol/µl)** | 1.0 | 500 Nm |
| **PCR-water** | 6.0 | |
| **Template (gDNA)** | 2.0 | |

**Table 7:** PCR temperature program used for quantification of *R. solani* AG3PT DNA via qPCR.

| Step | | Temperature [°C] | Time |
|---|---|---|---|
| **Initial denaturation** | | 94 | 5 min |
| **Denaturation** | x35 | 94 | 30 sec |
| **Annealing** | x35 | 57 | 30 sec |
| **Elongation** | x35 | 72 | 1 min |
| **Melt Curve** | | 60 to 95 | 0.5 increment for 5 sec |

*Calculation of data*

The quantity of *R. solani* AG3PT DNA in plant tissue was determined via absolute qPCR. For this purpose a 10-fold serial dilution (50 ng/µl-0.5 pg/µl) of gDNA from a pure culture of *R. solani* AG3PT isolate Ben3 was used to generate a calibration curve. The calibration curve was created by plotting the logarithm of the starting quantity of the template on the *x* axis and the threshold cycle ($C_t$) on the *y* axis (CFX Manager software 3.0, Bio-Rad). Quantity of *R. solani* AG3PT DNA in samples was calculated by subjecting the $C_t$ values of the samples to the equation of the calibration curve.

### 3.5.6.2 Quantitative real-time reverse-transcription PCR

*Reaction mix and temperature program*

The quantitative real-time reverse-transcription PCR (qRT-PCR) is an excellent method for the quantification of gene transcripts, however, it is sensitive to errors in application which might affect the outcome of analyses. Besides precaution considering the quality of RNA and cDNA as well as the design of primers, the choice of appropriate reference genes for normalisation of transcript abundances of the target genes is essential.

All reactions were performed in the Thermal Cycler CFX96 C1000 Touch (Bio-Rad) using the SsoAdvanced Universal SYBR Green supermix (Bio-Rad). The composition of the qRT-PCR reaction mix and the temperature protocol are listed in tables 8 and 9, respectively.

**Table 8:** Composition of 15 µl reaction mix for quantification of transcript levels of potato genes via qRT-PCR.

| | Volume [µl] | Concentration |
|---|---|---|
| **SsoAdvanced Universal SYBRGreen supermix (Bio-Rad)** | 7.5 | 1x |
| **Primer forward (5 pmol/µl)** | 1.0 | 333.3 nM |
| **Primer reverse (5 pmol/µl)** | 1.0 | 333.3 nM |
| **PCR-water** | 3.5 | |
| **Template (cDNA)** | 2.0 | |

**Table 9:** PCR temperature program for quantification of transcript levels of potato genes via qRT-PCR.

| Step | | Temperature [°C] | Time |
|---|---|---|---|
| **Initial denaturation** | | 95 | 30 sec |
| **Denaturation** | x 39 | 95 | 5 sec |
| **Annealing** | x 39 | depending on primer pair (57-61) | 30 sec |
| **Melt Curve** | | 65 to 95 | 0.5 increment for 5 sec |

*Calculation of data*

At first, qRT-PCR results were analysed using qbase (Biogazelle) software and the CFX Manager software 3.0 (Bio-Rad). Since both products provide the opportunities to insert individual primer efficiencies as well as evaluate reference genes and also lead to similar results, all following data analyses were conducted with the CFX Manager software 3.0 (Bio-Rad). In case of absence of outliers, values were generated from three technical replicates. The software uses following formulas to normalise the relative quantities of the target genes to the quantities of reference genes:

$$Relative\ quantity\ RQ\ (gene) = E^{(Cq\,(\min) - Cq\,(sample)}$$

E = Efficiency of primer (determined via calibration curve)

$C_t$ (min) = Average $C_t$ of the sample with lowest average $C_t$ for this gene

$C_t$ (sample) = Average $C_t$ of the sample for this gene

Then the normalised expression of the target gene is calculated based on the RQ values:

$$Normalised\ expression\ (target\ gene) = \frac{RQ\ sample\ (target\ gene)}{(RQsample\ (Ref1)x\ RQsample\ (Ref2)x\ \dots x\ RQsample\ (Refn)^{1/n}}$$

RQ = Relative quantity

Ref = Reference gene

n = Number of reference genes

## 3.6 Biochemical analyses

### 3.6.1 Extraction, determination, and quantification of proteins in roots and sprouts

In addition to the analysis of the transcript level of *PR* genes, their protein abundance was determined.

*Extraction*

Extraction buffer

- 56 mM $Na_2CO_3$
- 56 mM dithiothreitol (DTT)
- 2 % sodium dodecyl sulphate (SDS)
- 12 % sucrose
- 3 mM EDTA (Amresco)

A 100 mg aliquot of frozen homogenised tissue was dissolved in 500 µl of extraction buffer. After incubation at 80 °C for 10 min and a following centrifugation step (10 min at room temperature), the supernatant was transferred into a new tube.

*Protein quantification*

The total protein was quantified by BradfordRed protein assay (Expedeon Ltd., Cambridge, UK). Therefore, 2 µl of protein extract were mixed with 200 µl of Bradford reaction mixture (Expedeon Ltd.) and measured at 660 nm. For calibration, a dilution curve was generated using bovine serum albumin (BSA) (Sigma-Aldrich Chemie GmbH) as a standard [0, 0.5,-, 2.5 µg/mL BSA].

*Protein separation via gel electrophoresis*

Proteins were separated on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE, Tab. 10) according to Laemmli (1970). For detection of PR-1, PR-2, and PR-3, 5 µg of proteins, and for detection of PR-10, 10 µg were used for separation in polyacrylamide gel in 1 x SDS running buffer at 150 V for 70 min (Bio-Rad).

**Table 10:** Buffers, chemicals, and solutions used for SDS–PAGE.

| | **Component** | **Volume** |
|---|---|---|
| **Polyacrylamide gel** | Separation gel buffer | 5 Ml |
| | Millipore water (Immobilon-P, Millipore) | 20 mL |
| | Ammonium persulphate | 400 µl |
| | Acrylamid: Rotiphorese Gel 30 (37,5:1) | 15 mL |
| | Tetramethylethylenediamine (TEMED) | 20 µl |
| **10 x SDS running buffer** | Tris | 30.28 g |
| | Glycine | 144.0 g |
| | 10 % SDS | 100 mL |
| | | → in 1000 mL Millipore water |
| | protein marker | 5 µl |

*Western blot*

Solutions and chemicals used for Western blot are listed in table 11. The separated proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Immobilon-P, Millipore, Billerica, USA). For this purpose, six Whatman filter papers (Millipore) were incubated in

anode solution 1, three in anode solution 2 and nine in the cathode solution. The SDS gel was incubated for 5 min in anode solution 1, while the PVDF membrane was activated for 2 min in methanol (Carl Roth GmbH & Co. KG). Subsequently, the PVDF membrane was washed with water and equilibrated in anode solution 2 before the blot sandwich was constructed in the following order: batch of Whatman filter paper anode 1, batch of Whatman filter paper anode 2, PVDF membrane, SDS gel, batch of Whatman filter paper cathode. The blotting was carried out at 100 V for 60 min. Then, the PVDF membrane was rinsed thrice in TBST solution for 5 min and subsequently incubated in blocking solution at 4 °C overnight. Before probing with antibodies, the membrane was washed in TBST for 5 min. The membrane was incubated for 2 h at room temperature in the respective antibody solution [*Arabidopsis thaliana* for PR-1, tobacco for PR-2 and PR-3, and *Oryza sativa* for PR-10 (Agrisera, Vännäs, Sweden)]. Prior to and after a one-hour incubation in the second antibody solution [antibody Goat anti-Rabbit IgG (Agrisera), HRP conjugated], the membrane was rinsed thrice for 5 min with TBST. The immunodetection was carried out using Pierce ECL Western Blotting Substrate Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. After incubation of the PVDF membrane for 1 min in the solution provided by the kit, the immunoblot was developed using the Octoplus QPLEX Fluorescence Imager (NH DyeAGNOSTICS, Halle, Germany). The blots were stained in 1:50 (v:v) diluted amido black 10B stock solution (20 mM amido black 10B dissolved in 10 % acetic acid in methanol) in 10 % acetic acid in methanol for 10 min at room temperature. After destaining in 10 % acetic acid methanol solution for 10 min, the blots were dried at room temperature and subsequently captured using a flat-bed scanner. Band intensities were quantified using ImageJ software (http://imagej.nih.gov/ij/index.html) and normalised to the total protein stained amido black 10B signals.

**Table 11:** Composition of buffers and solutions used for Western blotting.

| | Component | Volume |
|---|---|---|
| **Anode solution 1** | 1 M Tris-HCl at pH 10.4 (Sigma-Aldrich Chemie GmbH) | 150 mL |
| | 100 % MeOH (Carl Roth GmbH & Co. KG) | 100 mL |
| | Millipore water | 250 mL |
| **Anode solution 2** | 1 M Tris-HCl at pH 10.4 (Sigma-Aldrich Chemie GmbH) | 12.5 mL |
| | MeOH (100 %) | 100 mL |
| | Millipore water | 387.5 mL |
| **Cathode solution** | 6-aminohexanoic acid | 2.6236 g |
| | 1 M Tris-HCl at pH 9.4 (Sigma-Aldrich Chemie GmbH) | 12.5 mL |
| | MeOH (100 %) | 100 mL |
| | Millipore water | 387.5 mL |
| **10x Tris buffered saline (TBS)** | Tris-HCl at pH8 (Sigma-Aldrich Chemie GmbH) | 100 mM |
| | 1.5 M NaCl (Serva Electrophoresis GmbH) | |
| **Tris buffered saline tween (TBST) solution** | 10 x TBS | 100 mL |
| | Millipore water | 900 mL |
| | TWEEN 20 | 500 µl |
| **Antibody solution 1** | BSA-TBST | 0.5 % |
| | 1:1000 antibody | |
| **Antibody solution 2** | BSA-TBST | 0.5 % |
| | 1:10,000 antibody | |
| **Blocking solution** | TBST | |
| | 1% BSA | |

### 3.6.2 Non-targeted analysis of plant secondary metabolites

Plant secondary metabolites were analysed in the cultivars ‘Arkula’ and ‘Granola’ to investigate whether there is a relation between the degree of resistance to black scurf disease and the content of plant secondary metabolites in roots and shoots.

*Extraction*

The extraction for the non-targeted metabolome analysis was carried out according to Errard et al. (2015). Therefore, 100 mg of ground frozen homogenised shoot and root tissue, mixed of four plants per replicate, were dissolved in 1.5 mL solvent solution [70 % of MeOH and 0.1 % of aqueous formic acid (FA) (Serva Electrophoresis GmbH)], incubated for 5 min at 1400 rpm and centrifuged (7 min, 4500 rpm). The supernatant was collected in a 10 mL flask

and the procedure was repeated four times. Subsequently, the collected supernatants were filled up with the solvent solution to a final volume of 10 mL in a volumetric flask. An aliquot was transferred into a spin filter [0.2 µm polytetrafluoroethylene (PTFE) membrane] and centrifuged (5 min, 3000 rpm).

*Determination and quantification*

Afterwards, the samples were separated on a 1290 Infinity UHPLC equipped with a C18-column (2.1 × 50 mm, 1.8 µm; Agilent Zorbax Entend-C18-Rapid resolution HT) and coupled with an Agilent 6530 QToF LC-MS (Agilent Technologies, Santa Clara, USA) at 4 °C. The column had a temperature of 30 °C. The separation was performed in a gradient mode of two solvent mixtures [solvent A: 0.01 % aqueous formic acid, solvent B: 0.01 % FA in acetonitrile (Carl Roth GmbH & Co. KG)]. The flow rate was set to 0.5 mL/min. Solvent B was increased from 2 to 5 % within 3 min and from 5 to 85 % within 7 min. An electronspray (ESI) source was used, and spectra were collected in positive and negative ionization modes (acquisition rate, 1 spectra/s) over an *m/z* 100-1700 range. The capillary voltage was set to 3.5 kV; the source temperature was set to 300 °C; and the nebuliser gas flow rate was 8 L/min at a pressure of 35 psi. The skimmer was set to 65 V and the fragmentor voltage to 175 V. Data generated by the UHPLC-QToF-MS analysis were processed with the Mass Hunter qualitative analysis 3.06.00 and Mass Profiler Professional (MPP; version 12.1, Agilent Technologies) using the molecular feature extraction with the following settings: small molecules, minimum 500 counts for feature extraction and 5000 counts for MPP analysis, ion species $[M + H]^+$, $[M - H]^-$, $[M + Na]^+$, M + NH4]$^+$, $[M + HCOO]^-$ including dimers ($[2M - H]^-$, $[2M + H]^+$) and trimers ($[3M - H]^-$, $[3M + H]^+$), $H_2O$ as neutral loss, a peak spacing tolerance of 0.5 *m/z* with a maximum mass error of 15 ppm. The quality score was based on mass accuracy, isotope abundance, and isotope spacing. Only data of a score of 80 % RD were processed using the MPP recursive workflow. Subsequently, formulas were generated using the above-mentioned ions and neutral loss with a match tolerance of 30 ppm and 0.5 min. Data were analysed with MPP and subjected to one-way ANOVA ($p \leq 0.01$; fold change ≥ 2). Putative identification was performed with Mass Hunter Metlin PCD (version 4.0, approximately 41000 compounds) and in-house databases for plant hormone metabolites (154 compounds), metabolism of amino acids (250 compounds), sugars (83 compounds), and phenylpropanoids (72 compounds) (Mass Hunter Metlin PCD compound identification settings: scoring; charge state, 10 ppm; mass error; allowed species, C H, O, N, S, P).

### 3.6.3 Extraction, determination, and quantification of carotenoids in roots and shoots

The extraction and determination of carotenoids was conducted according to the modified method of Errard et al. (2015).

*Extraction*

For the extraction, 25 mg (root) or 5 mg (shoot) of homogenised lyophilised ground tissue of four plants per replicate were extracted four times using 100 µl of methanol (Carl Roth GmbH & Co. KG). After incubation (5 min, 1400 rpm, room temperature), a buffer [100 µl 50 mM Tris-HCl (Sigma-Aldrich Chemie GmbH) at pH 8.0, 1 M NaCl (Serva Electrophoresis GmbH)] was added. Prior to and after adding 400 µl of chloroform the solution was incubated again (5 min, 1400 rpm, room temperature). Following centrifugation (5 min, 11000 rpm, room temperature), the lower phases were collected and evaporated under a stream of nitrogen (VLM GmbH, Bielefeld, Germany). The concentrated extract was dissolved in a solution of 20 µl dichlormethan and 80 µl isopropanol (Serva Electrophoresis GmbH), filtered through a 2 µM polytetrafluoroethylene (PTFE) membrane, transferred into a HPLC-vial, and covered with nitrogen until analysis.

*Determination and quantification*

For the determination and quantification of carotenoids, a UHPLC-DAD-QToF-MS (Ultrahigh-performance liquid chromatograph diode array detection, coupled to a time-of-flight mass spectrometry) analysis was conducted. The separation of carotenoids was performed on an Agilent Technologies 1290 Infinity Ultrahigh-performance liquid chromatograph (UHPLC) equipped with a C30-column (YMC Co. Ltd. Japan, YMC C30, 100 x 2.1 mm, 3 µm) and coupled with an Agilent Technologies 6230 time-of-flight (TOF) which is equipped with an atmospheric-pressure chemical ionization (APCI) ion source in positive ionization mode. The separation was performed in a gradient mode of two solvent mixtures [solvent A = methanol, methyl-tert-butyl ether, and water in 81/15/4 and B = methanol, methyl-tert-butyl ether, and water in 6/90/4] set to a flow rate of 0.2 mL/min. Solvent A was decreased from 100 % (10 min isocratic) to 0 % within one hour. 20 mM ammonium acetate (AppliChem GmbH) was added to the mobile phases in order to enhance ionisation. The gas temperature (325 °C) was set to a flow rate of 8 L/min, the vaporiser was set to 350 °C, and the nebuliser pressure was set to 35 psi. The analysis ran at 3500 V and at a fragmentor voltage of 175 V with a corona current of 6.5 µA.

The identification of carotenoids was achieved via high resolution masses, retention time, and co-chromatography with the reference substances chlorophyll a and b, the (*all-E*)-isomers for β-carotene, lutein, and zeaxanthin, and (*9Z*)-neoxanthin. Standard substances were used to generate external calibration curves for quantification via dose-response

curves. Therefore, stock solutions of standards [α-carotene, (*9Z*)-neoxanthin, zeaxanthin (Carote Nature GmbH, Switzerland), β-carotene, chlorophyll a and b (Sigma-Aldrich Chemie GmbH), lutein isolated form *Tagetes erecta* (Errard et al., 2015)] were prepared individually and concentrations were measured spectrophotometrically using specific wavelengths and respective extinction coefficients.

### 3.6.4 Extraction, determination, and quantification of glycoalkaloids in roots and shoots

*Extraction*

An aliquot of 5 mg of homogenised lyophilised ground root and shoot tissue of four plants per replicate was extracted five times with 1.5 mL of 90 % aqueous MeOH (Carl Roth GmbH & Co. KG). After incubation for 5 min at 1400 rpm at room temperature, the samples were centrifuged (4500 rpm) for 5 min. Subsequently, supernatants were collected and filled up to 10 mL with the extraction solution in a volumetric flask.

*Determination and quantification*

The samples were separated on a 1290 Infinity UHPLC equipped with a C18 (2.1 × 50 mm, 1.8 µm; Agilent Zorbax Entend-C18-Rapid resolution HT) coupled with an Agilent 6530 QToF LC-MS (Agilent Technologies). The column had a temperature of 30 °C. The separation of alkaloids was performed in a gradient mode of two solvent mixtures [solvent A: 0.01 % aqueous formic acid, solvent B: 0.01 % FA in acetonitrile (Carl Roth GmbH & Co. KG)]. The flow rate was set to 0.5 mL/min. The gradient used for solvent B was 3 % (0-2 min), 3-16 % (2-4 min), 16-22 % (4-13 min), 22-35 % (13-15 min), 35-60 % (15-16 min), 60-100 % (16-18 min). The capillary voltage was set to 3.5 kV; the source temperature was set to 300 °C; and the nebuliser gas flow rate was 8 L/min at a pressure of 35 psi. The skimmer was set to 65 V and the fragmentor voltage to 0 V.

The identification of glycoalkaloids was achieved via high resolution masses, retention time, and co-chromatography with the reference substances α-chaconine and α-solanine (PhytoLab GmbH & Co. KG, Vestenbergsgreuth, Germany). For quantification of glycoalkaloids, standard substances were used to generate external calibration curves.

### 3.6.5 Extraction, determination, and quantification of phenylpropanoids in roots and shoots

*Extraction*

Phenylpropanoids were extracted according to the modified method of Schmidt et al. (2010). An aliquot of 10 mg of lyophilised ground shoot or root tissue from a mixture of four plants per replicate was dissolved in 600 µl of 60 % aqueous MeOH, incubated for 40 min at 1400 rpm and 20 °C and then centrifuged (4500 rpm, 10 min, 20 °C). Afterwards, the supernatant was collected in a new reaction tube. This process was repeated twice with 300 µl of 60 % aqueous MeOH, once with 20 min incubation and once with 10 min incubation at 1400 rpm, and subsequent centrifugation (4500 rpm, 10 min, 20 °C) respectively. After combining all three supernatants, the extract was evaporated to dryness. Then the concentrated extract was resuspended in 200 µl of 10 % aqueous MeOH and transferred into a Corning® Costar® Spin-X® plastic centrifuge tube filter (Sigma-Aldrich Chemie GmbH) for centrifugation (3000 rpm, 5 min, 20 °C) and subsequent high-performance liquid chromatography (HPLC) analysis.

*Determination and quantification*

Phenylpropanoids present in the filtrate were analysed using a series 1100 HPLC (Agilent Technologies) equipped with a degaser, a binary pump, an autosampler, a column oven, and a photodiode array detector. The compounds were separated using an Ascentis® Express F5 column (150 mm × 4.6 mm, 5 µm, Supelco, Sigma-Aldrich Chemie GmbH) at a temperature of 25°C. Eluent A was 0.5 % acetic acid, and eluent B was 100 % acetonitrile. The gradient used for eluent B was 5-12 % (0-3 min), 12-25 % (3-46 min), 25-90 % (46-49.5 min), 90 % isocratic (49.5-52 min), 90-5 % (52-52.7 min), and 5 % isocratic (52.7-59 min). The separation was performed at a flow rate of 0.85 mL $min^{-1}$ and the determination was conducted at wavelengths of 280 nm, 320 nm, 330 nm, 370 nm and 520 nm. The hydroxycinnamic acid derivatives and glycosides of flavonoids were identified as deprotonated molecular ions and characteristic mass fragment ions according to Schmidt et al. (2010) and Neugart, Rohn, and Schreiner (2015) by HPLC-DAD-ESI-$MS^n$ using a Bruker amazon SL ion trap mass spectrometer in negative ionisation mode. Nitrogen was used as dry gas (10 L $min^{-1}$, 325 °C) and nebuliser gas (40 psi) with a capillary voltage of -3500 V. Helium was used as the collision gas in the ion trap. For quercetin, the mass optimisation for the ion optics of the mass spectrometer was performed at *m/z* 301 or arbitrarily at *m/z* 1000. The $MS^n$ experiments were performed in auto mode up to $MS^3$ in a scan from *m/z* 200-2000. Standard substances (Carl Roth GmbH) were used to generate external calibration curves (0.01 to 10 mg 100 $mL^{-1}$) for a semi-quantitative approach. Caffeoylquinic acids were

quantified using the standard for chlorogenic acid, quercetin-3-rutinoside-7-glucoside and quercetin-3-rutinoside (rutin) were quantified via quercetin-3-glucoside, kaempferol-3-rutinoside-7-glucoside and kaempferol-3-rutinoside (nicotiflorin) were quantified using the standard of kaempferol-3-glucoside (PhytoLab GmbH & Co. KG). The results are presented as mg $g^{-1}$ dry weight (DW). For identification, retention time (RT), mass spectra and absorption maxima were compared to data in the literature (Navarre et al., 2011).

### 3.7 *In vitro* assay for sensitivity *of Rhizoctonia solani* AG3PT to rutin, quercetin, nicotiflorin, kaempferol, 3-caffeoylquinic acid, and α-chaconine and α-solanine

Commercially available rutin, quercetin, nicotiflorin, kaempferol, 3-caffeoylquinic acid (neochlorogenic acid), α-chaconine, and α-solanine (PhytoLab GmbH & Co. KG) were tested regarding their impact on mycelial growth of *R. solani* AG3PT (isolate Ben3) *in vitro*. *R. solani* AG3PT (isolate Ben3) was grown on potato dextrose agar (PDA, Merck) at 20-22 °C. Stock solutions of neochlorogenic acid, flavonol glycosides as well as their aglycones were generated by dissolving them in minimum volume of ≥ 99.5 % ethanol and adjusting to the respective volume with distilled water. *In vitro* tests were conducted in Petri dishes (Ø 90 mm) containing 1 % agar (VWR International GmbH) and 0.39 % potato dextrose agar (PDA, Merck). After autoclaving (20 min, 121 °C) and cooling down, the antibiotics penicillin (100 mg/L, Carl Roth GmbH & Co. KG), tetracyclin hydrochlorid (10 mg/L, Carl Roth GmbH & Co. KG), streptomycin (50 mg/L, Sigma-Aldrich Chemie GmbH) and various concentrations of phenolic compounds (400, 200, 100, 50 µM) were incorporated into the medium by sterile filtration (Rotilabo-syringae filters, 45 µm, Carl Roth GmbH & Co. KG). Agar plates containing respective concentrations of the dissolving agent (ethanol) were used as control. Five day old mycelial disks (Ø 10 mm) of *R. solani* AG3PT were placed in the center of each Petri dish and incubated in the dark at 18 °C. Three replicates per substance and concentration were considered for this experiment. Growth was assessed after 72 and 168 hours of incubation (hoi) by measuring the colony diameter. Stock solutions (2000 µM) of α-chaconine and α-solanine were prepared by dissolving in minimum volume of 0.1 M HCl and adjusting to the respective volume with distilled water. *In vitro* tests were conducted in 12 well culture plates (Greiner Bio-One, Frickenhausen, Germany). Appropriate volumes of the stock solution were added to dry components of the medium (agar, PDA) and the pH was adjusted to 5.8. Thus, concentrations of alkaloids in the media were 50 µM to 200 µM, respectively. Additionally, the synergistic effect of both alkaloids was tested (50 µM of α-chaconine and α-solanine). After autoclaving (20 min, 121 °C) and cooling down, the antibiotics penicillin (100 mg/L), tetracyclin hydrochlorid (10 mg/L), streptomycin (50 mg/L) were added. Five day old mycelial disks (5 mm) of *R. solani* AG3PT were placed in the center of each well (Ø 20 mm) and

incubated in the dark at 18 °C. Culture wells without alkaloids served as control. Three replicates per substance and concentration were considered for this experiment. Growth was assessed after 24, 48 and, 72 hoi by measuring the colony diameter.

### 3.8 Statistics

Statistical analyses were carried out using STATISTICA software package version 12.0 (StatSoft Inc., Tulsa, OK, USA). Data of growth characteristics, *R. solani* AG3PT quantification, expression analyses and metabolite analyses were subjected to one-way or to factorial analysis of variance (ANOVA) ($p \leq 0.05$) as indicated throughout the document. Data regarding black scurf severity, incidence of tuber deformation and drycore were subjected to Mann Whitney U test ($p \leq 0.05$).

# 4. RESULTS

## 4.1 Degree of field resistance of ‘Arkula’ and ‘Granola’ to *Rhizoctonia solani* AG3PT

A selection of various potato cultivars was evaluated for three consecutive years (2013, 2014, 2015) regarding their susceptibility to *R. solani* AG3PT at different field locations in Germany. These trials were performed in cooperation with potato breeders. Based on these results, the cultivars 'Arkula' and 'Granola' were selected, representing a highly and less susceptible cultivar, for further investigation in field, greenhouse, and growth chamber experiments. Results of field experiments, which were conducted at the IGZ using these two cultivars, are presented in Fig. 10 for the years 2015 and 2016.

*Tuber yield and size*

Tuber size and marketable yield were determined by grading all harvested tubers according to square dimensions into size categories (chapter 3.1) and recording the fresh mass [FM (g)] and number of tubers per category. Tubers with a size between 35 and 55 mm are regarded as marketable. Thus, the percentage of the tuber fresh mass with a marketable size was calculated from the total fresh mass for all treatments (2015 each cultivar with and without *R. solani* AG3PT inoculation, 2016 seed tubers without additional inoculation). In 2015, ‘Granola’ had a significantly higher relative yield of tubers with marketable size compared to ‘Arkula’ (Fig. 10). In 2016, no significant differences were detected between the two cultivars. An additional inoculation with *R. solani* AG3PT had no significant influence on the fresh mass (FM) of harvest tubers with marketable size.

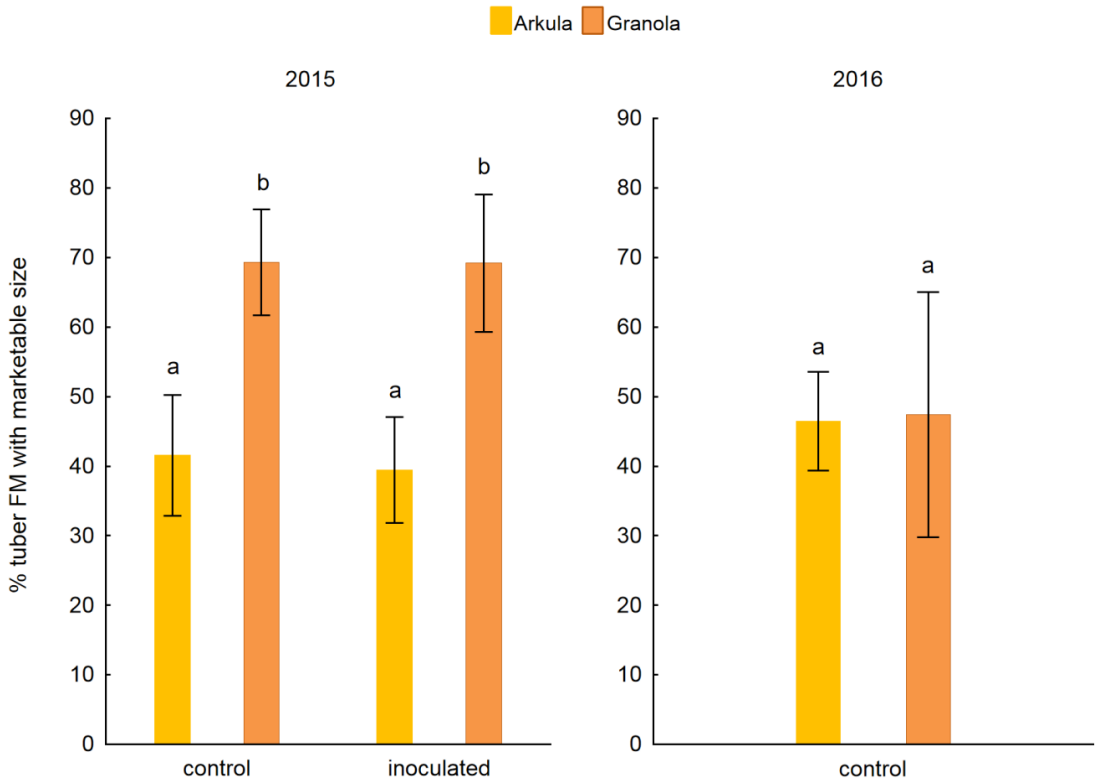


**Figure 10:** Tuber yield of the cultivars 'Arkula' and 'Granola' in treatments with (inoculated) and without (control) *Rhizoctonia solani* AG3PT inoculation harvested in the growing period 2015 and 2016. Values represent percentages of fresh mass (FM) of progeny tubers with a marketable size (< 30 mm, > 55 mm). The bars represent means of six (2015) or five (2016) replicates with standard deviation. Significant differences between cultivars are indicated by different letters (One-way ANOVA followed by Dunnett, $p \leq 0.05$). No significant differences between treatments (control, inoculated) were detected (One-way ANOVA followed by Dunnett, $p \leq 0.05$).

*Black scurf*

In 2015, the percentage of sclerotia infestation on harvested potato tubers (black scurf) was determined for treatments without and with additional inoculation of *R. solani* AG3PT. In 2016, seed tubers were not additionally inoculated. In both years, significant differences regarding the incidence of black scurf on harvested tubers were revealed between the two cultivars in control as well as inoculated treatments (see appendix Tab. 20, p. 147). Similarly, the evaluation of black scurf severity revealed significant differences between the cultivars. 'Arkula' showed a significantly higher black scurf severity than 'Granola' in treatments with and without additional inoculation (Fig. 11; see appendix Tab. 20, p. 147 for more details). Additional inoculation with the pathogen caused a significant increase of black scurf severity compared to non-inoculated treatments in 2015 for both cultivars (Fig. 11).

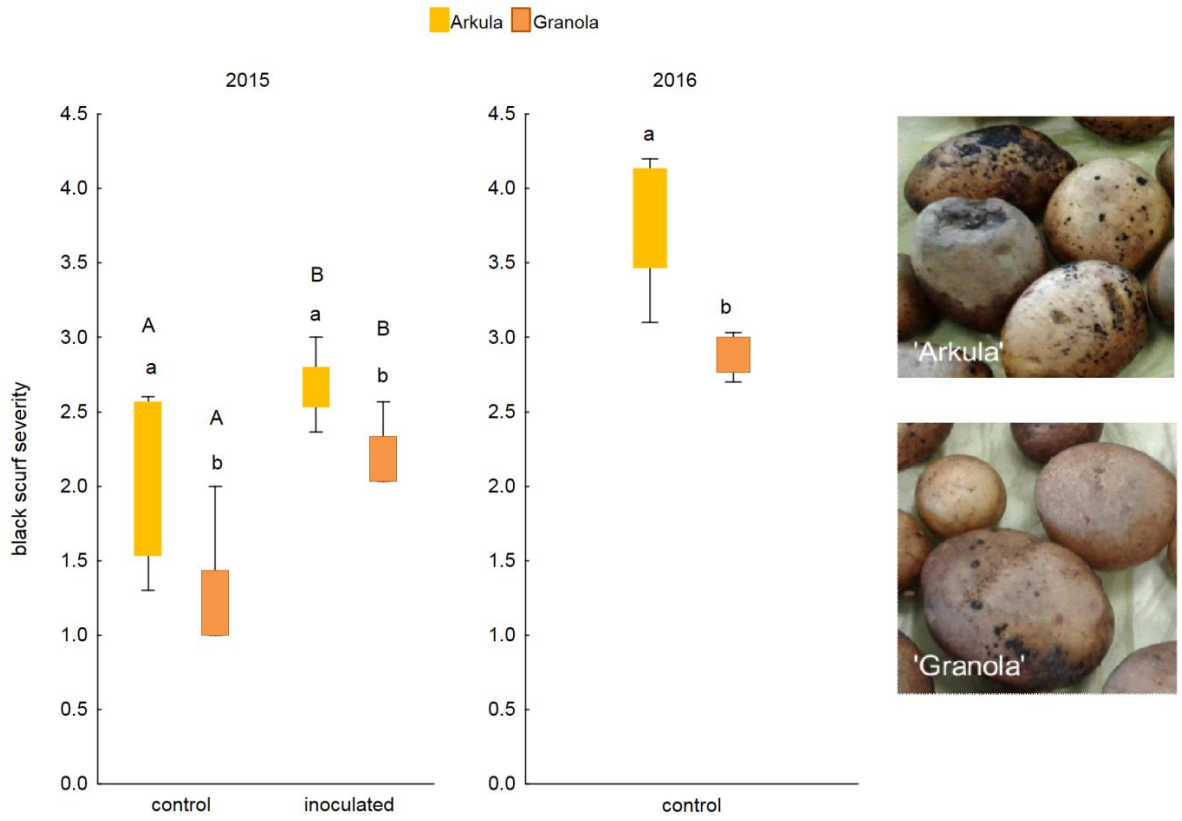


**Figure 11:** Severity of black scurf symptoms on tubers of the potato cultivars ‘Arkula’ and ‘Granola’ in treatments with and without *R. solani* AG3PT inoculation harvested in the growing period 2015 and 2016. Black scurf severity was evaluated by determining the percentage of tuber surface covered with sclerotia according to a 1 (0 %) to 9 (>45 %) scale. The box plots show the 50 % inter quartile range of the values and the median. Significant differences between cultivars are indicated by different lowercase letters (Mann Whitney U-Test, $p \leq 0.05$). In 2015, black scurf severity was evaluated under low (control, non-inoculated) and high pathogen pressure (inoculated with *R. solani* AG3PT) and significant differences between these treatments are indicated by uppercase letters (Mann Whitney U test, $p \leq 0.05$). Pictures show typical black scurf symptoms on tuber surface of 'Arkula' and 'Granola'.

*Deformation and drycore*

Regarding the occurrence of deformed tubers, significant differences were observed between the two cultivars (Fig. 12; appendix Tab. 20, p. 147). In both years, 'Granola' had significantly less deformed tubers than ‘Arkula’ (Fig. 12). This was also the case for treatments with additional *R. solani* AG3PT inoculation. A significant increase of deformed tubers in inoculated treatments was not observed. A cultivar-dependent difference regarding the occurrence of drycore was only detected in 2016, where ‘Arkula’ had a significantly higher percentage of tubers bearing this symptom compared to ‘Granola’ (Fig. 12; see appendix Tab. 20, p. 147). In addition, the incidence of drycore symptoms was significantly increased in the case of 'Arkula' in 2015 due to inoculation with *R. solani* AG3PT (Fig. 12).

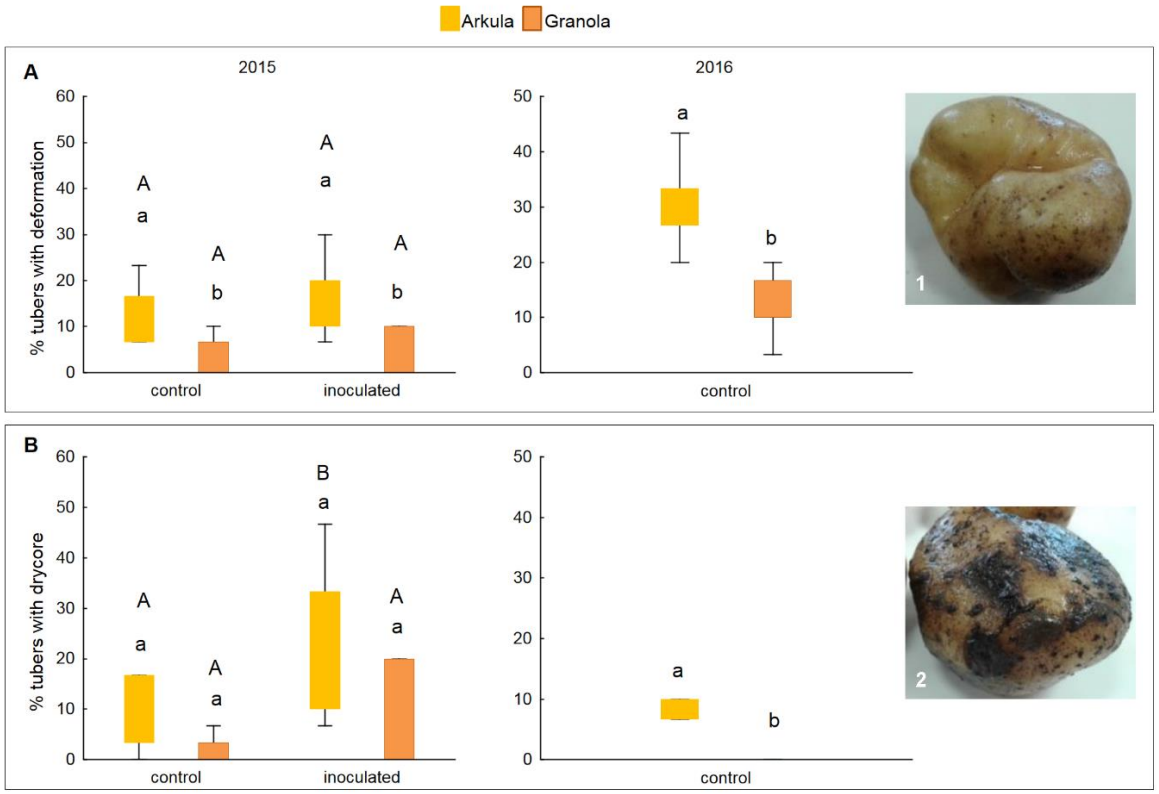


**Figure 12:** Percentage numbers of deformed tubers and tubers with drycore symptoms of the potato cultivars 'Arkula' and 'Granola' in treatments with and without *R. solani* AG3PT inoculation harvested in the growing period in 2015 and 2016. Values represent percentages of progeny tubers with deformation and drycore. The box plots show the 50 % inter quartile range of the values and the median. Significant differences between cultivars are indicated by different lowercase letters (Mann Whitney U test, $p \leq 0.05$). In 2015, disease symptoms were evaluated under low (control) and high pathogen pressure (inoculated with *R. solani* AG3PT) and significant differences between these treatments are indicated by uppercase letters (Mann Whitney U test, $p \leq 0.05$). Pictures show typical tuber deformation (1) and drycore symptoms (2) on tubers of 'Arkula'.

## 4.2 Colonisation of potato roots and sprouts with *Rhizoctonia solani* AG3PT

### 4.2.1 Microscopic observation of root colonisation

*Results of this chapter were published in Plant Pathology (Genzel et al., 2017).*

The colonisation of roots with *R. solani* AG3PT was observed via staining of fungal hyphae using an ink-vinegar solution (Fig. 13). Thus, different structures of pathogen hyphae were seen on the surface of roots and stolons. Runner hyphae, which are growing along the root surface, were observed. Moreover, extensive hyphal growth of *R. solani* AG3PT over the root surface and hyphal branching were detected at 3 dpi (Fig. 13). The formation of infection cushions and T-shaped hyphal branches were visible as well (Fig. 13). Additionally, the microscopic observation revealed that the root system is not homogeneously colonised by the pathogen. The initially extensive hyphal growth observed at 3 dpi and 6 dpi did not

proceed proportionally to the strong growth of the roots which was determined at 13 dpi (data not shown).

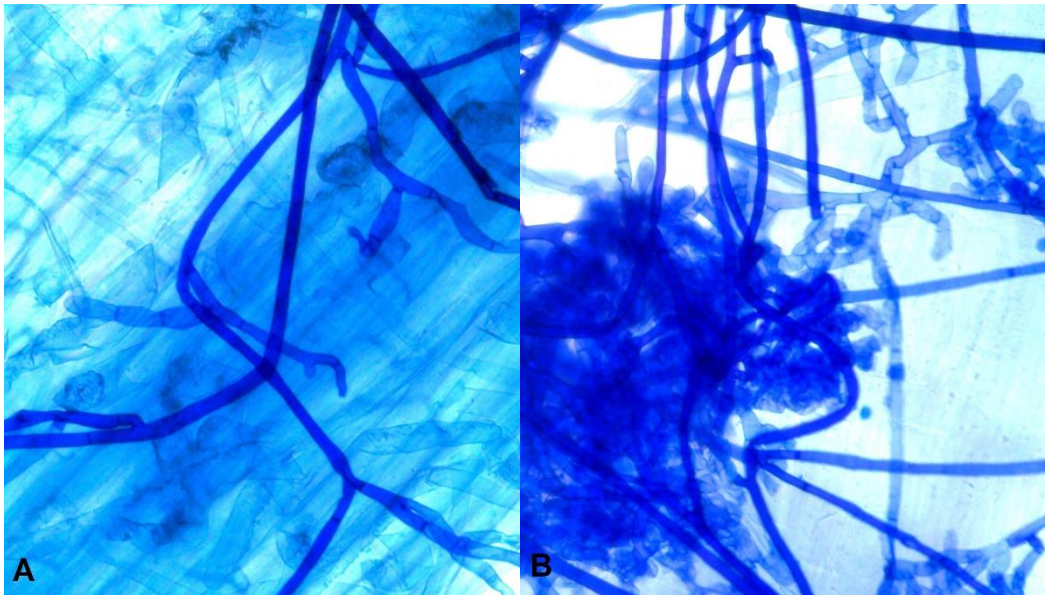


**Figure 13:** Microscopic observation of *Rhizoctonia solani*-hyphae (AG3PT isolate Ben3) stained with ink-vinegar (40x magnification). Branching hyphae on the root surface of potato 3 dpi (A). Cushion forming hyphae on a stolon 6 dpi (B).

### 4.2.2 Establishment of qPCR assay

*Results of this chapter were published in Plant Pathology (Genzel et al., 2017).*

Quantitative real-time PCR (qPCR) allows the quantification of *R. solani* AG3PT DNA in plant tissue and thus, the study of the pathogen density during the pathogenesis on potato. Therefore, it is a useful tool to investigate whether the degree of susceptibility to *R. solani* AG3PT is correlated to the pathogen density in plant tissue. Hence, the aim was to establish a qPCR assay applicable for rapid quantification of *R. solani* AG3PT DNA in potato roots, underground sprouts or shoots and tubers.

*Primer choice and validation*

In the beginning, appropriate primers specific to *R. solani* AG3PT and suitable for qPCR, had to be selected. Several primer pairs specific to *R. solani* AG3PT, which have already been published, were tested prior to the experiment (chapter 3.5.1). However, due to the occurrence of false negatives, they were not suitable for quantitative real-time PCR. Therefore, the primer pair (ITS L396 and ITS U114, Tab. 3, chapter 3.5.1) specific to the internal transcribed spacer (ITS) regions 1 and 2, which was kindly provided by Dr. Daniel

Rivard (McGill University, Canada), was used. The sequence of the PCR amplicon produced by the primers ITS U 114 (forward) and ITS L 396 (reverse) matched *R. solani* AG3PT isolates in the GenBank NCBI database (Fig. 14).

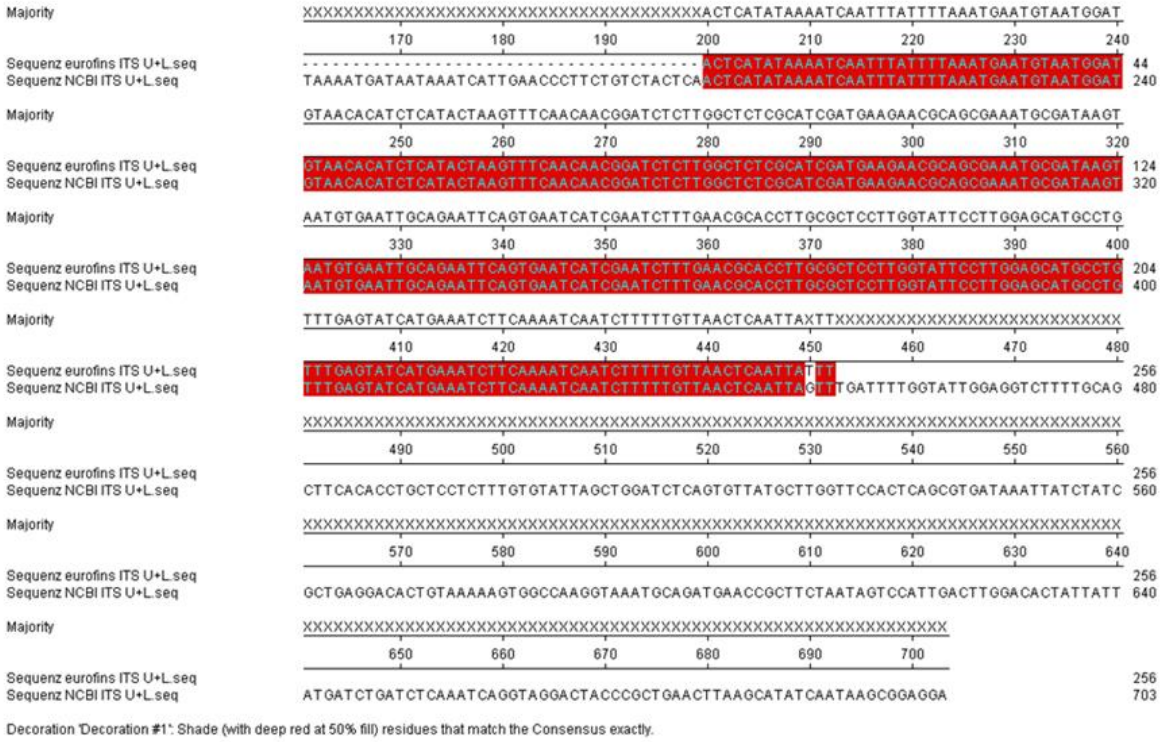


**Figure 14:** Clustel W alignment of sequenced PCR amplicon, generated using primers ITS U 114 (forward) and ITS L 396 (reverse), to the sequence *of Rhizoctonia solani* AG3PT (GenBank NCBI).

The assay sensitivity was tested using a 10-fold dilution calibration series of DNA of *R. solani* AG3PT isolate Ben3. The calibration curve showed that an amount as low as 0.5 pg DNA of *R. solani* AG3PT can be reliably detected in 25 ng of plant DNA using this PCR assay (Fig. 15). The variation between technical replicates was below 0.20 cycles. However, starting quantities with less than 0.5 pg DNA produced high variation in cycle number. Samples without *R. solani* AG3PT DNA gave no fluorescence signal. Melt curve data analysis revealed one single peak at 83 °C and the absence of amplification products of no template controls. In order to test if the presence of potato DNA hampers amplification of ITS primers, dilution calibration curves were generated with and without additional potato DNA (25 ng). A comparison of mean threshold cycle ($C_t$) values and of the slopes for their respective equations did not reveal distinct differences in primer efficiency due to the presence of plant DNA (Fig. 15, Tab. 12). A strong negative linear relationship between $C_t$ value and log 10 of diluted *R. solani* AG3PT DNA was revealed in absence and in presence of potato DNA (Fig. 15).

**Table 12:** Threshold cycle ($C_t$) values from serial dilution of *Rhizoctonia solani* AG3PT DNA in presence and absence of potato DNA (25 ng).

| Starting quantity of fungal DNA [ng] | $C_t$ mean without plant DNA | $C_t$ mean with plant DNA |
|---|---|---|
| **50** | 12.58 | 12.72 |
| **5** | 16.12 | 16.29 |
| **0.5** | 19.70 | 20.15 |
| **0.05** | 23.42 | 23.76 |
| **0.005** | 27.75 | 28.11 |
| **0.0005** | 31.23 | 31.94 |

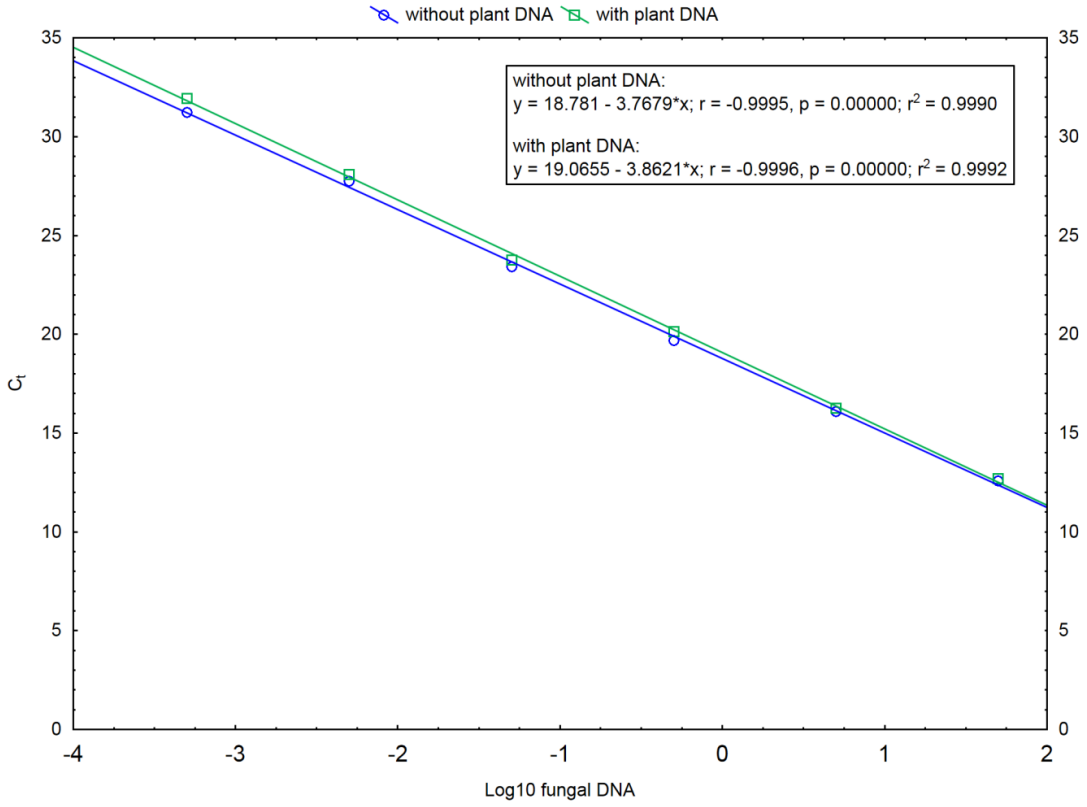


**Figure 15:** Real-time quantitative polymerase chain reaction (qPCR) curves of six-point serial dilution of *R. solani* AG3PT DNA without and with additional potato DNA (25 ng).

### 4.2.3 Quantification of *Rhizoctonia solani* AG3PT in plant tissue

#### 4.2.3.1 Quantity of fungal DNA in plant tissue during early infection stages

*Results of this chapter were published in Plant Pathology (Genzel et al., 2017).*

In addition to the study of the defence response, these root and sprout samples were also analysed regarding a successful colonisation with *R. solani* AG3PT. Therefore, the quantity of pathogen DNA was determined in roots and sprouts of 'Arkula' via absolute quantification at a timescale of 3, 6, and 13 days post inoculation (dpi). Results regarding the study of defence gene expression are described in chapter 4.3.4. Fungal DNA was detected in all samples of inoculated treatments, while there was no fungal DNA detectable in control treatments. Two-way ANOVA ($p = 0.05$, factors: organ and time point) revealed significant differences in the quantity of *R. solani* AG3PT DNA between root and sprout tissue ($p = 0.004$) and between time points ($p = 0.042$) (Fig. 16). An amount of 23.3 pg of *R. solani* AG3PT DNA per ng extracted root DNA was determined at 3 dpi. At 6 dpi, a significantly increased amount of 73.0 pg of *R. solani* AG3PT DNA per ng extracted root DNA was determined, whereas a lower level (25.3 pg) was detected at 13 dpi (Fig. 16). Regarding the sprouts, no significant differences in the quantity of fungal DNA were observed between the time points (Fig. 16). At 6 dpi, a significantly higher density of fungal DNA was found in roots compared to the sprouts.

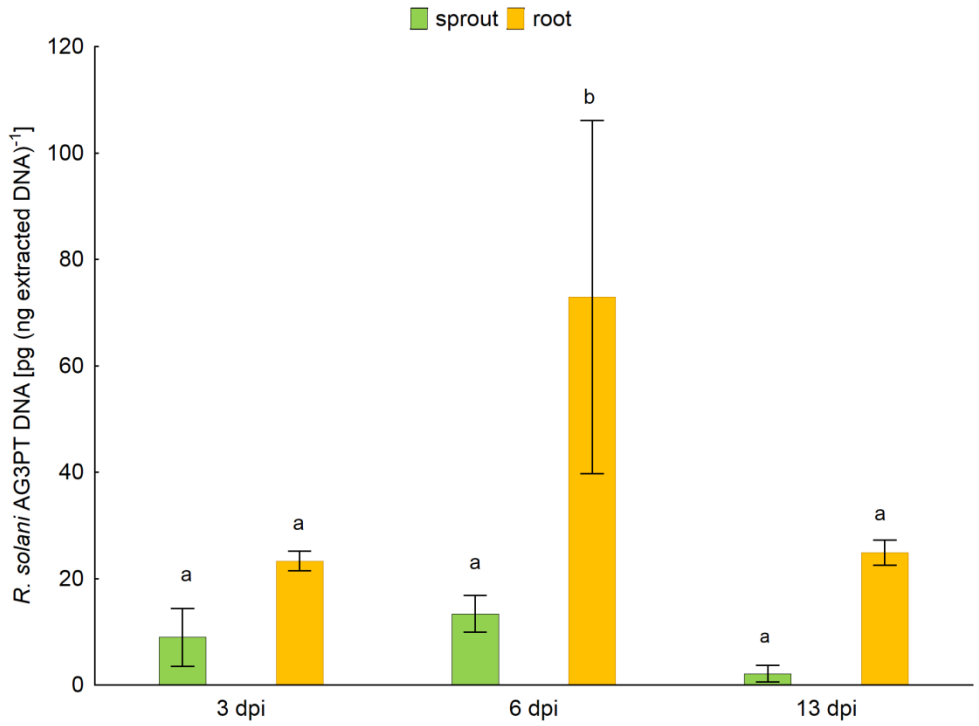


**Figure 16:** Quantity of *R. solani* AG3PT DNA in roots and sprouts of cultivar 'Arkula'. Values represent means of three replicates with standard deviations at three different time points post inoculation [3, 6, 13 days post inoculation (dpi)]. Different letters indicate significant differences between time points and between organs (two-way ANOVA followed by Fisher's LSD, $p \leq 0.05$).

#### 4.2.3.2 Quantity of fungal DNA in potato tissue dependent on cultivar susceptibility

A greenhouse experiment was conducted to test whether, the quantity of *R. solani* AG3PT DNA in potato tissue differs dependent on cultivar susceptibility. Within the time course of the experiment (7 weeks after pathogen inoculation), *R. solani* AG3PT was not once detected in non-inoculated control plants of either cultivar. Three-way ANOVA ($p \leq 0.05$, factors: organ, time point and cultivar) revealed significant effects of the factors organ ($p = 0.027469$), time point ($p < 0.000$), and a combined effect of cultivar, organ, and time point ($p = 0.010001$). At 3 wpi, the quantitative real-time PCR analysis showed a significantly higher quantity of *R. solani* DNA per ng extracted DNA in roots of 'Arkula' (91.4 pg) compared to 'Granola' (32.7 pg) (Fig. 17). However, considering the amount of pathogen DNA in shoots, there was no difference between the two cultivars detectable. At 5 wpi, a decrease in the quantity of fungal DNA in relation to extracted DNA in roots and shoots of both cultivars was noticed. At this time point no significant differences in *R. solani* AG3PT density in roots were revealed between the two cultivars (‘Granola’, 7.4 pg *R. solani* DNA per ng extracted DNA; Arkula, 4.2 pg *R. solani* DNA per ng extracted DNA). Similarly, no significant difference in pathogen density was observed in shoot tissue at 5 wpi. Moreover, the cultivars did not differ significantly regarding the amount of *R. solani* DNA in either root or shoot tissue at 7 wpi (Fig. 17).

The roots of 'Arkula' were significantly more densely colonised at 3 wpi (Fig. 17) compared to shoots. Although not significant, this difference was also observed at 5 and 7 wpi. In contrast to 'Arkula', a similar amount of *R. solani* DNA was found in roots and shoots for 'Granola' at all time points (3, 5, 7 wpi) (Fig. 17). 'Arkula' had already formed tubers at 5 and 7 wpi. The quantity of *R. solani* DNA in tuber tissue was significantly lower compared to roots at 5 wpi (data not shown).

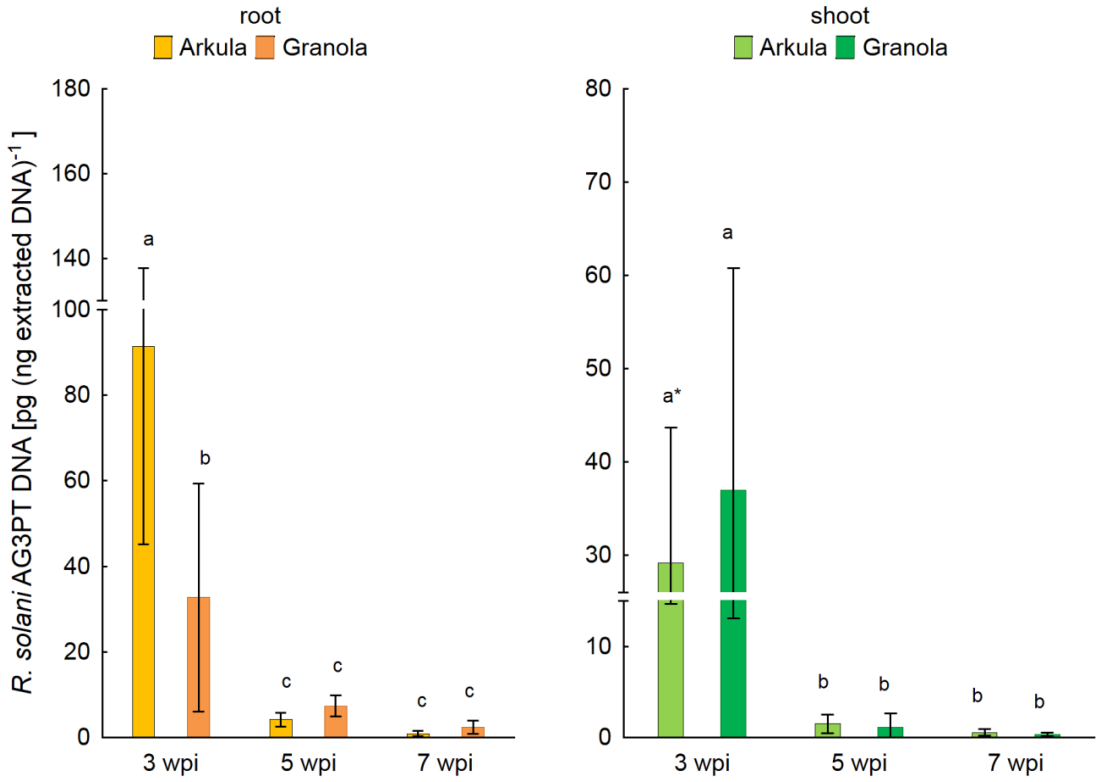


**Figure 17:** Quantity of *R. solani* AG3PT DNA in roots and shoots of the two potato cultivars 'Arkula' and 'Granola'. Values represent means of four replicates with standard deviations at three different time points post inoculation. Different letters indicate significant differences between cultivars and time points [3, 5, 7 weeks post inoculation (wpi)], differences between organs (roots, shoots) are indicated by asterisks (three-way ANOVA followed by Fisher's LSD, $p \leq 0.05$).

Additionally, samples of root and shoot tissue of 'Arkula' and 'Granola', which were collected in the growth chamber experiment at 10 dpi, were analysed regarding the density of *R. solani* AG3PT. Two-way ANOVA ($p \leq 0.05$, factors: cultivar and organ) revealed significant effects of the cultivar ($p = 0.003016$) and the organ ($p = 0.000011$) on the quantity of *R. solani* AG3PT DNA. In shoots of 'Arkula', a significantly higher quantity of pathogen DNA was detected compared to 'Granola' (Fig. 18). Moreover, roots of both cultivars were more extensively colonised than the shoots at this time point.

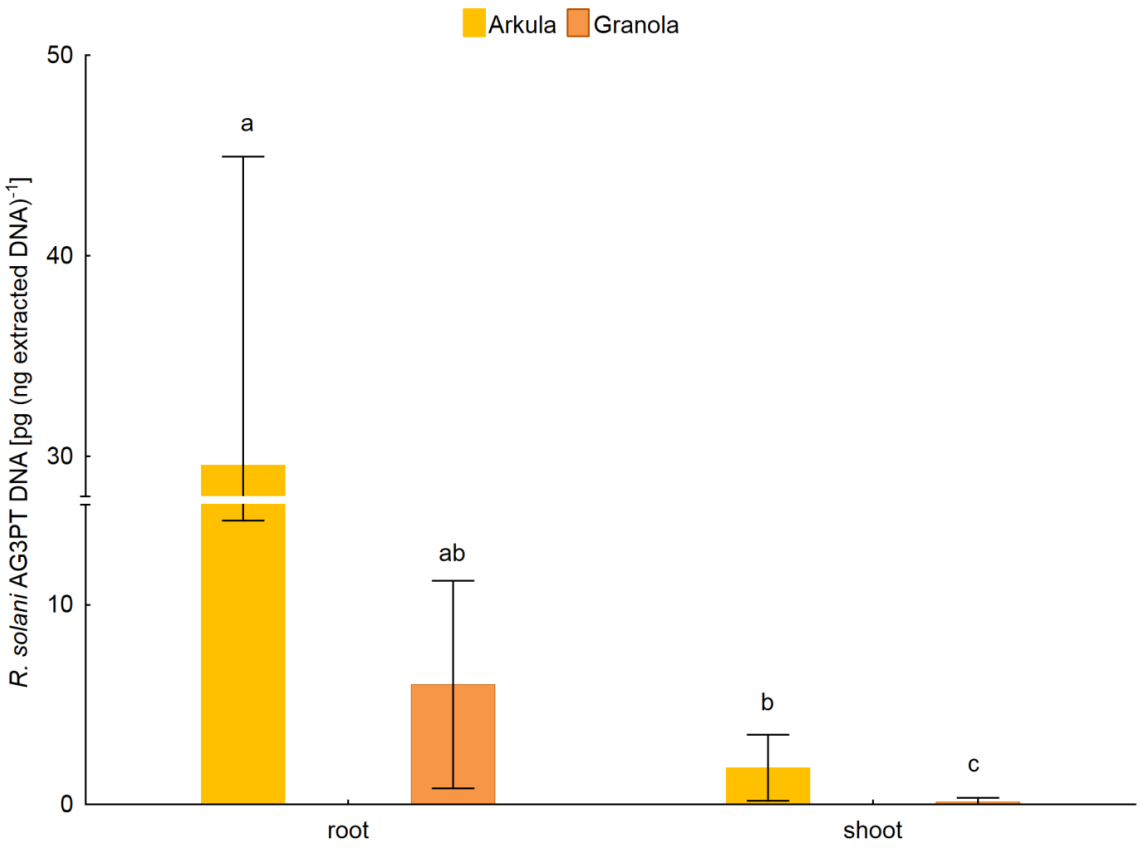


**Figure 18:** Quantity of *R. solani* AG3PT DNA in root and shoot tissue of the potato cultivars 'Arkula' and 'Granola' at 10 days post inoculation (dpi). Values represent means of six replicates with standard deviations. Different letters indicate significant differences between cultivars and organs (two-way ANOVA followed by Fisher's LSD, $p \leq 0.05$).

#### 4.2.3.3 *Rhizoctonia solani* AG3PT density dependent on root sections

Sampled roots were vertically divided into upper, central, and lower sections of equal size for assessment of *R. solani* AG3PT density dependent on the root section. Two-way ANOVA ($p \leq 0.05$, factors: cultivar and section) revealed significant differences in pathogen density between root sections at 3 wpi. Thus, a significantly lower quantity of pathogen DNA in the lower root section of 'Arkula' compared to the upper section was determined (Fig. 19). However, at 5 wpi and 7 wpi no significant differences in the quantity of fungal DNA were detected between the root sections (Fig. 20). For 'Granola', no significant differences between root sections were detected at any time point (Fig. 19 and 20).

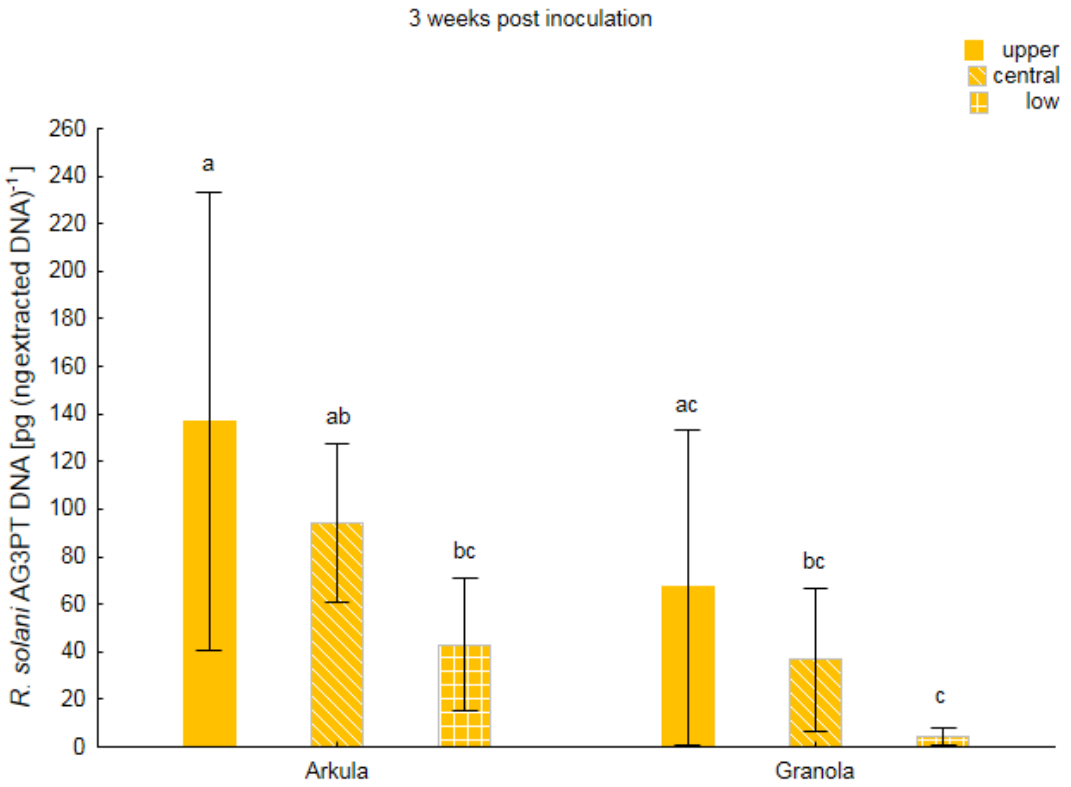


**Figure 19:** Quantity of *R. solani* AG3PT DNA in different root sections of potato in the cultivars 'Arkula' and 'Granola' at 3 weeks post inoculation (wpi). Values represent means of four replicates with standard deviations. Different letters indicate significant differences between sections and cultivars (two-way ANOVA followed by Fisher's LSD, $p \leq 0.05$).

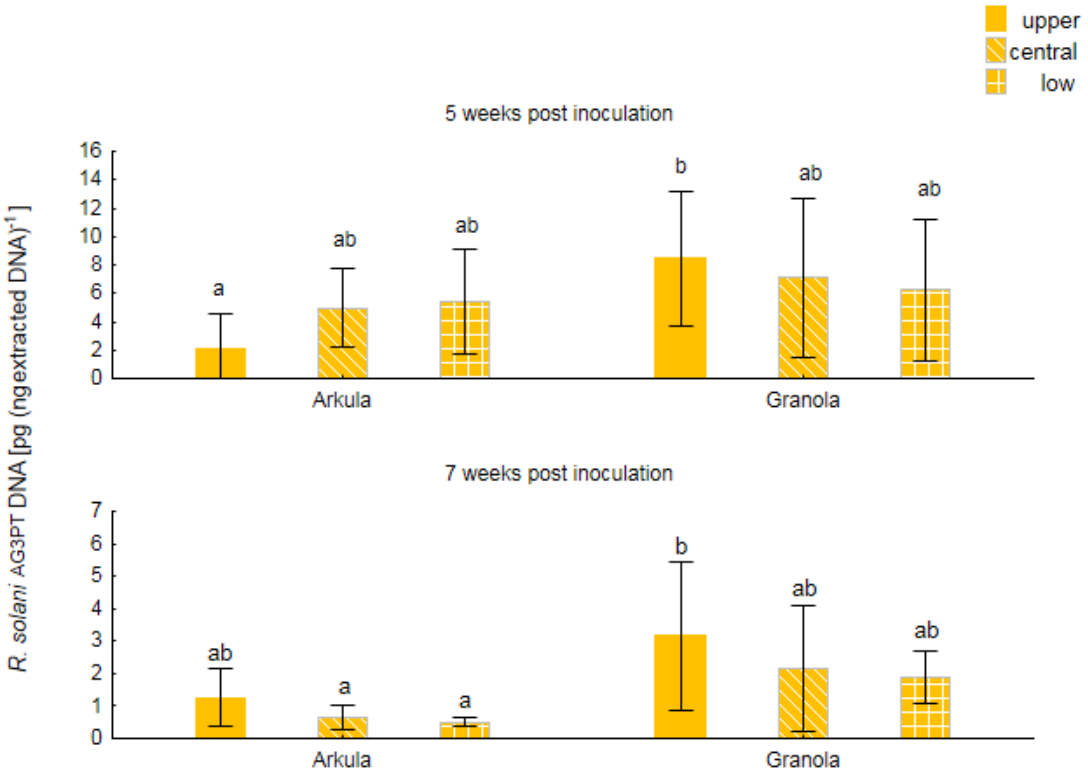


**Figure 20:** Quantity of *R. solani* AG3PT DNA in different root sections of potato in the cultivars 'Arkula' and 'Granola' at 5 and 7 weeks post inoculation (wpi). Values represent means of four replicates with standard deviations. Different letters indicate significant differences between sections and cultivars (two-way ANOVA followed by Fisher's LSD, $p \leq 0.05$).

## 4.3 Expression of defence-related potato genes

The study was carried out to get an impression of the potato plant's response to an inoculation with *R. solani* AG3PT and to investigate the relationship between the expression of basal defence genes and the manifestation of a certain degree of resistance to *R. solani* AG3PT. Therefore, a set of common defence-related genes (*PR-1*, *PR-2*, *PR-3*, *PR-10*, *GST*, *PAL*, *PI2*) was chosen for expression analysis in roots, emerging sprouts, and shoots of potato. Research regarding plant responses to necrotrophic pathogens is still underrepresented and sometimes contradictory. An important role of salicylic acid (SA) during plant defence has been primarily associated with biotrophic pathogens. However, recent research has shown that SA might also be important in resistance to necrotrophic pathogens. Thus, a set of defence-related genes, dependent on SA as well as on jasmonic acid (JA) or ethylene (ET), had been selected (Tab. 14). The first study was carried out to test whether an inoculation with *R. solani* AG3PT affects the expression of the selected defence-related potato genes. Furthermore, we hypothesised that a higher degree of resistance is related to a higher expression level of defence-related genes. Therefore, the transcript level of defence-related genes was determined in two cultivars which differ in their degree of field resistance to *R. solani* AG3PT in a subsequent study. To verify successful inoculation and colonisation by the pathogen, the quantity of *R. solani* AG3PT DNA was determined in roots and sprouts or shoots. Detailed results regarding pathogen density are presented in 4.2.3.

### 4.3.1 Establishment of experimental set-up and choice of sampling dates

Pre-experiments were run prior to the main experiments to test various sampling dates and sample preparation methods. Via microscopy, the time point of the first fungal colonisation of the emerging sprout had been observed and was thus chosen as the earliest sampling date. In order to conduct studies on plant-pathogen interactions, pathogen-free plant material is mandatory. Since conventional seed tubers of potato might be latently infested with various plant pathogens, we decided to use plant material derived from *in vitro* cultures. For the first study, pathogen-free potato mini tubers were utilised to create conditions similar to conventional potato cultivation. In regard to practicability and time requirements, *in vitro*-derived plantlets were used in subsequent studies. Results of gene expression analyses conducted on mini tubers of 'Arkula' were consistent with results of experiments on plantlets, so that the suitability of *in vitro* plantlets for gene expression analyses could be confirmed. Moreover, Bienkowski et al. (2010) showed the suitability of potato plantlets for plant-pathogen interaction assays.

### 4.3.2 RNA extraction

The extraction of RNA using the Rneasy Plant Mini Kit (Qiagen) produced RNA of good quality and purity with absorbance ratios at 260/230 and 260/280 above 2.0. The absence of gDNA was confirmed by performing qRT-PCRs with negative reverse transcriptase controls.

### 4.3.3 Optimisation and validation of primer suitability

All primers used for expression analyses were examined regarding their PCR product size, PCR efficiency (Fig. 21), and melt curve peak. All PCR products were of correct size. Only primers with efficiency between 90 and 110 % and one distinct melt curve peak were selected for further analysis (Tab. 3 and 4).

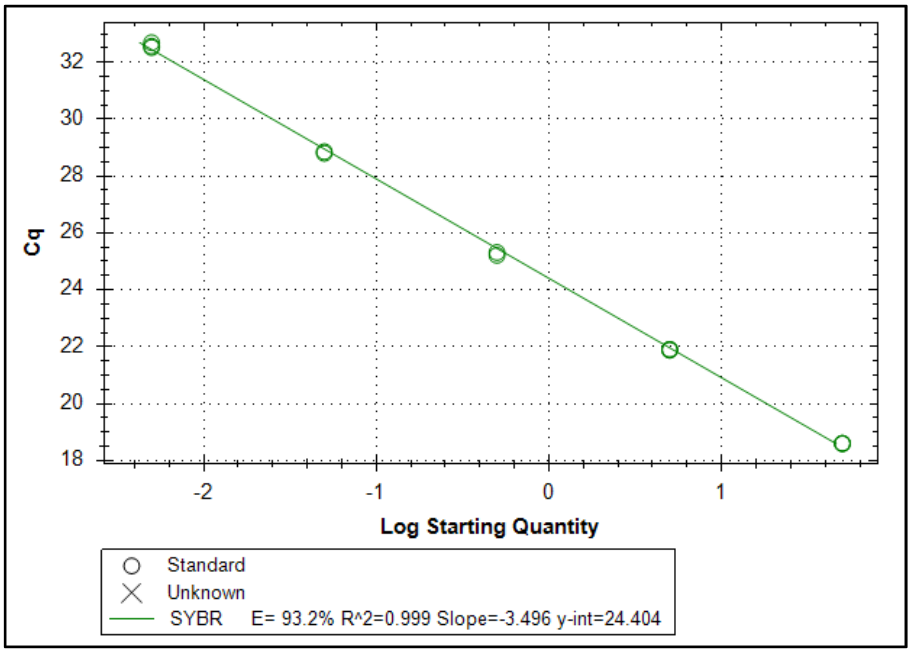


**Figure 21:** Testing of PCR efficiency via dilution calibration curve exemplarily shown for target gene *GST*. The logarithm of the starting quantity is plotted against the threshold cycle $C_t$ (also known as quantification cycle $C_q$). Efficiency was calculated with the Bio-Rad CFX Manager Software 3.0.

*Choice of reference genes used for normalisation*

An assortment of primers for various potato reference genes was tested prior to the main experiments. After checking melt curve data and primer efficiency, several primer pairs had to be excluded. For instance, the primers designed for genes encoding cyclophilin (*CYC*) and adenine phosphoribosyltransferase (*APRT*) produced two melt peaks (Fig. 22), so that they were excluded from further analyses.

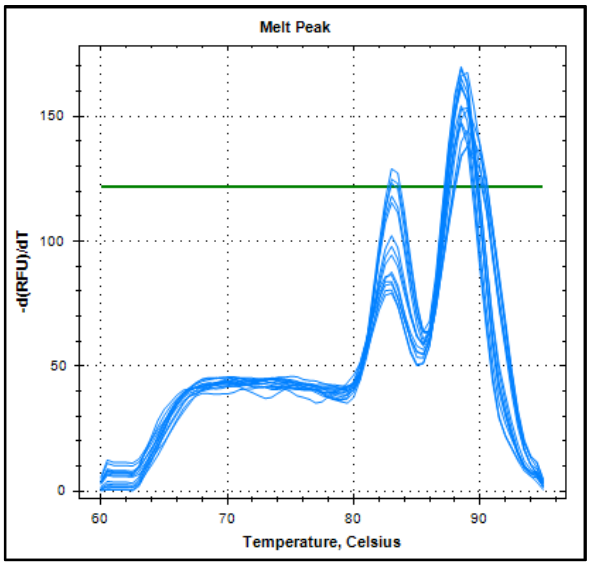


**Figure 22:** Unacceptable melt peak of primers for cyclophilin generated using CFX Manager Software 3.0 (Bio-Rad).

The primers designed for 18S rRNA (*18S*) were excluded as well, because they led to amplification products in no template controls (water control), probably caused by primer dimers. Since the primers used to amplify fragments of the genes for actin (*ACT*), for the elongation factor 1-α (*TEF1*), and for the cytoplasmatic ribosomal protein L2 (*L2*) produced appropriate melt curve peaks, no primer dimers, and showed PCR efficiencies between 90 and 110 %, they were tested in further experiments. Finally, stability values were acceptable for *ACT* and *TEF1* (Tab. 13), while *L2* had to be excluded.

**Table 13:** Stability values [M (internal control gene measure) and CV (coefficient of variation)] of references genes *ACT* and *TEF1*. The root and sprout samples were taken from non-inoculated and inoculated potato cultivar 'Arkula' at three different time points post inoculation.

| Gene | M | CV |
|---|---|---|
| ***ACT*** | 0.5065 | 0.1715 |
| ***TEF1*** | 0.5065 | 0.179 |

### 4.3.4 *Rhizoctonia solani* AG3PT inoculation leads to increased expression of defence-related genes in 'Arkula'

*Results of this chapter were published in Plant Pathology (Genzel et al., 2017).*

#### 4.3.4.1 Transcript level

In order to analyse the impact of *R. solani* AG3PT on the potato's defence, the transcript level of putative SA-dependent and putative JA-dependent (Tab. 14) defence-related genes of potato was determined using quantitative real-time reverse transcription PCR (qRT-PCR).

**Table 14:** Signalling pathways putatively involved in regulation of selected defence-related genes. References are given.

| Gene | Putative pathway | Reference |
|---|---|---|
| ***PR-1*** **(pathogenesis-related protein 1)** | SA | Vleeshouwers et al., 1999<br>van Loon et al., 2006 |
| ***PR-2*** **(basic glucan endo-1,3-betaglucanase)** | JA | Kasprzewska, 2003 |
| ***PR-3*** **(acidic class II chitinase ChtA2)** | SA | Büchter et al., 1997<br>Kasprzewska, 2003 |
| ***PR-10*** **(pathogenesis-related protein 10)** | JA, SA, ABA | Wang et al., 1999<br>Liu and Ekramoddoullah, 2006 |
| ***PI2*** **(proteinase-inhibitor II)** | JA, (ABA) | Lawrence et al., 2014 |
| ***GST*** **(glutathione S-transferase)** | SA | Ghanta et al., 2011 |
| ***PAL*** **(phenylalanine ammonia-lyase)** | JA, ABA, precursor of SA | Gundlach et al., 1992<br>Mauch-Mani and Slusarenko, 1996<br>Asselbergh et al., 2008<br>Kim and Hwang, 2014 |

The selection of defence-related genes was based on literature research (Tab. 14). Since pathogenesis-related (PR) proteins are known to play a role in plant defence, several members of this group were chosen.

The $C_t$ values were analysed using qbase (Biogazelle) software and the CFX Manager software 3.0 (Bio-Rad). Transcript level of selected defence-related genes was studied in roots and underground sprouts which were sampled at a time course (3, 6 and 13 dpi) after inoculation with the fungus in two independent experiments. Three-way ANOVA (factors: pathogen treatment, organ, and time point) revealed significant effects of the pathogen treatment (experiment 1: $p = 0.0002$; experiment 2: $p < 0.0000$), of the organ (experiment 1: $p < 0.0000$; experiment 2: $p < 0.0000$), and of the time point (experiment 1: $p < 0.000$; experiment 2: $p < 0.0000$) on gene expression in both experiments. Within the time course of 13 dpi, a significant up-regulation of all tested defence-related genes in treatments inoculated with *R. solani* AG3PT was noticed. However, the results of the two experiments were inconsistent. Thus, a significant effect of the *R. solani* AG3PT inoculation on the transcript level of the defence-related genes *PR-1*, *PR-2*, *PR-3*, and *PR-10* was found in root and sprout tissue at 3 and 6 dpi in both experiments, whereas the transcript level of the genes *GST* and *PAL* was only affected in the first experiment. The expression level of the genes *PR-1* and *PR-2* was significantly increased in roots and sprouts treated with *R. solani* AG3PT in both experiments (Fig. 23A), except for the gene *PR-1*, whose expression was not significantly altered due to the pathogen treatment at 3 dpi in either root or sprout in experiment 1 (Fig. 23A). A significant increase of *PR-3* transcripts in the presence of *R. solani* AG3PT was revealed in roots and sprouts in both experiments at 3 and 6 dpi, except for the roots at 6 dpi in experiment 1 and for the sprouts at 3 dpi in experiment 2. In both experiments, the expression level of the gene *PI2* was significantly increased by the pathogen at 3 dpi in the roots but not in the sprouts. In addition, pathogen treatment resulted in a significant up-regulation of *PR-10* at 3 and 6 dpi in roots and sprouts in both experiments. However, the experiments occasionally produced inconsistent results. The expression level of the gene *PR-10* was not affected by the pathogen treatment in either roots or sprouts at 3 dpi in experiment 2, in sprouts at 6 dpi in experiment 2 and in roots at 6 dpi in experiment 1. Inconsistencies were also observed for the genes *GST* and *PAL*. A significant effect of the pathogen was only observed in experiment 1, for *GST* in sprouts and for *PAL* in roots and sprouts at 3 dpi.

A significant influence of the time point on the expression level was observed as well. In experiment 1, the genes *PR-3* and *PAL* were significantly up-regulated in non-inoculated roots at 6 dpi compared to 3 dpi, whereas the gene *PR-2* was down-regulated in sprouts. No changes in expression level were revealed in experiment 2 until 6 dpi, except for the decreased transcript level of the gene *PI2* observed in roots at 6 dpi compared to 3 dpi.

Within the time course of the experiment (from 3 to 13 dpi) the expression level of *PR-2*, *PAL* and *PR-10* significantly increased in the roots in both treatments (with and without *R. solani* AG3PT inoculation) in experiment 1. In the same experiment, transcript levels of *PR-3*, *PR-1* and *PI2* were significantly increased at 13 compared to 3 dpi in non-inoculated roots (Fig. 23A & B). In experiment 2, transcript levels of the genes *PR-1* and *PAL* were significantly increased at 13 dpi compared to 3 dpi in non-inoculated roots. In contrast to the root samples, a significantly decreased transcript level of *PR-2*, *GST,* and *PAL* in both treatments (with and without *R. solani* AG3PT inoculation) and of *PR-3*, *PR-10* in inoculated treatments was detectable in sprouts over the time course of the experiment 1. No significant effects of the time on the gene expression level in inoculated sprouts were found in experiment 2. The defence-related gene *PI2* was up-regulated in roots and sprouts in both experiments at 13 dpi compared to 3 dpi. While this difference was significant in *R. solani* AG3PT-inoculated as well as non-inoculated roots and/or sprouts of experiment 1, in experiment 2 no significant time-dependent change was detectable at 13 dpi in either root or sprout samples for *PI2* (Fig. 23B).

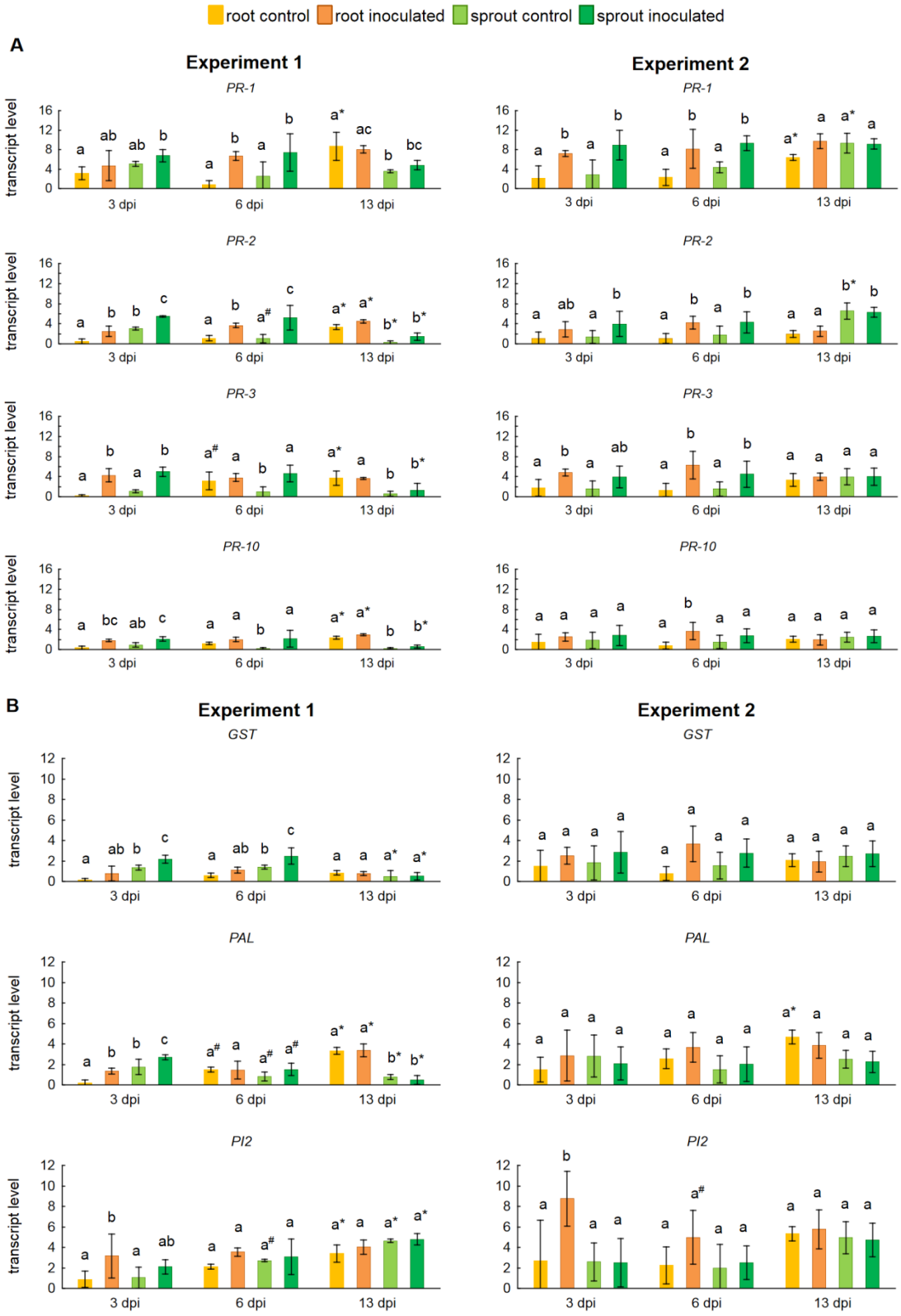


**Figure 23:** Quantitative expression of defence-related genes *PR-1*, *PR-2*, *PR-3*, *PR-10* (A) and *GST*, *PAL*, *PI2* (B) in potato roots and sprouts inoculated and non-inoculated (control) with *Rhizoctonia solani* AG3PT at 3, 6 and 13 dpi. Expression was normalised to reference genes *ACT* and *TEF1*. Normalised data are presented as means of three replicates with standard deviations. Different letters indicate significant differences between treatments (inoculated, control) and organs (root, sprout). Significant differences between time points 3 and 6 dpi are indicated by # and between 3 and 13 dpi by asterisks (three-way ANOVA followed by Fisher's LSD, $p \leq 0.05$). Data were obtained from two individual experiments.

#### 4.3.4.2 Comparison of transcript and protein level of PR-1, PR-2, PR-3, and PR-10

In order to verify the effect of *R. solani* AG3PT on expression of those *PR* genes, which had been detected at transcript level, a protein gel blot analysis was conducted for PR-1, PR-2, PR-3, and PR-10. Band intensities of PR proteins were normalised to total protein load of the respective sample. Similar to the analysis of transcript levels, three-way ANOVA (factors: pathogen treatment, organ, and time point) revealed a significant impact of pathogen treatment ($p = 0.000122$), organ ($p = 0.000284$), and time point ($p = 0.018854$) on the abundance of PR proteins (data not shown).

In roots, an increase in protein abundance of PR-1 was observed over time and an elevated effect of the inoculation was observed at 13 dpi. The abundance of PR-2 decreased within the time course of the experiment, however, pathogen treatment resulted in an increased abundance of PR-2 at 3 and 6 dpi. Similar to PR-1, the levels of the proteins PR-3 and PR-10 generally increased during the experiment. An effect of *R. solani* AG3PT on the protein level of PR-3 was found at 6 dpi, where protein abundance in roots of inoculated plants was higher than in control plants. In sprouts however, the abundance of PR-1 and PR-3 decreased over the time course of the experiment. No treatment effect was found for expression of PR-1, while PR-3 levels were significantly higher in sprouts of inoculated plants at 3 and 13 dpi. In addition, the protein level of PR-2 was significantly increased in inoculated compared to control sprouts at 6 dpi. Results of transcript and protein quantification were compared in a heat map showing normalised values of transcript and protein abundances (Fig. 24). The ontogenetic increase in abundance of PR-1, PR-3, and PR-10 in roots and their decreasing abundance in sprouts was found to overlap at transcript and protein level to a great extent. In contrast to the results of the protein analysis, PR-2 transcript abundance increased in roots and decreased in sprouts over time. Pathogen-induced changes in expression levels of PR-2 and PR-3 in roots and PR-3 and PR-10 in sprouts were similar at transcript and protein level. In general, the pathogen-induced changes were more pronounced at transcript level than at protein level.

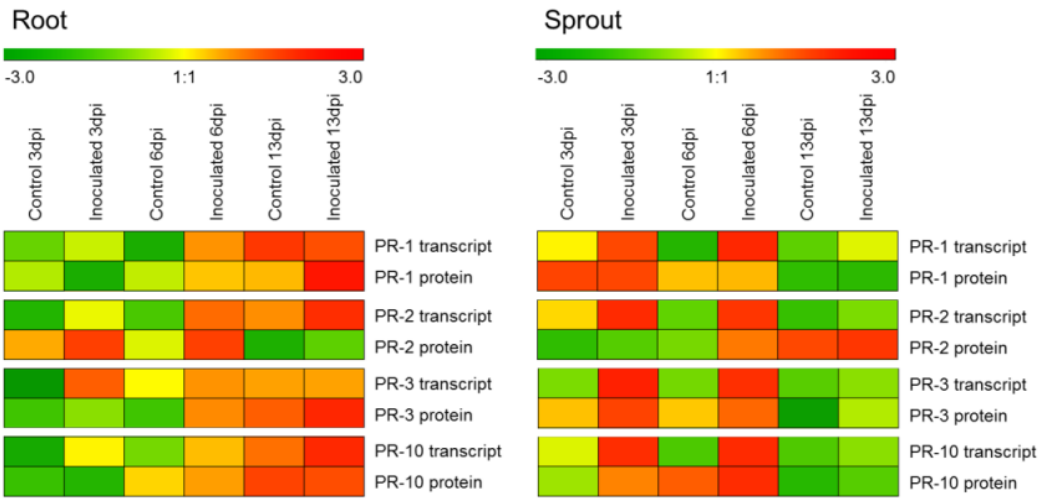


**Figure 24:** Normalised transcript and protein abundances for PR-1, PR-2, PR-3, and PR-10 in roots and sprouts of plants inoculated and non-inoculated (control) with *R. solani* AG3PT at 3, 6 and 13 dpi. Rows display levels of transcript and proteins using colour coding based on relative scale (-3.0 to +3.0).

### 4.3.5 Expression of defence-related genes in potato cultivars with different degrees of resistance to black scurf disease

#### 4.3.5.1 Transcript level

Quantitative real-time reverse-transcription PCR (qRT-PCR) was used to analyse the expression of the pathogenesis-related (*PR*) genes *PR-1*, *PR-2*, *PR-3*, *PR-10* and the defence-related genes *GST*, *PAL,* and *PI2* in the potato cultivars 'Arkula' and 'Granola', which had shown different levels of susceptibility to *R. solani* AG3PT in the field. The gene expression analyses (including RNA extraction, cDNA synthesis and qRT-PCR) were run according to the established method described in 3.5.6. The stability values of reference genes for the normalisation of the target gene expression were in an acceptable range (Tab. 15).

**Table 15:** Stability values [M (internal control gene measure) and CV (coefficient of variation)] of references genes *ACT* and *TEF1*. The root and shoot samples were taken from non-inoculated and inoculated potato cultivars ‘Arkula’ and ‘Granola’.

| Gene | M | CV |
|---|---|---|
| ***ACT*** | 0.8336 | 0.2824 |
| ***TEF1*** | 0.8336 | 0.2877 |

In this study, *in vitro*-derived plantlets of 'Arkula' and 'Granola' were inoculated with *R. solani* AG3PT (isolate Ben3) and at 10 days post inoculation (dpi) whole roots and shoots were sampled. The experiment was performed twice. Two-way ANOVA (factors: cultivar, pathogen treatment) revealed significant differences in the transcript level of defence-related genes between the two cultivars in both experiments (experiment 1: $p = 0.000008$, experiment 2: $p = 0.000692$). Generally, a significantly higher transcript level of defence-related genes was detected in 'Granola' compared to 'Arkula'. In both experiments, the constitutive expression of *PR-2*, *PR-3*, and *PAL* was significantly higher in roots and shoots of the less susceptible cultivar 'Granola' compared to 'Arkula' (Fig. 25). Furthermore, a significantly higher constitutive transcript level of *PR-1*, *PR-10,* and *GST* was found in shoots and of *PI2* in roots of 'Granola' compared to cultivar 'Arkula' (Fig. 25). The transcript level of *PAL* was also significantly higher in inoculated shoots of 'Granola' compared to 'Arkula' in both experiments. In addition, *PR-2* and *PR-3* were significantly higher expressed in pathogen-treated roots and shoots of 'Granola' in the first experiment. In contrast, in experiment 2 there was no significant difference between the cultivars detectable in inoculated treatments regarding these two genes.

Pathogen treatment led to an up-regulation of the defence-related genes *PR-2* and *PI2* in roots of the cultivar 'Arkula' in both experiments at 10 dpi. In inoculated shoots of 'Arkula', the genes *PR-2*, *PR-3*, *PR-10,* and *PAL* were significantly higher expressed compared to control shoots in both experiments. In addition, the transcript level of *PR-10* was significantly increased in inoculated roots of 'Arkula' in the experiment 1, whereas the genes *PR-3* and *GST* showed an increased transcript level in inoculated roots of the experiment 2 (Fig. 25). In the second experiment, the transcript levels of *PR-1* and *GST* were significantly increased due to the inoculation in shoots of the cultivar 'Arkula' as well. In contrast, an increased transcript level in *R. solani* AG3PT-inoculated plants of 'Granola' was only detected for *PR-2* in roots in the second experiment (Fig. 25A). Interestingly, the transcript level of *PR-1* was significantly decreased in inoculated shoots of 'Granola' compared to the control in the first experiment (Fig. 25A). The gene *GST* was also significantly down-regulated in inoculated shoots and roots of 'Granola' in the first experiment. Although not statistically supported, this trend was seen again in the second experiment.

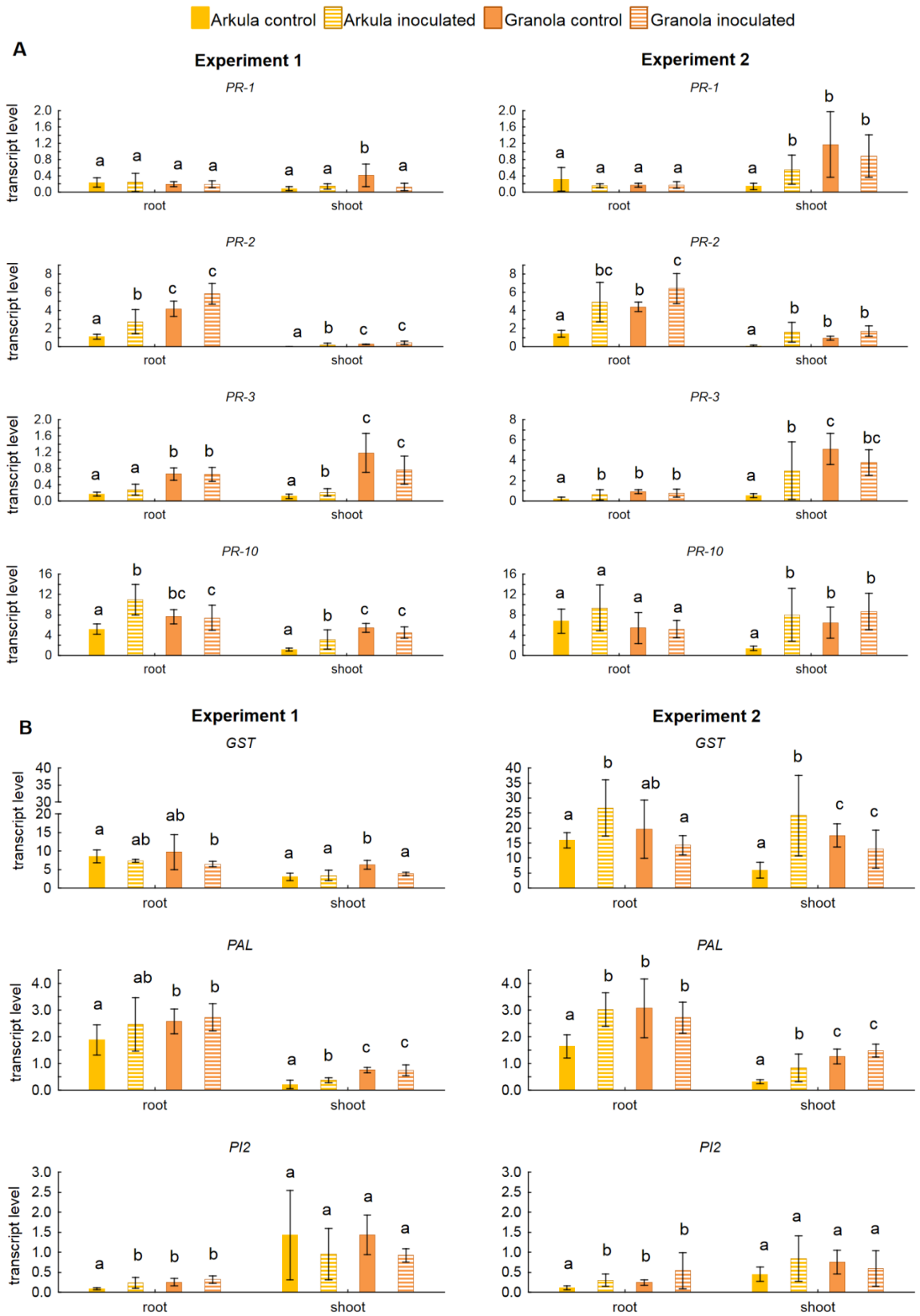


**Figure 25:** Transcript level of defence-related genes *PR-1*, *PR-2*, *PR-3*, *PR-10* (A) and *GST*, *PI2*, *PAL* (B) determined in potato roots and shoots of cultivars 'Arkula' and 'Granola' without (control) and with inoculation of *R. solani* AG3PT at 10 dpi. Target genes were normalised to reference genes *ACT* and *TEF1*. Data are presented as means of six or five replicates with standard deviations. Different letters indicate significant differences between treatments (inoculated, control) and cultivars (two-way ANOVA followed by Fisher's LSD, $p \leq 0.05$). Data were obtained from two individual experiments.

### 4.3.5.2 Comparison of transcript and protein level of PR-1, PR-2, PR-3, and PR-10

In both cultivars, protein abundances of PR-1, PR-2, PR-3, and PR-10 were additionally determined in root and shoot samples of the first experiment. Similar to the results of the qRT-PCR, a significantly higher constitutive abundance of PR-2 was determined in roots and shoots of the less susceptible cultivar 'Granola' (Fig. 26). Moreover, the protein PR-1 was only detectable in shoots of 'Granola' but not in shoots of highly susceptible 'Arkula'. This is in accordance with a significantly higher constitutive transcript level of *PR-1* in shoots of 'Granola'. In contrast to results of the qRT-PCR, there was no significant difference in protein abundance of PR-3 between the two cultivars (Fig. 26). Surprisingly PR-10 was not detectable at protein level in shoots of either cultivar.

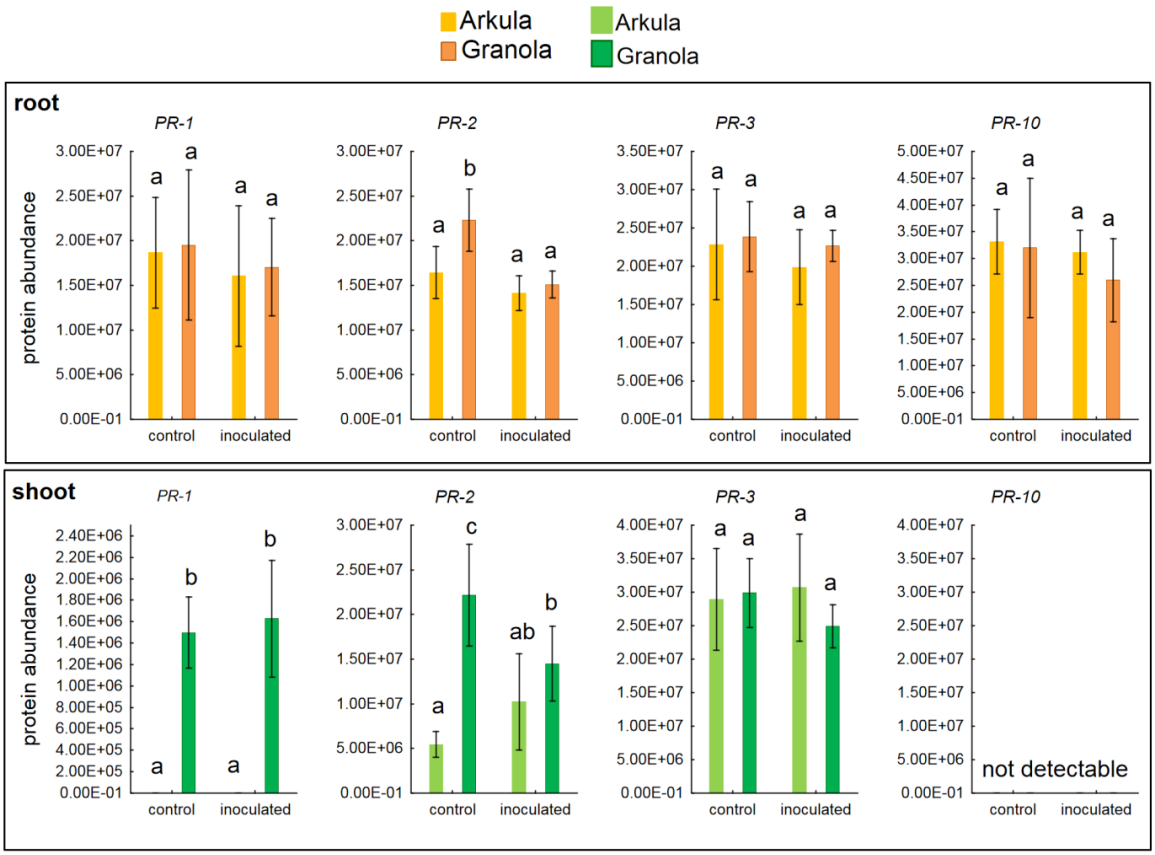


**Figure 26:** First experiment in the growth chamber: Protein abundance of PR-1, PR-2, PR-3, PR-10 in potato roots and shoots of 'Arkula' and 'Granola' without (control) and with inoculation of *R. solani* AG3PT at 10 dpi. Band intensities were quantified using ImageJ software (http://imagej.nih.gov/ij/index.html) and normalised to the total protein stained amido black 10B signals. Data are presented as means of six or five replicates with standard deviations. Different letters indicate significant differences between treatments (inoculated, control) and cultivars (two-way ANOVA followed by Fisher's LSD, $p \leq 0.05$).

A significant effect of the pathogen treatment on the abundance of PR proteins was only detected for 'Granola'. Thus, a significant decrease of PR-2 accumulation was revealed in inoculated roots and shoots compared to the control. However, at transcript level, the fungal

treatment had no significant effect on the expression of the gene *PR-2* in 'Granola' in this experiment. *R. solani* AG3PT-mediated changes in *PR* gene expression determined via qRT-PCR and via Western blot analysis were compared in a heat map (Fig. 27). Colour coding represents expression changes based on the ratio of the transcript or protein abundance in inoculated plants to the transcript or protein abundance in control plants. In roots, there was no significant influence of the pathogen treatment on the expression of *PR-1* detectable, either at protein or at transcript level. Unfortunately, the results of the Western blot analysis corresponded poorly to the results of the qRT-PCR analysis. An increase of *PR-2* expression in inoculated shoots of 'Arkula' compared to the control was observed with both methods, however, only statistically supported at transcript level. The transcript level of *PR-10* was significantly increased in inoculated roots and shoots of 'Arkula' compared to the respective controls. Yet, the PR-10 protein was neither detectable in shoots of 'Arkula' nor of 'Granola'. In summary, changes in expression levels of *PR* genes due to pathogen treatment were less pronounced at protein level.

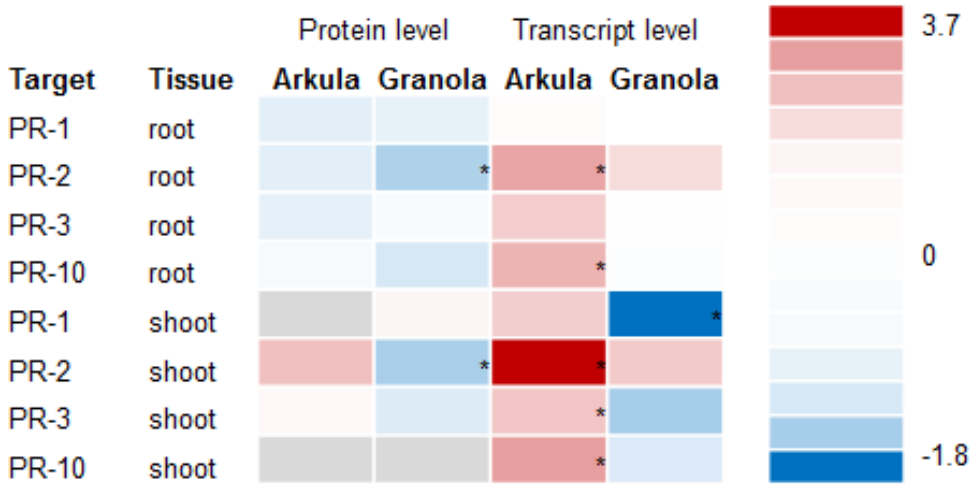


**Figure 27:** Expression changes of PR proteins calculated as ratio (*R. solani* AG3PT-inoculated/non-inoculated) and shown for protein and transcript level. Transcript level was determined via qRT-PCR and normalisation of $C_t$ values of *PR* genes to $C_t$ values of reference genes *ACT* and *TEF1*. Protein abundance was determined via Western blot analysis. Band intensities were quantified using ImageJ software (http://imagej.nih.gov/ij/index.html). Data were transformed into Log2 fold changes (ratio inoculated/non-inoculated) of expression. Colours represent either up (red) or down-regulation (blue) according to the colour coding on the right. Significant differences between treatments (non-inoculated, inoculated) are indicated by asterisks (two-way ANOVA followed by Fisher's LSD test, $p \leq 0.05$). Non-detectable proteins are indicated by grey fields.

### 4.3.6 Impact of *Rhizoctonia solani* AG3PT on plant growth

The influence of an infection with *R. solani* AG3PT on plant growth of potato and disease symptoms was determined recording fresh mass (FM) of roots, shoots, leaves, and tubers as well as the incidence of lesions and sclerotia formation in the growth chamber and the

greenhouse experiment. In the greenhouse experiment, no effect of the pathogen treatment on growth characteristics was observed for ‘Granola’, whereas the leaf mass and tuber mass of the cultivar ‘Arkula’ were significantly reduced due to inoculation with *R. solani* AG3PT at 7 wpi (Fig. 28). Moreover, ANOVA analysis (factors: cultivar, pathogen treatment) revealed significant differences in growth characteristics between both cultivars (Fig. 28). At 3 wpi, inoculated ‘Arkula’ had a significantly higher shoot mass than ‘Granola’. In contrast, at 7 wpi non-inoculated ‘Granola’ showed a significantly higher shoot mass than ‘Arkula’. The leaf mass of ‘Arkula’ was also significantly higher, for non-inoculated treatments at 5 and 7 wpi and for inoculated treatments at 3 and 5 wpi. However, ‘Granola’ had a higher root mass than ‘Arkula’ at 7 wpi in both treatments. Interestingly, non-inoculated and inoculated plants of ‘Arkula’ had already produced tubers at 3 wpi, whereas no tubers were found in the case of ‘Granola’ at any time point.

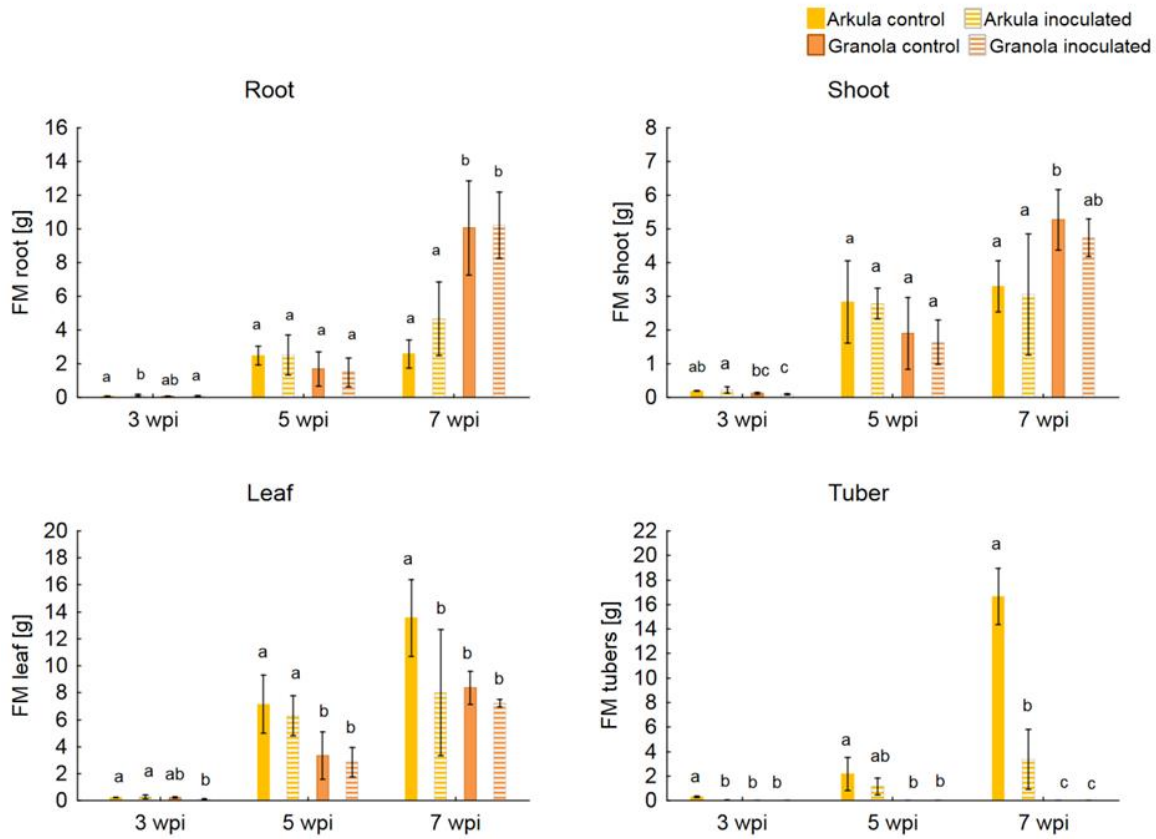


**Figure 28:** Effect of *Rhizoctonia solani* AG3PT on fresh mass (FM) of root, shoot, leaf, and tuber at three sampling dates (3, 5 and 7 weeks post inoculation, wpi) of the cultivars 'Arkula' and 'Granola' grown under greenhouse conditions. Values represent means of four replicates with standard deviation. Different letters represent significant differences between cultivars and treatments (control, inoculated) per time point (two-way ANOVA followed by Fisher’s LSD, $p \leq 0.05$).

In the growth chamber experiment, no significant effect of the pathogen on plant growth characteristics was detectable for either cultivar at 10 dpi. However, comparing growth characteristics of the cultivars significant differences were detected at this time point (Fig. 29). For instance, the root biomass of 'Granola' was again significantly higher compared to 'Arkula', while leaf biomass and tuber number were significantly higher for 'Arkula' than 'Granola'.

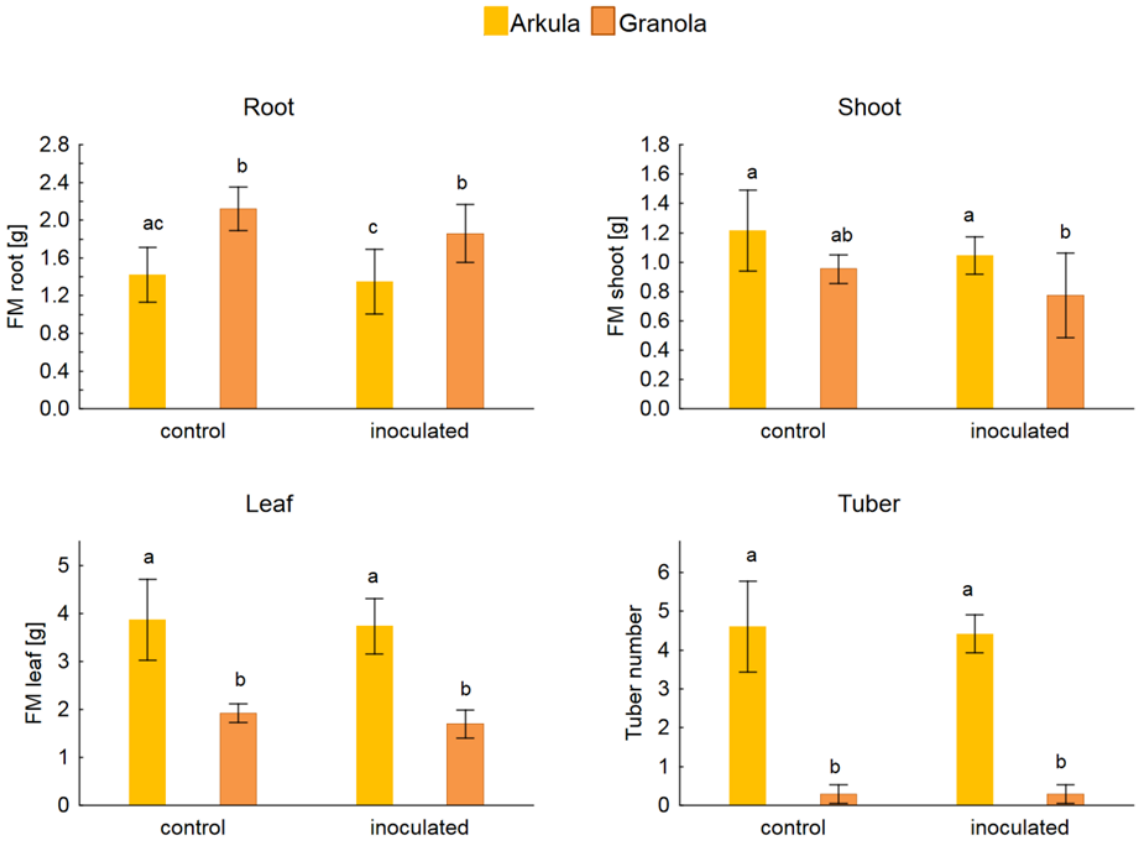


**Figure 29:** Impact of *R. solani* AG3PT infection on fresh mass (FM) of root, shoot, leaf, and, tuber at 10 days post inoculation (dpi) of the cultivars 'Arkula' and 'Granola' grown in the growth chamber. Values represent means of six replicates with standard deviation. Different letters represent significant differences between cultivars and treatments (control, inoculated) (two-way ANOVA followed by Fisher's LSD, $p \leq 0.05$).

In addition to plant growth characteristics, disease symptoms caused by *R. solani* AG3PT were assessed on shoots of both cultivars grown in the greenhouse as well as in the growth chamber at all sampling dates. However, no significant differences were observed comparing the incidence of symptoms on both cultivars. Besides, the formation of sclerotia on roots of both cultivars was occasionally detected at all three sampling dates in the greenhouse and the growth chamber.

## 4.4 Content of plant secondary metabolites in potato cultivars with different degrees of resistance to black scurf disease

### 4.4.1 Non-targeted analysis of plant secondary metabolites

A non-targeted analysis using UHPLC-ToF-MS was conducted to compare metabolite profiles in roots and shoots of two potato cultivars with different degrees of susceptibility to *R. solani* AG3PT in the field, before and after inoculation with the pathogen.

In general, a diversity of plant metabolites produced by 'Arkula' and 'Granola' was revealed with a number of differences between cultivars, organs, and treatments. The principle component analysis showed different clustering between cultivars and treatments in roots and shoots (Fig. 30A and B). Comparing mass spectra with the literature (Friedmann, 2006; Tai et al., 2014) allowed the identification of several agents of the alkaloid biosynthesis pathway (Tab. 16). In addition to glycoalkaloids, several phenylpropanoid compounds, e.g. quercetin and kaempferol associated, were identified (Tab. 16).

**Table 16:** Metabolites with *m/z* matching with known glycoaklaloids and phenylpropanoids. Compounds in the Mass Hunter Metlin PCD in-house databases that matched the *m/z* are listed along with their monoisotopic mass of the $[M+H]^+$ or $[M - H3O + H]^+$ molecular ion.

| Associated pathway | Compound | Monoisotopic mass |
|---|---|---|
| Alkaloid biosynthesis | α-solanine | 868.5053 $[M + H]^+$ |
| | β-solamarine | |
| | solasonine | 866.48956 $[M - H_3O + H]^+$ |
| | β-tomatidine | |
| | teinemine | 398.34222 $[M - H_3O + H]^+$ |
| **Phenylpropanoid biosynthesis** | caffeate (3,4-Dihydroxy-trans-cinnamate) | 163.03912 $[M - H_3O + H]^+$ |
| | coumaric acid (hydroxycinnamate) | 147.04436 $[M - H_3O + H]^+$ |
| | ferulic acid (4-hydroxy-3-methoxycinnamic acid) | 177.0553 $[M - H_3O + H]^+$ |
| | sinapic acid (3,5-Dimethoxy-4-hydroxycinnamic acid) | 207.06557 $[M - H_3O + H]^+$ |
| | kaempferol 3-[2Gal-(6'''-feruloylglucosyl)-robinobioside] | 933.26556 $[M + H]^+$ |
| | quercetin-3-rutinoside (rutin) | 610.1534 $[M + H]^+$ |

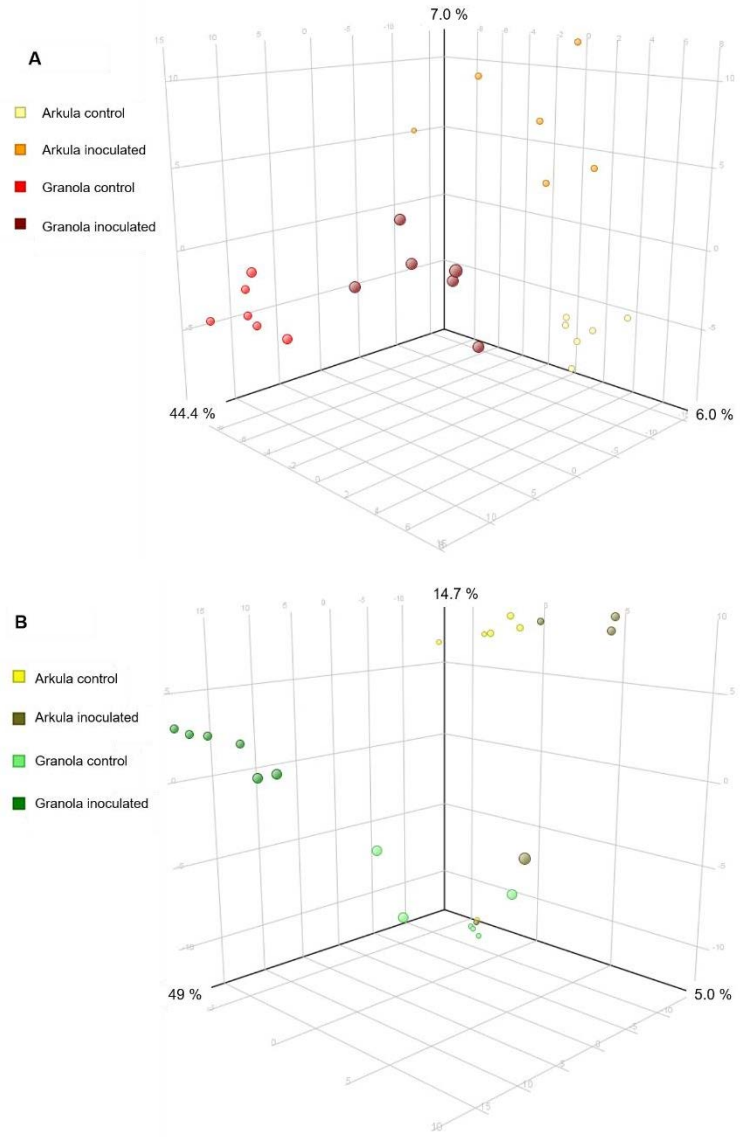


**Figure 30:** Principal component analysis ($p \leq 0.01$, fold change $\geq 2$) based on metabolites differentially present in *Rhizoctonia solani* AG3PT-inoculated and non-inoculated (control) roots (A) and shoots (B) of cultivars 'Arkula' and 'Granola' at 10 days post inoculation. Generated in positive ionization mode.

### 4.4.2 Targeted analyses of selected plant secondary metabolites and their biosynthetic genes

These analyses aimed at studying a possible relation between the contents of selected secondary plant metabolites and the degree of resistance to *R. solani* AG3PT in potato. Thus, the cultivars 'Arkula' (highly susceptible) and 'Granola' (less susceptible) were compared regarding their contents of carotenoids, of phenylpropanoids and, of the glycoalkaloids α-chaconine and α-solanine. Furthermore, transcript levels of selected biosynthetic key enzymes were investigated. The following figure (Fig. 31) summarises compounds which were investigated using targeted analyses and briefly illustrates connections between different pathways where transcript accumulation for the corresponding enzymes was analysed.

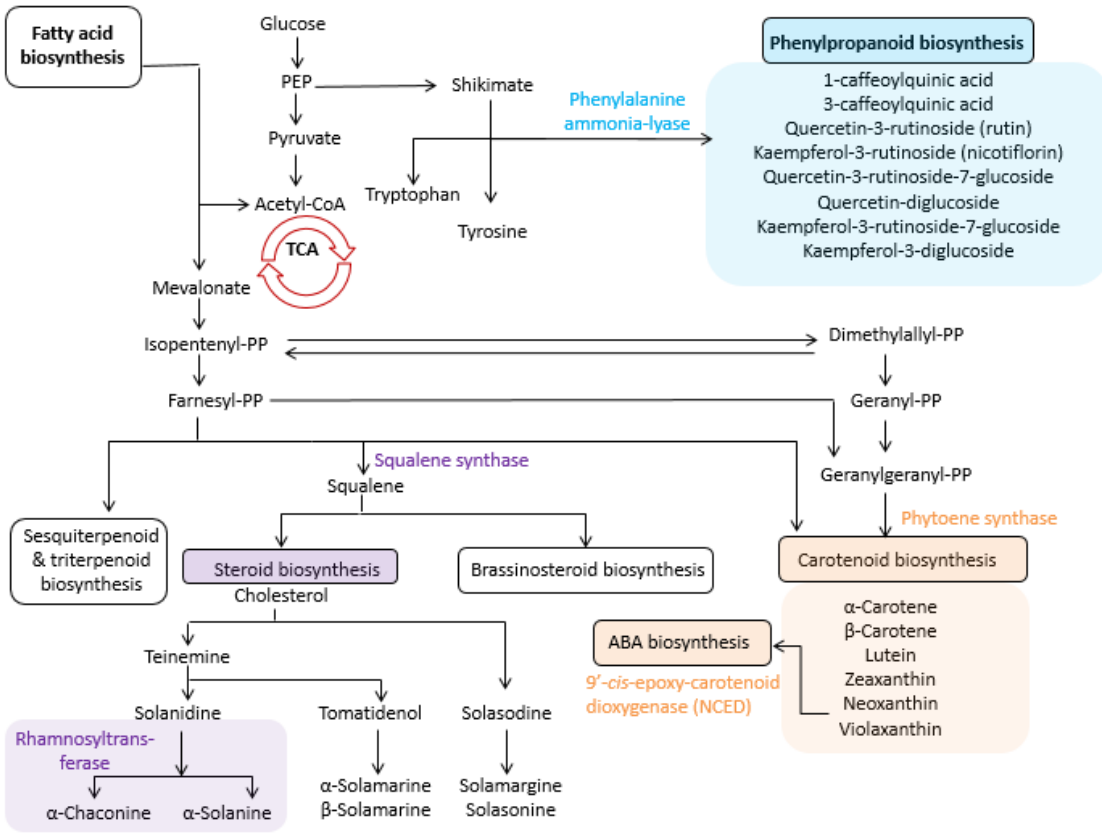


**Figure 31:** Simplified visualisation of biosyntheses of phenylpropanoid compounds, carotenoids, and steroidal glycoalkaloids of potato. Highlighted compounds and enzymes were investigated in the potato cultivars 'Arkula' and 'Granola' prior to and after inoculation with *R. solani* AG3PT.

*Carotenoids*

Isoprenoid compounds are a family of highly diverse low-molecular-mass products (Bouvier et al., 2005). In our study, members of this family, the carotenoids (this chapter) and steroidal glycoalkaloids (next chapter), were investigated in the potato cultivars 'Arkula' and 'Granola'. In the process of determining the content of carotenoids in both cultivars, it was observed that 'Arkula' and 'Granola' showed a different response to an inoculation with *R. solani* AG3PT (Fig. 32). Thus, genes for enzymes which are key elements in the carotenoid biosynthesis pathway were chosen for further analysis via qRT-PCR (Fig. 33). This included a gene for a phytoene synthase (*PSY*), key enzyme in the carotenoid biosynthesis. Additionally, the expression of a 9-*cis*-epoxy-carotenoid dioxygenase-encoding gene (*NCED*), which is a carotenoid cleavage oxygenase and an important enzyme of the abscisic acid (ABA) biosynthesis, and the expression of an ABA receptor gene (*ABAR*) were studied.

Analysis of carotenoids revealed significant differences between cultivars regarding the constitutive content and the content of carotenoids after pathogen treatment (Fig. 32) The constitutive contents of the carotenoids β-carotene, lutein, neoxanthin, and violaxanthin were significantly higher in roots and shoots of 'Arkula' compared to 'Granola'. Additionally, zeaxanthin was significantly higher in shoots of 'Arkula' (Fig. 32). However, at 10 dpi with *R.*

*solani* AG3PT, α-carotene and zeaxanthin were higher in inoculated roots of ‘Granola’. Besides, the chlorophylls a and b were significantly higher in shoots of ‘Arkula’ compared to shoots of ‘Granola’. There were no further differences between cultivars in inoculated treatments detectable. The comparison of non-inoculated and *R. solani* AG3PT-inoculated treatments revealed a significant increase in the carotenoid content in the case of ‘Granola’, while there was a decrease observed for ‘Arkula’. In inoculated roots of ‘Granola’, β-carotene, lutein, zeaxanthin, neoxanthin, and violaxanthin were significantly higher compared to non-inoculated roots. In contrast, lutein, neoxanthin, and violaxanthin were significantly decreased in inoculated roots of ‘Arkula’. In shoots, only the contents of zeaxanthin and violaxanthin were significantly altered in ‘Granola’ (Fig. 32).

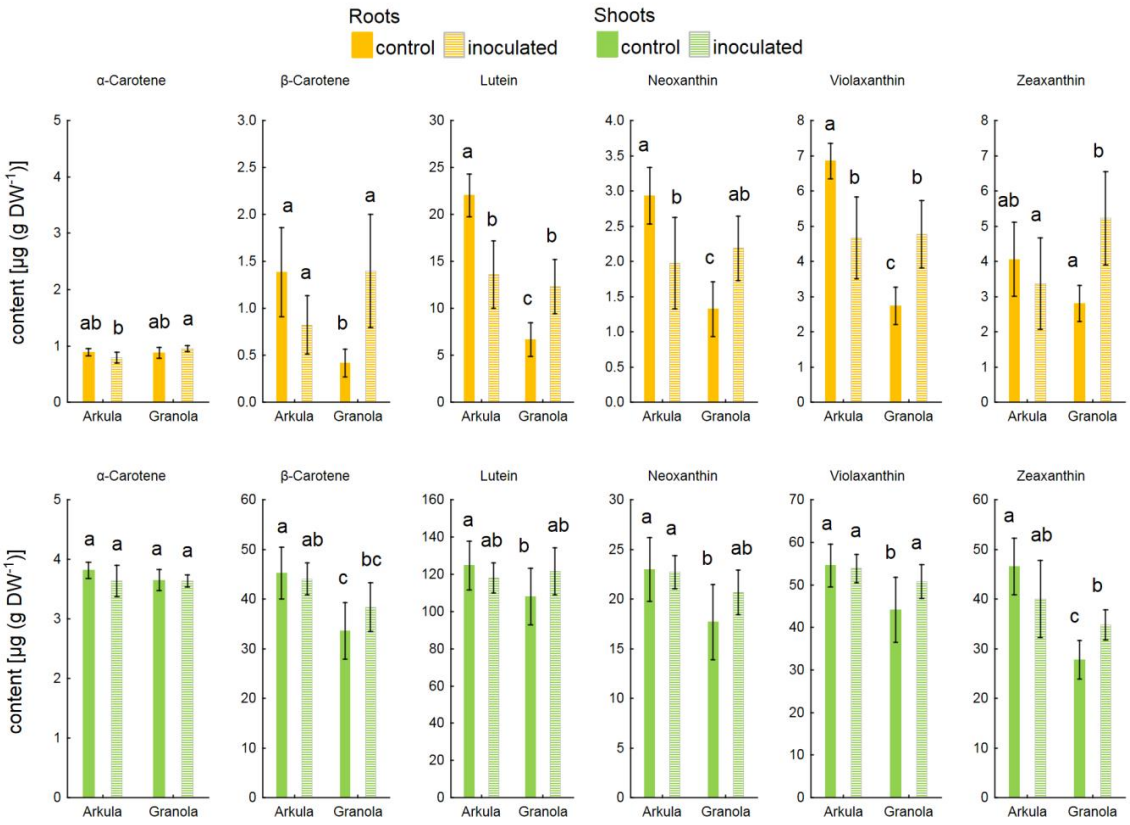


**Figure 32:** Determination of content of carotenoids in roots and shoots of the potato cultivars 'Arkula' and 'Granola' non-inoculated and inoculated with *Rhizoctonia solani* AG3PT. Significant differences between cultivars and treatments (inoculated, control) are indicated by different letters (two-way ANOVA followed by Fisher’s LSD test, $p \leq 0.05$). Carotenoid concentration is represented as means [µg x $g^{-1}$ of dry weight (DW)] of five/six replicates with standard deviation.

The qRT-PCR analysis revealed no significant differences between the cultivars regarding the transcript level of *ABAR* (Fig. 33), either in roots or in shoots. However, the gene *PSY* was significantly higher expressed in 'Granola' compared to 'Arkula' (Fig. 33) in pathogen-treated as well as non-treated roots and shoots. In contrast, the transcript level of the gene *NCED* was significantly higher in roots of 'Arkula' compared to ‘Granola’.

The impact of the pathogen treatment on the expression of these biosynthetic genes was investigated as well. Thus, a significantly higher transcript level of the gene *ABAR* was detected in pathogen-treated shoots of 'Arkula' compared to the non-treated control at 10 dpi (Fig. 33). In addition, the transcript level the gene *NCED* was significantly decreased in inoculated roots of 'Arkula' (Fig. 33). No significant changes in the transcript level of the genes *PSY*, *ABAR*, and *NCED* were observed for ‘Granola’.

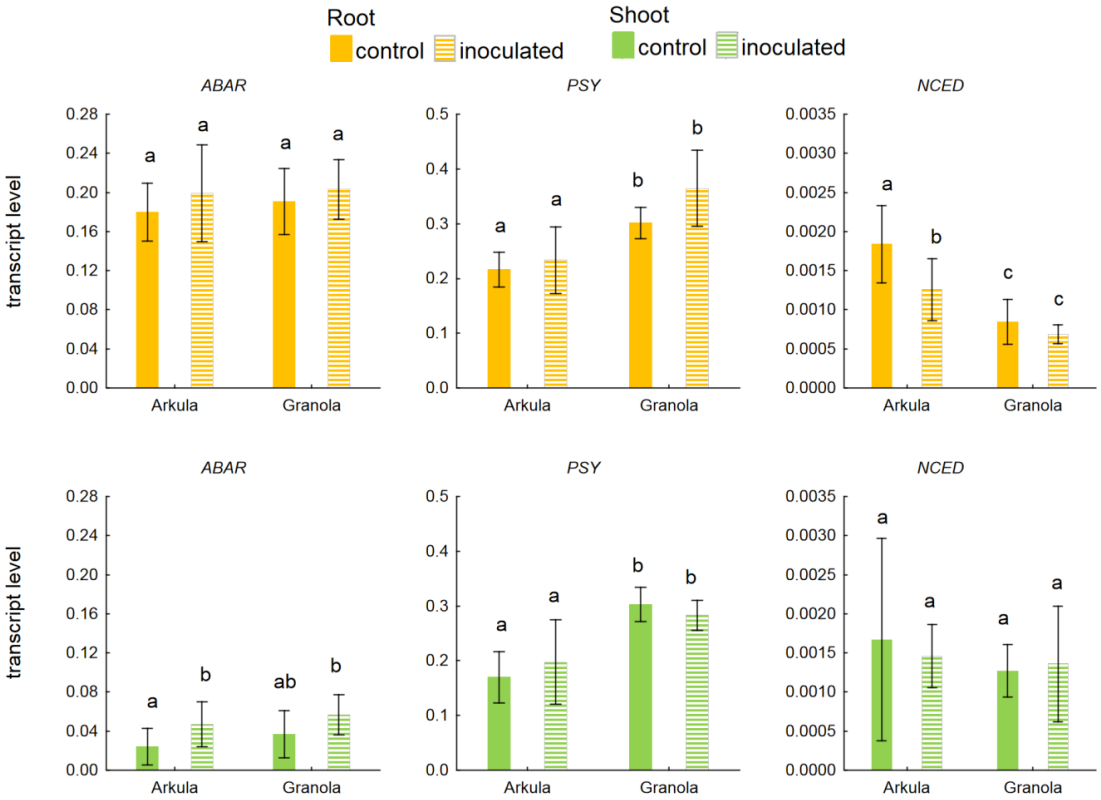


**Figure 33:** Determination of transcript levels of biosynthetic genes connected to the carotenoid and ABA metabolism encoding a phytoene synthase (*PSY*), an ABA receptor (*ABAR*), and a 9-*cis*-epoxy-carotenoid dioxygenase (*NCED*) in roots and shoots of potato cultivars 'Arkula' and 'Granola' non-inoculated and inoculated with *R. solani* AG3PT. Significant differences between cultivars and treatments (inoculated, control) are indicated by different letters (two-way ANOVA followed by Fisher’s LSD test, $p \leq 0.05$). Transcript levels represent means (n = 6) of the respective target gene expression normalised to the expression of reference genes *ACT* and *TEF1* including standard deviation.

*Glycoalkaloids*

In addition to carotenoids, the content of the two potato glycoalkaloids α-chaconine and α-solanine as well as transcript levels of two key biosynthetic genes were determined. First, a gene encoding a rhamnosyltransferase (*SGT3*), an enzyme catalysing the formation of α-chaconine and α-solanine, was selected for gene expression analysis. In addition, the expression of a gene coding for a squalene synthase (*SQS*) was investigated. Squalene is a precursor of potato steroids and brassinosteroids (Fig. 31).

The analysis of the glycoalkaloids revealed significant differences between the two cultivars. Thus, a significantly higher content of α-chaconine was determined in roots and shoots of 'Granola' compared to 'Arkula' in pathogen-treated and non-treated plants (Fig. 34). In shoots, the concentration of α-solanine was significantly higher in 'Granola' than in 'Arkula' in both treatments as well. Moreover, the content of α-solanine was significantly higher in *R. solani* AG3PT-inoculated roots of 'Granola' compared to inoculated roots of 'Arkula'. A comparison of pathogen-treated and non-treated samples revealed a significant effect of an inoculation with *R. solani* AG3PT on the content of glycoalkaloids in roots. Thus, a significantly decreased concentration of α-chaconine and α-solanine was determined in inoculated roots of 'Arkula' compared to the respective control (Fig. 34). In contrast, the content of these two glycoalkaloids significantly increased in roots of 'Granola' after inoculation with the pathogen.

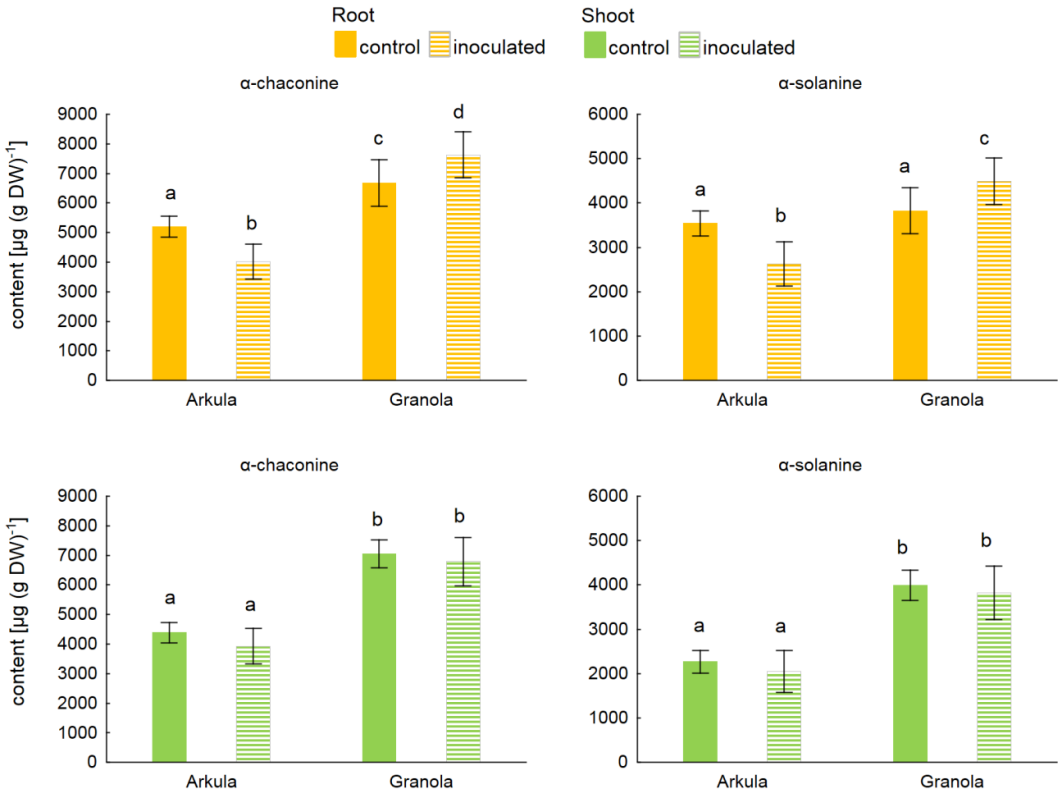


**Figure 34:** Determination of the content of glycoalkaloids (α-chaconine, α-solanine) in roots and shoots of the potato cultivars 'Arkula' and 'Granola' non-inoculated and inoculated with *R. solani* AG3PT. Significant differences between cultivars are indicated by by different letters (two-way ANOVA followed by Fisher's LSD test, $p \leq 0.05$). Glycoalkaloid concentration is represented as means [µg x $g^{-1}$ of dry weight (DW)] of five/six replicates with standard deviation.

Compatible with these results, the transcript levels of genes encoding enzymes involved in the biosynthesis of glycoalkaloids were higher in 'Granola' than in 'Arkula'. More specifically, the constitutive transcript levels of the genes *SQS* and *SGT3* were significantly higher in

roots and shoots of 'Granola' compared to 'Arkula' (Fig. 35). Moreover, the expression of *SGT3* was significantly higher in inoculated roots and shoots of ‘Granola’ than ‘Arkula’. Comparing the transcript levels of non-inoculated and inoculated plants, significant differences were revealed. The expression of *SQS* was significantly increased in roots and shoots of both cultivars at 10 dpi with *R. solani* AG3PT (Fig. 35). In contrast, the expression level of *SGT3* decreased significantly in roots of ‘Granola’ after pathogen treatment.

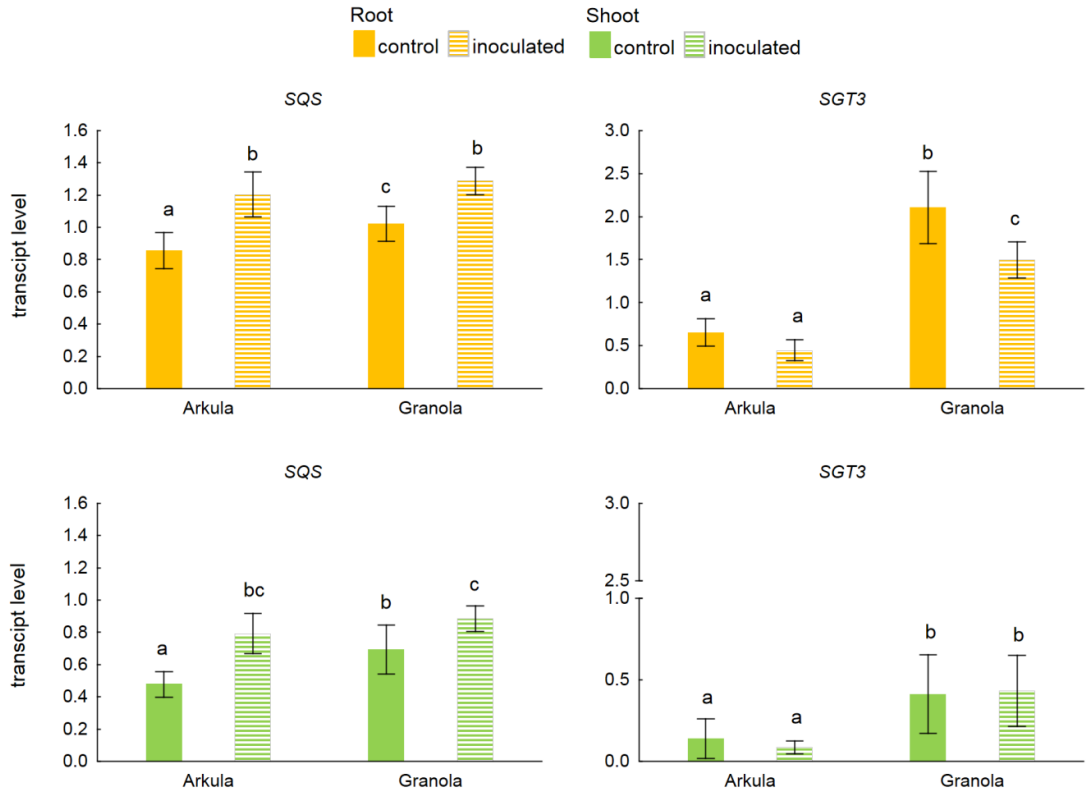


**Figure 35:** Determination of transcript levels of a squalene synthase gene (*SQS*) and a rhamnosyltransferase gene (*SGT3*) in roots and shoots of the potato cultivars 'Arkula' and 'Granola' non-inoculated and inoculated with *R. solani* AG3PT. Significant differences between cultivars and treatments (inoculated, control) are indicated by different letters (two-way ANOVA followed by Fisher’s LSD test, $p \leq 0.05$). Transcript levels represent means ($n = 6$) of the target gene expression normalised to the expression of the reference genes *ACT* and *TEF1* including standard deviation.

*Phenylpropanoids*

In addition to carotenoids and alkaloids, compounds of the phenylpropanoid pathway were investigated. Several phenylpropanoids were detected in roots and shoots of both cultivars using high-performance liquid chromatography (HPLC). Hydroxycinnamic acid derivatives were monitored at 320 nm and flavonol glycosides were monitored at 370 nm. Phenylpropanoids were identified according to retention time (RT), mass spectra, and absorption maxima described in the literature (Navarre et al., 2011).In roots of both cultivars, 1- and 3-caffeoylquinic acid and rutin were detected. Nicotiflorin was only found in roots of 'Granola'. Furthermore, 'Arkula' showed a significantly lower content of 3-caffeoylquinic acid

in pathogen-treated and non-treated roots compared to 'Granola' (Fig. 36). In addition, the contents of rutin and 1-caffeoylquinic acid were significantly lower in inoculated roots of ‘Arkula’ compared to ‘Granola’. Besides these compounds, quercetin-3-rutinoside-7-glucoside, quercetin-diglucoside, kaempferol-3-rutinoside-7-glucoside, and kaempferol-3-diglucoside were identified in shoots of both cultivars. 'Arkula' had significantly higher constitutive concentrations of quercetin-diglucoside and kaempferol-3-diglucoside, while 'Granola' showed higher concentrations of rutin and nicotiflorin (Fig. 36). In *R. solani* AG3PT-inoculated shoots, significantly higher contents of 3-caffeoylquinic acid, rutin, nicotiflorin, and quercetin-3-rutinoside-7-glucoside were determined in ‘Granola’ compared to ‘Arkula’. In contrast, shoots of inoculated ‘Arkula’ contained more quercetin-diglucoside than shoots of inoculated ‘Granola’. In the case of ‘Arkula’, the inoculation with *R. solani* AG3PT significantly affected the contents of the detected phenylpropanoids. Thus, a significant reduction of 1-caffeoylquinic acid and rutin was determined in roots and of quercetin-3-rutinoside-7-glucoside and quercetin-diglucoside in shoots (Fig. 36).

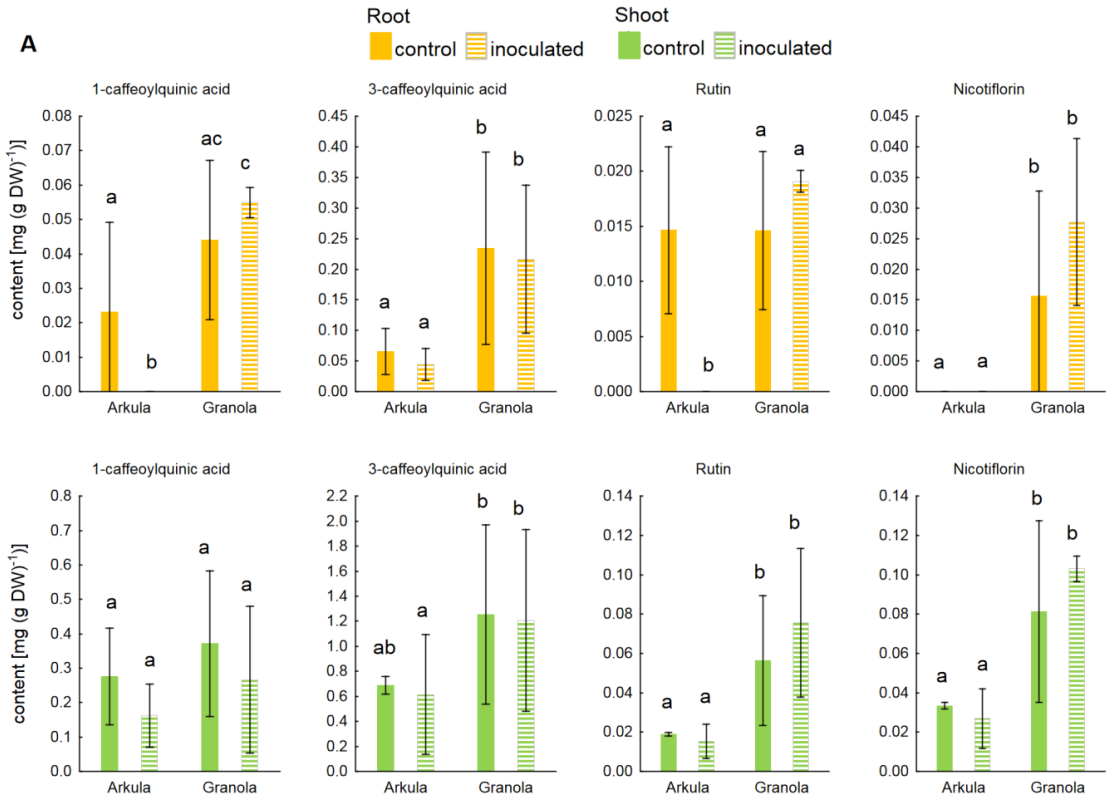


**Figure 36A:** Determination of the content of phenylpropanoids in roots and shoots of the potato cultivars 'Arkula' and 'Granola' non-inoculated and inoculated with *R. solani* AG3PT. Significant differences between cultivars and treatments (control, inoculated) are indicated by different letters (two-way ANOVA followed by Fisher’s LSD test, $p \leq 0.05$). The content of phenylpropanoid compounds was quantitated either as chlorogenic acid equivalents, quercetin-3-glucosid equivalents or as kaempferol-3-glucosid equivalents. Values represent means [mg x $g^{-1}$ dry weight (DW)] of five/six replicates with standard deviation.

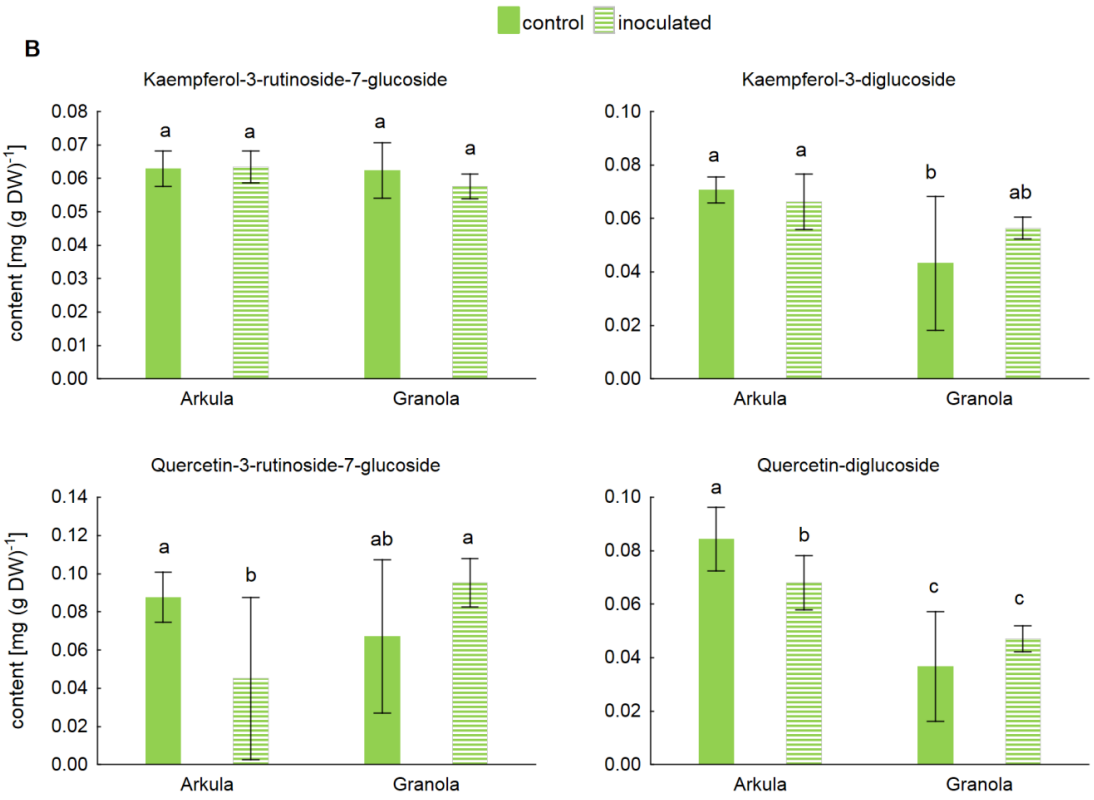


**Figure 36B:** Determination of the content of phenylpropanoids in shoots of the potato cultivars 'Arkula' and 'Granola' non-inoculated and inoculated with *R. solani* AG3PT. Significant differences between cultivars and treatments (control, inoculated) are indicated by different letters (two-way ANOVA followed by Fisher's LSD test, $p \leq 0.05$). The content of phenylpropanoid compounds was quantitated either as chlorogenic acid equivalents, quercetin-3-glucosid equivalents or as kaempferol-3-glucosid equivalents. Values represent means [mg x $g^{-1}$ dry weight (DW)] of five/six replicates with standard deviation.

### 4.4.3 Sensitivity of *Rhizoctonia solani* AG3PT to secondary metabolites of potato

To test whether phenolic acids or flavonoids identified in roots and shoots of the two cultivars have a direct toxic effect on *R. solani* AG3PT, *in vitro* culture tests were conducted. Therefore the fungus was grown on culture medium which was enriched with 400, 200, 100, and 50 µM of nicotiflorin, rutin, quercetin or kaempferol respectively. Additionally, the effect of 3-caffeoylquinic acid was tested (800, 400, 200, 100, and 50 µM). Since the standards were dissolved in ethanol, medium of control treatments was supplemented with the equivalent concentration of ethanol. Concentrations of 400 and 200 µM nicotiflorin significantly reduced the mycelial diameter (cm) of *R. solani* AG3PT compared to the respective control after 72 and 168 hours of incubation (hoi) (Fig. 37A). However, 3-caffeoylquinic acid, quercetin, and rutin had no growth inhibitory effect on the pathogen after 72 hoi and 168 hoi. In contrast, adding kaempferol, 3-caffeoylquinic acid, quercetin or rutin resulted in a significantly increased mycelium diameter compared to the respective control dependent on the concentration and incubation time. Thus, mycelium diameter was higher when the fungus

was grown on medium containing either 200 µM kaempferol, 400 µM 3-caffeoylquinic acid or 50 to 100 µM rutin (Fig. 37).

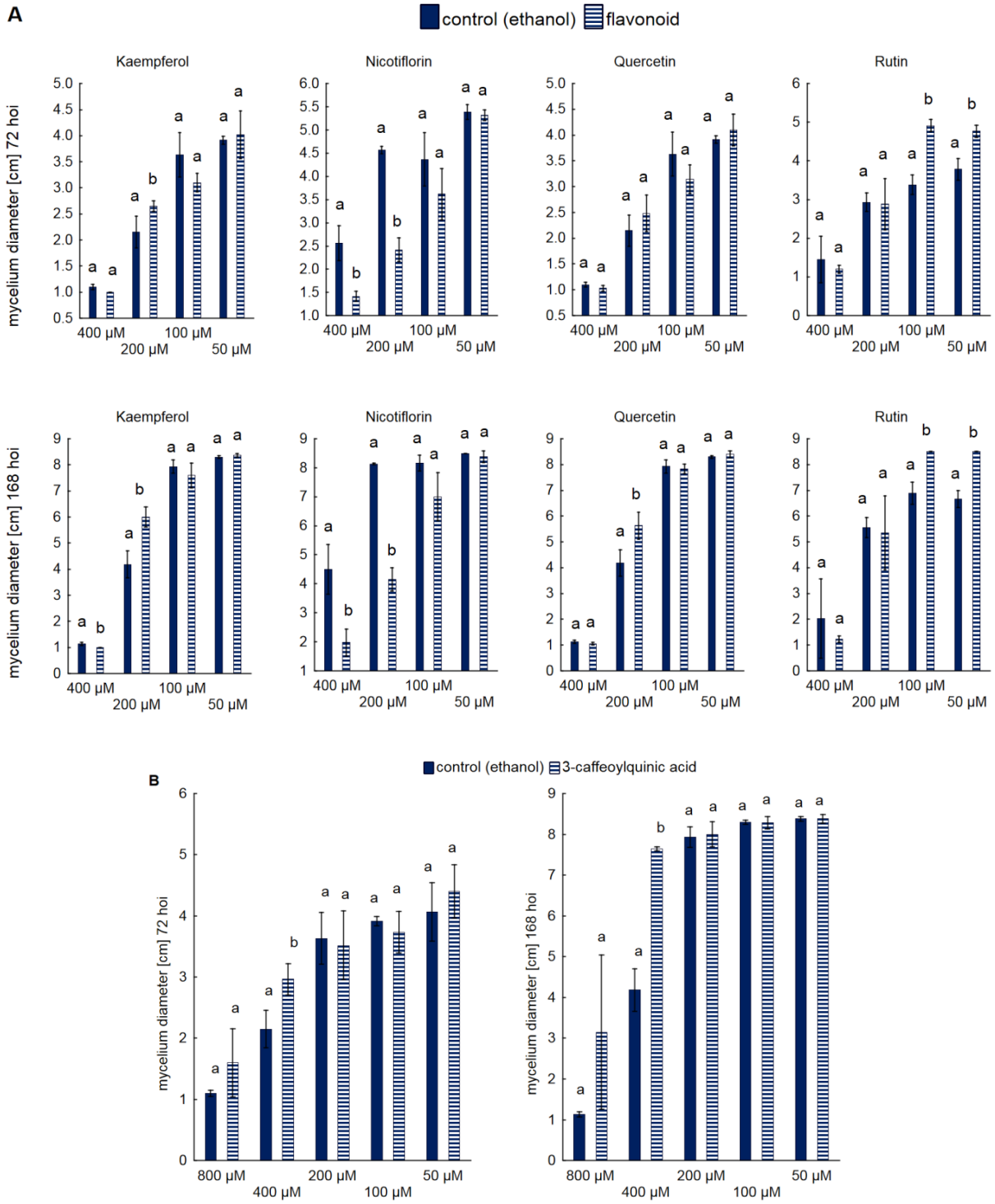


**Figure 37:** Effect of kaempferol, nicotiflorin, quercetin, rutin (A), and 3-caffeoylquinic acid (B) on the growth of *Rhizoctonia solani* AG3PT *in vitro*. Mycelium diameter (cm) was measured after 72 and 168 hours of incubation (hoi) on medium supplemented with different concentrations of either of the compounds (800, 400, 200, 100, 50 µM). Significant differences between control medium (ethanol) and medium containing phenylpropanoids are indicated by different letters (one-way ANOVA followed by Dunnett's test, $p \leq 0.05$).

Furthermore, the direct inhibitory effect of α-chaconine and α-solanine on *R. solani* AG3PT was tested. Both alkaloids completely inhibited mycelial growth at concentrations of 200 and 100 µM after 24 and 48 hoi (Fig. 38). A significantly reduced mycelial growth was observed at 50 µM. In addition, a synergistic effect of both alkaloids was detected. Thus, a total inhibition of mycelial growth was determined on media containing 50 µM α-chaconine and 50 µM α-solanine after 24 and 48 hoi.

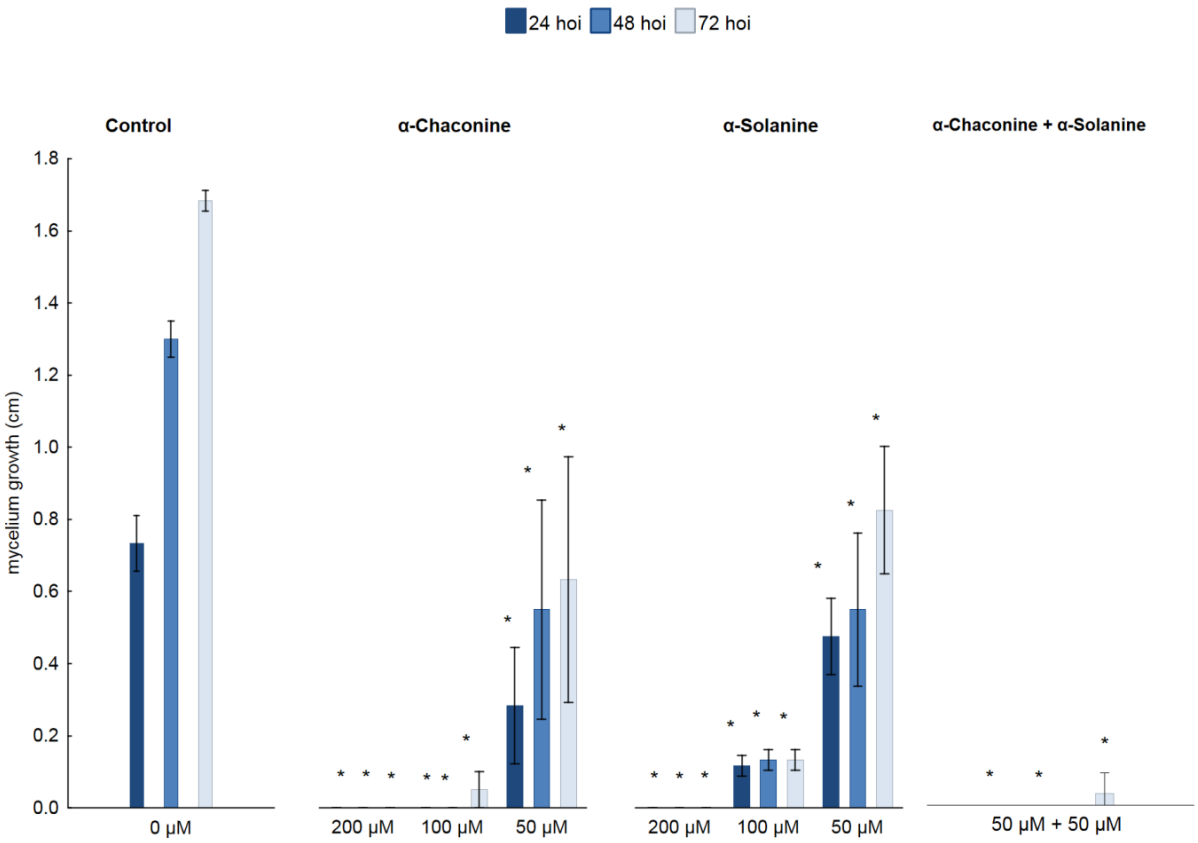


**Figure 38:** Effect of α-chaconine and α-solanine on growth of *R. solani* AG3PT *in vitro*. Mycelium growth (cm) was measured after 24, 48, and 72 hours of incubation (hoi). Growth was measured on medium supplemented with different concentrations of either of the two glycoalkaloids (200, 100, 50 µM). In addition, the synergistic effect of α-chaconine and α-solanine was tested on medium containing 50 µM of each compound. Significant differences between control medium (0 µM) and medium containing glycoalkaloids are indicated by asterisks (one-way ANOVA followed by Dunnett's test, $p \leq 0.05$).

### 4.4.4 Connection of analysed metabolites and their biosynthetic genes

The following maps (Fig. 39 & 40) illustrate connections between all metabolites and enzymes investigated in the two cultivars. Significant changes in transcript levels or in contents of plant secondary metabolites following *R. solani* AG3PT inoculation are represented by colour coding. This illustration clearly shows, that the content of several metabolites (α-chaconine, α-solanine, rutin, lutein, zeaxathin, neoxanthin, violaxanthin) is significantly decreased in inoculated roots of 'Arkula', whereas there is no significant change,

or in the case of carotenoids even an increase, detectable in roots of 'Granola'. In shoots, a significantly decreased content of phenolic compounds was determined in 'Arkula', but not in 'Granola' as well.

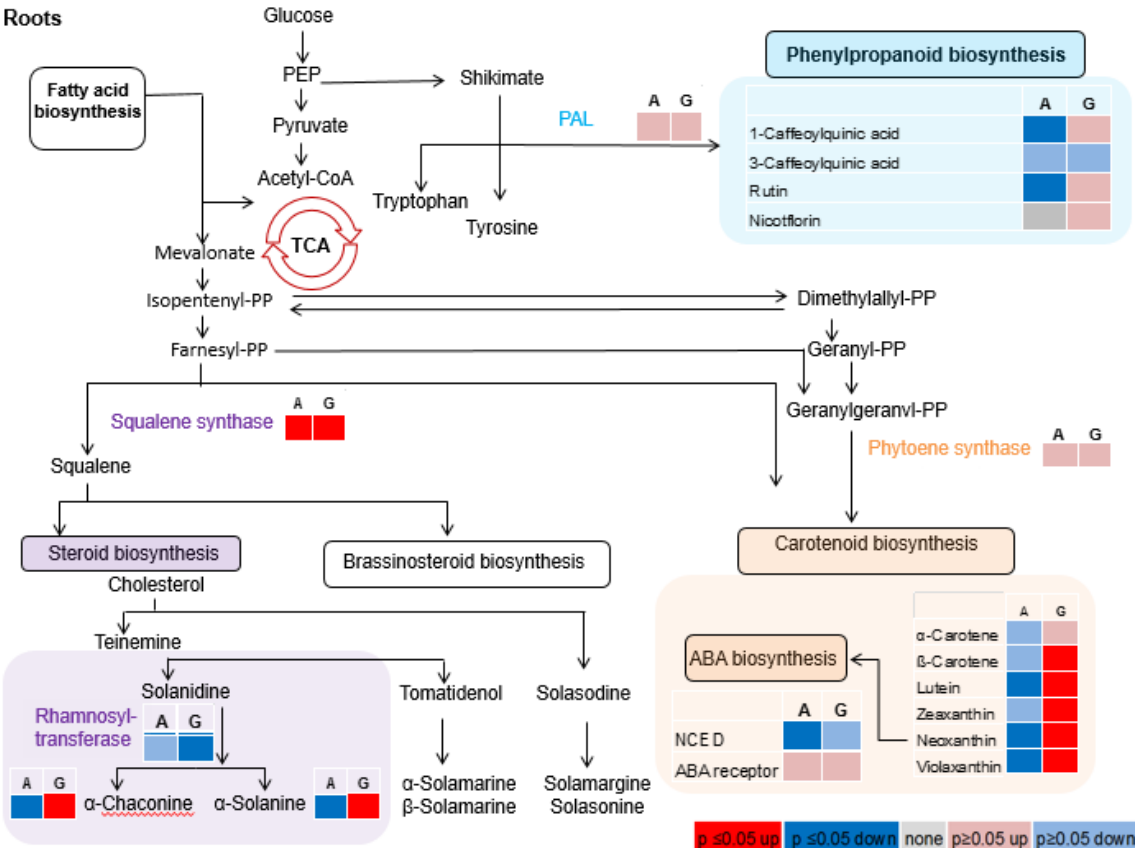


**Figure 39:** Simplified visualisation of biosyntheses of phenylpropanoid compounds, carotenoids, and steroidal glycoalkaloids of potato. Highlighted compounds and enzymes (transcription of the corresponding genes) were investigated in 'Arkula' (A) and 'Granola' (G) prior to and after inoculation with *Rhizoctonia solani* AG3PT. The map shows changes in transcript levels and in concentration of analysed compounds (ratio inoculated/control) in roots at 10 dpi with *R. solani* AG3PT illustrated by colour coding. Colours represent either up- or down-regulation according to the colour coding beneath the map, with significant differences between treatments (control, inoculated) indicated by darker colours (two-way ANOVA followed by Fisher's LSD test, $p \leq 0.05$).

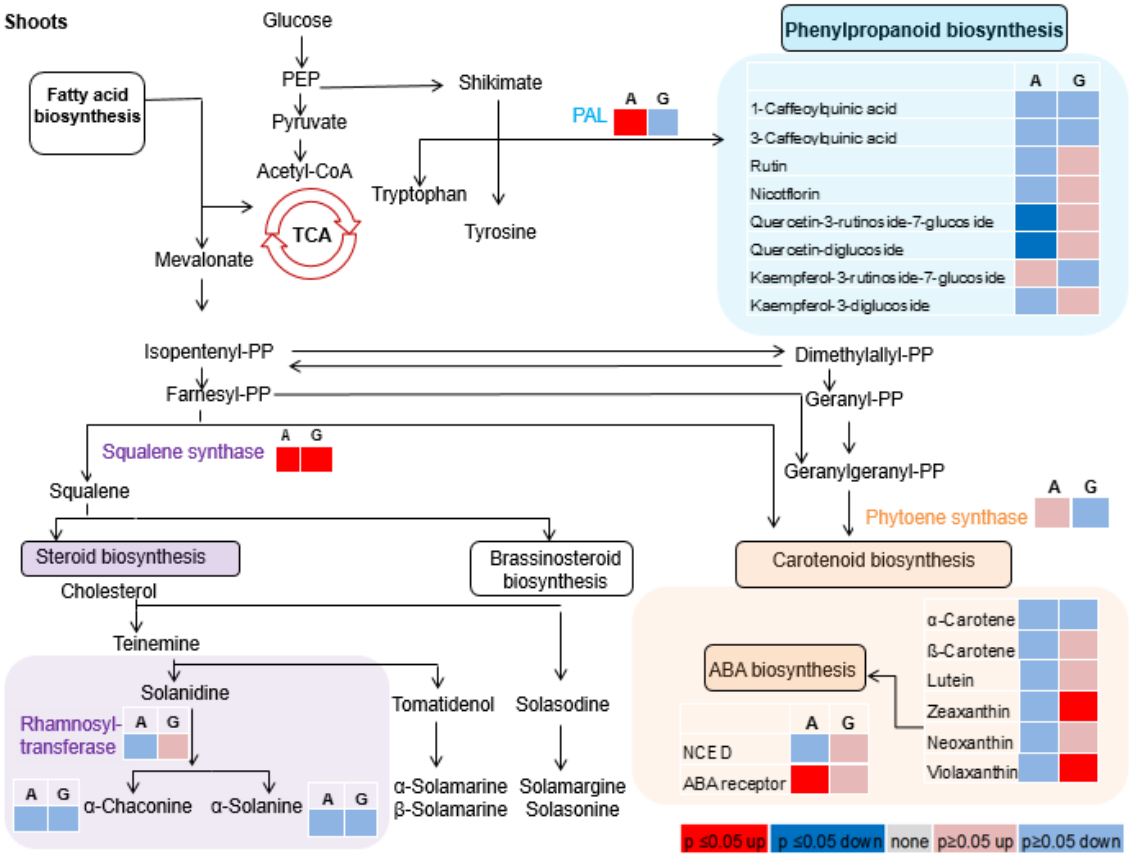


**Figure 40:** Simplified visualisation of biosyntheses of phenylpropanoid compounds, carotenoids, and steroidal glycoalkaloids of potato. Highlighted compounds and enzymes (transcription of the corresponding genes) were investigated in 'Arkula' (A) and 'Granola' (G) prior to and after inoculation with *R. solani* AG3PT. The map shows changes in transcript levels and in the concentration of analysed compounds (ratio inoculated / control) in shoots at 10 dpi with *R. solani* AG3PT illustrated by colour coding. Colours represent either up- or down-regulation according to the colour coding beneath the map, with significant differences between treatments (control, inoculated) indicated by darker colours (two-way ANOVA followed by Fisher's LSD test, $p \leq 0.05$).

# 5. DISCUSSION

## 5.1 'Arkula' and 'Granola' show different degrees of resistance to *Rhizoctonia solani* AG3PT in the field

Several studies described that potato cultivars show different degrees of field resistance to *R. solani* AG3PT (Buhr, 1989; Bains et al., 2002; Djébali and Belhassen, 2010). However, cultivars with complete resistance against this pathogen have not been identified until now (Jeger et al., 1996; Tsror, 2010). To investigate the interaction between potato and *R. solani* AG3PT in more detail, cultivars which differ in their degree of field resistance to this pathogen are of high advantage. In the beginning of the current study, field trials were conducted in cooperation with potato breeders at different locations in Germany to evaluate various potato cultivars regarding their degree of field resistance to *R. solani* AG3PT. Based on these field experiments, the cultivars 'Arkula' and 'Granola' were selected as cultivars with different degrees of field resistance to *R. solani* AG3PT. According to The European Cultivated Potato Database (https://www.europotato.org), the cultivar 'Arkula' is described as a very early maturing, high yielding cultivar which shows medium high resistance to *Fusarium* dry rot and *Erwinia* blackleg and a medium high to high resistance to late blight on tubers. In contrast, Ros et al. (2008) described 'Arkula' as susceptible to the late blight-causing pathogen *Phytophthora infestans*. 'Granola' is medium early to late maturing, shows a medium high to high yield and a high to very high resistance to *Fusarium* dry rot, high resistance to *Erwinia* blackleg but only low to medium high resistance to late blight on tubers (https://www.europotato.org). Regarding *R. solani*, it is stated that 'Arkula' is moderately resistant and 'Granola' highly resistant to stem canker. However, there is no information given in regard to *R. solani*-induced black scurf. Repeated field experiments at the research station of the IGZ confirmed a higher susceptibility of 'Arkula' compared to 'Granola' against *R. solani* AG3PT based on the number of deformed progeny tubers and the percentage of sclerotia infestation of tubers.

It is worth mentioning that conventionally produced seed tubers, which have been used in these field experiments, can be already infested with *R. solani* or other pathogens. Therefore, tubers in non-inoculated treatments are exposed to a lower inoculum density than *R. solani* AG3PT-inoculated tubers. The additional inoculation with *R. solani* AG3PT had no significant influence on the percentage of tubers with marketable size (35-55 mm). Consistent with these results, Lehtonen et al. (2009) did not observe a significant effect of a *R. solani* inoculation on the amount of tubers with marketable size. However, alterations in the distribution of tuber sizes and reduction of the marketable yield due to an infection with *R. solani* AG3PT have been repeatedly described in the literature (Banville, 1989; Buhr, 1989, Wilson et al., 2008). This is caused by an infection of stems, stolons, and roots which can

impair the nutrient flow to the tubers, so that tuber size and number are affected (Hide et al., 1973; Carling et al., 1989; Djébali and Belhassen, 2010). Presumably, yield reduction is dependent on the severity of the infection. Accordingly, Ritchie et al. (2013) observed an increasing decline of tuber yield with increasing inoculum density. Regarding black scurf severity, significant differences between the two cultivars were detected in both years, in treatments with and without additional *R. solani* AG3PT inoculation. This is in accordance with previous studies (Daami-Remadi et al., 2008; Djébali and Belhassen, 2010; Sedláková et al., 2013). However, studies conducted by Buhr (1989) showed that significant differences in black scurf severity between various cultivars are hardly reproducible. It was suggested that black scurf severity is not always suitable for an evaluation of the degree of susceptibility, because growing conditions during late vegetation period and especially the harvest time have a much greater impact on the level of black scurf (Dijst, 1985, 1990). Similarly, Djébali and Belhassen (2010) found that the incidence of black scurf was dependent on an interaction between cultivar and harvest time. According to Dijst et al. (1986), the duration between haulm destruction and harvest is related to the incidence of black scurf. Presumably, prolonging the time between haulm death and harvest increases black scurf severity (Bains et al., 2002). This was taken into account when determining the time point of the harvest. Therefore, harvest took place four weeks after haulm death, separately for each cultivar because ‘Arkula’ and ‘Granola’ belong to different maturity classes. The inoculation with *R. solani* AG3PT significantly increased black scurf severity for both cultivars in 2015. This is in accordance with previous observations which revealed that an increased soil-borne inoculum density leads to significantly elevated levels of black scurf severity on harvest tubers (Ritchie et al., 2013).

Tuber deformations can result from abiotic factors like temperature and water stress but also from biotic factors like *Rhizoctonia* disease (Hiller, 1985). Additionally, cultivar-dependent differences regarding the incidence of tuber deformation have been previously described (Campion et al., 2003). In our study, significant differences between the two cultivars were detected. ‘Arkula’ bore significantly more deformed tubers than 'Granola' in 2015 and 2016. This is in accordance with Buhr (1989) who reported that differences between cultivars regarding this symptom were reproducible, in particular within the group of medium early maturing cultivars like ‘Granola’. The author also observed a very low increase of deformation upon inoculation with *R. solani* in the case of 'Granola'. Similarly, we observed no significant increase of deformation in *R. solani* AG3PT-inoculated treatments of either ‘Arkula’ or ‘Granola’. Yet, several studies described that deformation of progeny tubers can be caused by *R. solani* infection (Buhr, 1989; Scholte, 1989; Jeger et al., 1996; Campion et al, 2003).

Besides deformation, the occurrence of drycore symptoms on harvest tubers has been associated with an *R. solani* infection (Keiser, 2012). In our study, an increased incidence of drycore symptoms due to pathogen treatment was observed for ‘Arkula’ in 2015. In addition, the incidence of drycore was higher for ‘Arkula’ than for ‘Granola’, however, only significantly in 2016. Variability regarding the incidence of drycore might be traced back to different soil types used as well as different weather conditions in 2015 and 2016. It is assumed that wet soil conditions promote the incidence of drycore symptoms (Schwinn, 1961). However, Keiser (2012) did not observe any connection between soil conditions and the incidence of drycore symptoms.

Evaluation of potato cultivars regarding their degree of susceptibility to *R. solani* AG3PT is challenging and due to its vulnerability towards various abiotic influences not always distinctly possible. For instance, the soil temperature affects disease severity considerably leading to high variability between years dependent on weather conditions (Hide and Firmager, 1989). Buhr (1989) also reported a high dependence of black scurf severity on environmental conditions. To compare the resistance level of different cultivars, it would be favourable to work with seed tubers of the same origin. Physiology and anatomy of tubers and plants greatly impact the black scurf severity (Spencer and Fox, 1979; Dijst, 1988). Thus, storage conditions, physiological age, and tuber-borne pathogens have a huge impact on the quality of seed tubers and the progression of the disease (Buhr, 1989). In 2015, seed tubers were obtained from different potato breeding companies which might have compromised the comparability. Seed tubers planted in 2016 were produced at the IGZ to standardise the quality of the planting material. Despite these impediments, results of both field experiments confirmed a higher disease severity in case of 'Arkula' compared to 'Granola'. Thus, the two selected cultivars seemed to be suitable representatives of higher and lower resistance to *R. solani* AG3PT and were subsequently used for comparative analyses with regard to pathogen colonisation (chapter 4.2.3), defence gene expression (chapter 4.3.5) and the content of plant secondary metabolites (4.4).

### 5.2 *Rhizoctonia solani* AG3PT colonises ‘Arkula’ more extensively than ‘Granola’

While disease severity can be also influenced by tolerance of a particular cultivar, pathogen density in host tissue is a parameter which defines the susceptibility or resistance of cultivars to specific pathogens, because higher plant innate immunity level generally results in reduced colonisation density. In the pathosystem potato-*Verticillium dahliae*, the response of potato cultivars to an infection was assessed based on the pathogen density in plant tissue (Atallah et al., 2007). Besides, Sayler and Yang (2007) observed a higher colonisation of a susceptible rice cultivar by *R. solani* AG-1IA compared to a partially resistant cultivar.

Therefore, we analysed whether a potato cultivar with lower disease indices is also less colonised by *R. solani* AG3PT compared to a cultivar showing more symptoms in order to distinguish between tolerance and resistance.

The quantification of fungal biomass using methods like microscopy or the measurement of fungal-derived biomolecules (ergosterol) is time-consuming or non-specific (Tellenbach et al., 2010). We solely used microscopy to investigate spreading of *R. solani* AG3PT hyphae over the root surface. Thus, a quick colonisation of potato roots by *R. solani* AG3PT, which increased during early post-inoculation phases until 6 dpi, was detected. Extensive hyphal growth of *R. solani* AG3PT over the root surface and strong hyphal branching represent the initial step in the infection process (Gonzalez et al., 2011). Usually, T-shaped hyphal branches, which were repeatedly observed at different sampling dates, precede the formation of infection structures. In the case of *R. solani*, these structures can include swollen hyphae, appressoria, repetitive T-shaped branches, or infection cushions (Gonzalez et al., 2011). In order to rapidly quantify *R. solani* AG3PT in infected potato tissue, we established a qPCR assay. Quantitative PCR allows specific detection and accurate quantification of fungal DNA also in small samples. After establishment of a qPCR bioassay, this assay was applied to compare two cultivars with putatively different degrees of field resistance to *R. solani* AG3PT ('Arkula' as highly susceptible, 'Granola' as less susceptible) regarding the pathogen colonisation densities in roots and underground shoots during a time course of seven weeks post inoculation. The cultivars were selected based on results of field experiments (chapters 4.1 and 5.1). A significantly lower pathogen density was detected in roots of ‘Granola’ compared to cultivar ‘Arkula’ during early plant-pathogen interaction. This confirms that ‘Granola’ does not only show less symptoms, but that it is also more resistant to the pathogen than ‘Arkula’. These findings are in accordance with results obtained in studies comparing hosts with different degrees in resistance to a pathogen (Mercado-Blanco et al., 2003; Gayoso et al., 2007; Sayler and Yang, 2007). For instance, severity of *Verticillium* wilt symptoms correlated with the amount of fungal DNA detected in olive stems (Mercado-Blanco). Similarly, *V. dahliae*-infected pepper plants showing different degrees of disease severity also contained different amounts of pathogen DNA (Gayoso et al., 2007).

During the growing season, *R. solani* is presumably present on all belowground plant parts of potato. In our studies, we focused on the analysis of the pathogen density on roots during early phases of plant-pathogen interaction. The results indicate that ‘Granola’ was able to limit fungal growth on roots to a greater extent than 'Arkula' during early infection stages. Presumably, this may also occur during the growing season, resulting in a lower pathogen density e. g. on stolons of a cultivar with a higher resistance level, which leads to lower black scurf severity on harvested tubers as has been found for ‘Granola’ in our field experiments. Inability to inhibit pathogen growth, which might result from a delayed activation of defence

responses or from low transcript levels of *PR* genes during early infection stages, can cause susceptibility of a plant to a pathogen (Okubara et al., 2014).

No differences between the cultivars were, however, observed regarding the colonisation density of underground shoots. Additionally, the fungal DNA/extracted DNA ratio was decreased in both cultivars at 5 and 7 wpi compared to 3 wpi, in roots as well as in shoots. Similarly, Mercado-Blanco et al. (2003) described a progressive decrease of *V. dahliae* DNA in roots of several olive cultivars at different time intervals post inoculation. The decrease in the amount of fungal DNA could also be associated with the developmental stage of the plant. Expression of resistance is dependent on plant development, often resulting in a higher susceptibility of younger compared to older plants (Develey-Rivière and Galiana, 2007). The expression of *PR* genes like chitinases for instance increases with plant age independently of pathogen infection (Develey-Rivière and Galiana, 2007; Kasprzewska, 2003).

Another explanation for the distinct decrease of pathogen density found in the roots of both cultivars at 5 wpi compared to 3 wpi might be the heterogeneous distribution of the pathogen on the roots. In order to investigate colonisation processes more closely, roots were divided vertically into three sections. Thus, a heterogeneous distribution of the pathogen, with a higher density of fungal DNA in upper parts of the roots compared to lower parts, was revealed. This effect was more pronounced at 3 wpi compared to later sampling dates at 5 and 7 wpi. The strong increase in root biomass from 3 to 5 wpi has to be considered as well. Due to a rapid, strong increase of the plant biomass, the amount of plant DNA increased in relation to the pathogen DNA (whole root was sampled). In addition, Hofman and Jongebloed (1988) supposed that potato tissue is not progressively colonised by *R. solani* AG3PT, but that colonisation is rather limited to infection sites. At later infection stages, the authors observed the formation of brown, non-infectious mycelium which might present some kind of resting form. In pre-experiments, we also noticed brown mycelium on the surface of potato sprouts. Taking the assumption of Hofman and Jongebloed (1988) into account, the growing potato roots are not further colonised by the fungus at later infection stages. Therefore, a lower quantity of pathogen DNA is detectable at lower sections of the root. Nevertheless, results of the qPCR reveal, especially during early infection stages, a lower pathogen density in plant tissue of ‘Granola’ compared to the cultivar ‘Arkula’. In general, low levels of pathogen DNA in plants indicate a limited spreading of the pathogen due to the induction of plant mechanisms which contribute to resistance (Vandemark and Barker 2003). This raises the question whether ‘Granola’ actually shows a higher innate immunity level, which might be indicated by a higher expression of defence-related genes or a higher biosynthesis of plant secondary metabolites. These issues will be addressed in detail in the following two chapters.

## 5.3 The role of basal defence gene expression in the pathosystem potato-*Rhizoctonia solani* AG3PT

### 5.3.1 *Rhizoctonia solani* AG3PT triggers up-regulation of defence-related genes in highly susceptible 'Arkula' at early post-inoculation phases

Changes in defence gene expression in potato after inoculation with *R. solani* AG3PT have already been shown by Lehtonen et al. (2008) via microarray analysis, however, only in sprouts. A previous report of Okubara and Paulitz (2005) indicated that an activation of JA- and ET-dependent pathways is generally associated with defence against necrotrophic pathogens and that, particularly in roots, the activation of SA-dependent pathways is rather unlikely. Yet, most experiments are mainly focused on the model plant Arabidopsis (Wang et al., 2002; Devoto and Turner, 2003). However, in an interaction between *S. lycopersicum* and *R. solani* AG8 up-regulation of *PR-1*, a target gene of SA signalling, has been observed in roots (Gao et al., 2006; Fu and Dong, 2013). Foley et al. (2013) suggested that rather the interplay of various hormones (SA, ET, JA, auxin, ABA) is crucial for the resistance of Arabidopsis to *R. solani* than only one single hormone. Due to these contradictory findings a set of common defence-related genes associated with the SA- (*PR-1*, *PR-3*, *PR-10*, *GST*, *PAL*) as well as JA-pathway (*PR-2*, *PAL*, *PI2*) was selected for gene expression analyses.

The first study was conducted to investigate *R. solani* AG3PT-induced changes in the expression level of these genes in the highly susceptible 'Arkula' at a time course (3, 6 and 13 dpi). Studies of interactions between potato and *R. solani* AG3PT are primarily concentrated on sprouts, while there is only one *in vitro* study considering the roots which are also colonised by *R. solani* AG3PT (Gallou et al., 2009). Thus, we aimed to characterise defence responses, particularly in potato roots, to the pathogen *R. solani* AG3PT. Lehtonen et al. (2008) showed that an initial infection of the basal sprout part leads to an induction of defence responses in the apex of the inoculated potato sprout. We hypothesised that an initial infection of a sprout would induce defence responses in all sprouts and in roots as well. Thus, samples of all sprouts and whole roots were taken at the mentioned time points and studied regarding the expression of the selected defence-related genes. This experiment was carried out twice. In both experiments, the results revealed that the necrotrophic fungus *R. solani* AG3PT significantly triggers an up-regulation of the genes *PR-1*, *PR-2*, *PR-3, PR-10,* and *PI2* during early interaction phases (3 and 6 dpi) in this highly susceptible cultivar. In both experiments, the gene *PI2* was only increased in the roots at 3 dpi. In contrast, *PR-1* and *PR-2* were consistently up-regulated in roots and sprouts during early post-inoculation phase (3 and 6 dpi) in both experiments. Similarly, Gallou et al. (2009) detected higher expression levels of the genes *PR-1* and *PR-2* in roots of potato plantlets inoculated with *R. solani* AG3PT in an *in vitro* culture system at 2 and 3 dpi. Moreover, both genes were

induced in tomato roots due to an inoculation with the pathogen *Fusarium oxysporum* (Aimé et al., 2013) and *PR-1* was induced in response to *R. solani* AG8 at early post-inoculation period (Gao et al., 2006; Fu and Dong, 2013). The gene *PR-1* encodes extracellular PR proteins which are potentially involved in the SA-dependent signalling pathway. The basic PR-2 protein is assumed to be dependent on JA or ET (Kasprzewska, 2003). Furthermore, the transcript level of *PR-3* was higher in roots and sprouts colonised by *R. solani* AG3PT in both experiments at early post-inoculation phase, except for root samples at 6 dpi of experiment 1 and sprout samples at 3 dpi in experiment 2. A simultaneous up-regulation of genes encoding for the enzymes β-1,3-glucanase (*PR-2*) and chitinase (*PR-3*) in potato roots and sprouts in response to *R. solani* AG3PT is in accordance with results of expression analyses of sprouts conducted by Lehtonen et al. (2008). Moreover, Kombrink et al., (1988) revealed a pronounced elevation of chitinase and β-1,3-glucanase enzyme activity level in potato leaves infected with *P. infestans*.

Co-expression of defence-related genes presumably increases resistance towards pathogens and may result in a delayed symptom development (Büchter, et al. 1997; Takemoto et al., 1997). The synergistic action of different enzymes may be necessary for a fast destruction of hyphae of *R. solani*. In addition, newly synthesised chitin in cell walls of young hyphae is more sensitive to enzymatic degradation (Lorito et al., 1998). However, *R. solani* has been shown to activate enzymes such as plant cell wall degrading cellulases (Jabaji-Hare et al., 1999). Degraded plant cell wall products can in turn act as damage-associated molecular patterns and induce the up-regulation of SA-dependent basic defence genes like *PR-1* and genes encoding enzymes for phytoalexin accumulation such as *PAL*. Gallou et al. (2009) detected a co-expression of the genes *PR-1*, *PR-2*, *GST*, and *PAL* in roots at 2 dpi. In our study, a significant increase of *GST* due to *R. solani* AG3PT was only observed in sprouts in one of the experiments at 3 and 6 dpi. Moreover, the transcript level of *PAL* was only increased in roots of the first experiment. PAL provides, *inter alia*, precursors for the biosynthesis of SA (Mauch-Mani and Slusarenko, 1996). However, Wildermuth et al. (2001) described an alternative pathway through isochorismate synthase (ICS). They supposed that SA synthesis via PAL occurs in cells at the infection site where it leads to a limitation of pathogen growth, while SA synthesis mediated by the ICS pathway takes place in adjacent and distant cells leading to responses typical of ‘Systemic Acquired Resistance’. Consistent with this, in the pathosystem *Arabidopsis thaliana-Botrytis cinerea*, SA-dependent gene products seem to be more relevant for limitation of fungal growth at the infection site than for systemic resistance as well (Ferrari et al., 2003).

Initially, we hypothesised that the necrotroph *R. solani* AG3PT would induce mainly JA- or ET-related defence responses in potato tissue. However, our results showed that defence-related genes associated with the SA-pathway are induced as well. This underlines the

important role of SA for the induction of defence responses in potato roots and sprouts against the soil-borne pathogen *R. solani* AG3PT. Similarly, Xing et al. (2016) detected the induction of JA-, ET- as well as SA-dependent pathways in roots of *Brassica oleracea* L. var. *capitata* due to the infection with *F. oxysporum* f. sp. *conglutinans*, a hemibiotrophic soil-borne pathogen. Interestingly, neither in roots nor in sprouts, our study did reveal an up-regulation of any of the tested defence-related genes at 13 dpi with *R. solani* AG3PT. In response to pathogen attack, plants reallocate energy resources from growth to defence pathways, although defence is costly for the plant and might weaken the plant's fitness (Walters and Heil, 2007; Kliebenstein and Rowe, 2008). Accordingly, Derksen et al. (2013) hypothesised that susceptible plants might not be able to maintain an enhanced defence status over longer periods as it was the case for the highly susceptible potato cultivar 'Arkula'.

Unfortunately, results regarding the expression of *PR-3*, *PR-10*, *PAL*, and *PI2* were inconsistent for the two experiments. Although the tuber material used for this study was cautiously chosen and treated equally from mother plant cultivation over harvest and storage until planting, differences in physiological tuber age between repeated experiments, which also affects the process of rooting and sprouting, cannot be excluded (Fernie and Willmitzer, 2001; Lehesranta et al., 2006). Differences in the physiological age of the tubers used in the experiments might be a reason for variations in the time course of plant-pathogen interaction and consequently for variations in expression of defence-related genes (Kasprzewska, 2003). Inconsistencies between both experiments were also observed for *PR-1*, *PR-2*, *PR-3*, *PR-10*, and *PI2* with respect to the time-dependent (3 to 13 dpi) basic expression level in roots. In contrast, *PAL* showed a consistently higher expression level in roots at 13 dpi compared to 3 dpi in both experiments. As mentioned above, the altered expression levels of this defence-related gene might be dependent on plant developmental stages. Nevertheless, the results revealed that an infection with *R. solani* AG3PT increases the expression level of SA-dependent and JA/ET-dependent defence-related genes, in roots as well as sprouts of the susceptible cultivar 'Arkula'. Thus, an infection of potato sprouts with the soil-borne pathogen *R. solani* AG3PT induces systemic defence responses associated with SA-, as well as JA/ET-pathways in sprouts and roots of potato. However, 'Arkula' was not able to maintain the elevated expression level of defence-related genes in roots and sprouts.

### 5.3.2 Relationship between the degree of field resistance to black scurf disease and expression of defence-related genes in potato

Until now, potato cultivars with complete resistance to *R. solani* AG3PT have not been found. However, field experiments have repeatedly shown that potato cultivars differ in their degree of resistance to this pathogen (Bains et al., 2002; Daami-Remadi et al., 2008; Djébali and

Belhassen, 2010). Yet, the underlying mechanisms of these different degrees of resistance have not been identified (Bradshaw and Mackay, 1994). Research on potato-pathogen interactions had been mainly focused on *Phytophthora infestans* and *Verticillium dahliae* (Vleeshouwers et al., 2000; Hoegen et al., 2002; Ros et al., 2008; Derksen et al., 2013). However, there are only a few studies on interactions between potato and the soil-borne pathogen *R. solani*, although *Rhizoctonia* diseases on potato are occurring worldwide (Lehtonen et al., 2008; Aliferis and Jabaji, 2012). We aimed to investigate defence responses in two potato cultivars with different degrees of resistance to *R. solani* AG3PT. Based on results of repeated field experiments conducted at the research stations of the IGZ, the cultivar 'Arkula' was selected as less resistant and 'Granola' as a cultivar with higher resistance level (chapters 4.1 and 5.1). Since gene-for-gene resistance is rarely described for interactions between plants and necrotrophic fungi (Wang et al. 2014), differences in susceptibility to *R. solani* AG3PT might rather be based on quantitative resistance, involving multiple mechanisms like pathogenesis-related (PR) proteins and secondary plant metabolites, than on qualitative resistance. It is assumed that cultivars with higher resistance level to a pathogen respond earlier and to a higher extent than susceptible ones (Tonón et al., 2002; Mazid et al., 2011). Therefore, we hypothesised that the cultivar with higher level of field resistance would bear a higher expression level of defence-related genes and a higher content of plant secondary metabolites compared to the cultivar with a lower level of field resistance to black scurf disease. For this purpose, expression levels of seven defence-related genes were determined in roots and shoots of both cultivars, in absence and presence of *R. solani* AG3PT.

It has been suggested that the constitutive expression of *PR* genes is associated with non-specific resistance of *Solanum* species to *P. infestans* (Vleeshouwers et al., 2000). We investigated constitutive transcript levels of the four pathogenesis-related (*PR*) genes *PR-1*, *PR-2*, *PR-3,* and *PR-10* and additionally the transcript levels of the genes *GST*, *PAL,* and *PI2* in a comparative analysis using the potato cultivars 'Arkula' and 'Granola'. All four analysed *PR* genes were significantly higher expressed in roots and shoots of more resistant 'Granola'. Several studies report a connection between quantitative resistance and constitutive expression level of *PR* genes (van Loon et al., 2006). Vleeshouwers et al. (2000) described that the genes *PR-1*, *PR-2*, and *PR-5* were constitutively higher expressed in cultivars resistant to *P.infestans* than in susceptible cultivars. Besides, Derksen et al. (2013) observed an elevated expression of *PR-1* and *PR-2* in a cultivar with moderate resistance to *V. dahliae* compared to a susceptible cultivar. It is known that PR proteins possess an important role in constitutive as well as induced basal defence (Jashni et al., 2015). Various attempts were made to enhance disease resistance against pathogens by introducing *PR* genes like chitinases into plants (Broglie et al., 1991; Nishizawa et al., 1999; Tabaeizadeh et al., 1999).

For instance, transgenic tobacco plants, which were constitutively expressing a bean endochitinase gene, showed an enhanced resistance against *R. solani* (Broglie et al., 1991). The genes *PR-2* and *PR-3*, which were significantly higher expressed in ‘Granola’, encode for the enzymes β-1,3-glucanase and chitinase. As a part of a broad generalised plant defence mechanism present in many higher plants, these enzymes contribute to the suppression of fungal pathogens (Tonón et al., 2002). Both 1,3-β-glucans and chitin are cell wall components of many fungi and indeed the enzymes β-1,3-glucanase and chitinase have the capability to degrade fungal cell walls. Therefore, these plant hydrolases have the potential to improve resistance of the plants against fungal pathogens. In our study, constitutive transcript levels of *PAL* and *PI2* were also significantly higher in 'Granola' compared to 'Arkula'. In accordance with these findings, Derksen et al. (2013) determined a significantly higher expression of *PAL* in roots and leaves of a potato cultivar with moderate resistance to *V. dahliae* compared to a susceptible cultivar. PAL is a key enzyme in the phenylpropanoid pathway (Derksen et al., 2013). Phenylpropanoid compounds like flavonoids possess important antimicrobial properties and function either as phytoanticipins (preformed) or phytoalexins (induced) (Shadle et al., 2003). Presumably, enhanced resistance towards pathogens is based on co-expression of defence-related genes and may result in lower disease severity (Takemoto et al., 1997). Hence, the co-expression of various defence-related genes in the cultivar ‘Granola’ might be one reason for a higher resistance level to black scurf.

Furthermore, the two cultivars showed different responses to an infection with *R. solani* AG3PT. More specifically, the expression of the analysed defence-related genes was differently affected in ‘Arkula’ compared to ‘Granola’. In accordance with the first study on defence gene expression in *R. solani* AG3PT-inoculated mini tubers of 'Arkula', a significant up-regulation of defence-related genes was revealed in roots (*PR-2*, *PI2)* and shoots (*PR-2*, *PR-3*, *PAL, PR-10*) of inoculated plantlets of 'Arkula' compared to respective non-inoculated treatments. Interestingly, except for *PR-2* in one out of two experiments, none of the tested genes was significantly up-regulated in 'Granola' due to the infection with the pathogen. Surprisingly, a significant down-regulation of *PR-1* and *GST* was observed in shoots of 'Granola' at 10 days post inoculation in one of the experiments. Similar observations have been made in the case of a tomato line tolerant to *V. dahliae* (Robb et al., 2007). The authors observed an induction of several defence-related genes in a susceptible line of tomato (high disease severity) at 10 days post inoculation with *V. dahliae*, while there was no change or even a down-regulation for some of these genes observed in a tolerant line (low disease severity). At the same time, susceptible and tolerant lines contained similar amounts of pathogen DNA. Another study also described that a tolerant potato line showed a decreased expression of defence-related genes like chitinase in an interaction with *V. dahliae* (Tai et al.,

2013). However, we assume that a lower black scurf disease severity of ‘Granola’ is rather based on quantitative resistance than tolerance because a lower amount of the pathogen’s DNA was detected in this cultivar compared to ‘Arkula’, the cultivar showing a higher black scurf disease severity in the field.

Although pathogen treatment did not increase the transcript level of *PR-2*, *PR-3*, and *PAL* in 'Granola', the expression level was still higher than in inoculated 'Arkula'. So in turn, these observations suggest that *PAL*, *PR-2*, and *PR-3* are involved in defence against *R. solani* AG3PT, however, the constitutive expression might be more relevant. Consistent with these findings, Derksen et al. (2013) reported that, although the inoculation of potato with *V. dahliae* did not increase *PAL1*, *PAL2*, and *PR-2* in a moderately resistant cultivar, these genes were still higher expressed than in the susceptible cultivar. An increased *PAL* activity or transcript level in plants infected with a pathogen, as has been observed for 'Arkula' challenged with *R. solani* AG3PT, has been repeatedly described before (Wen et al., 2005; Singh et al., 2014; Kim and Hwang, 2014). However, opposite observations, where no significant change in *PAL* expression was observed, have been made as well (Niehl et al., 2006). Besides, Kröner et al. (2011) revealed that an increased PAL activity after pathogen treatment was positively correlated with resistance of potato to *Pectobacterium atrosepticum* but negatively correlated with resistance to *P. infestans*. This indicates a pathosystem-dependent relation between PAL activity and resistance. Nicholson and Hammerschmidt (1992) suggested that an increased expression level of *PAL* after pathogen infection should not be taken as an indicator for plant resistance, but rather as the result of wounding during pathogen attack. They state that increases in the activity of PAL in potato tuber tissue were related to wounding after inoculation with *P. infestans* and not to the infection. A study on the pathosystem maize-*Helminthosporium maydis* revealed an increased level of PAL in a susceptible host-pathogen interaction, but no change in a resistant interaction (Nicholson and Hammerschmidt, 1992). These findings are in favour of our observations. Thus, an increase of the transcript level of *PAL* in 'Arkula' might have been caused by wounding due to the infection with *R. solani* AG3PT. 'Granola' however, might have been able to limit pathogen infection and wounding, so that the level of *PAL* did not increase. Moreover, the content of phenylpropanoids like rutin and 1-caffeoylquinic acids decreased in the case of ‘Arkula’ after inoculation with *R. solani* AG3PT (chapter 4.4.2.3). This suggests that the increased transcript level of the studied *PAL* gene, which was observed for ‘Arkula’, does not lead to elevated biosynthesis of these phenylpropanoids. Several isoforms and genes of *PAL* have been shown to exist at least for *Arabidopsis thaliana*. These different *PAL* genes are presumably assigned to the biosynthesis of different metabolite pools (Lillo et al., 2008). This might explain observations we made in the case of ‘Arkula’.

*Accordance of transcript and protein abundances?*

Concomitantly to the analyses of transcript levels, we determined abundances of the proteins PR-1, PR-2, PR-3, and PR-10 in roots and sprouts. It has been shown that alterations in transcription do not necessarily result in altered protein accumulations (Maier et al., 2009). Biological and methodological factors can have a great impact on the comparison of results of transcript levels and protein abundances. In the first study, we were able to confirm the results of the pathogen-induced up-regulation of *PR* genes on protein level to a great extent. Similar to RNA accumulation analyses, we determined a significantly higher abundance of PR-2 at 6 dpi in roots of the inoculated treatment. Moreover, significantly increased abundances were detected for *PR-2* at 6 dpi, *PR-3* at 3 and 6 dpi and *PR-10* at 3 dpi in inoculated sprouts at transcript and protein level compared to the respective controls. However, in case of *PR-1*, the accumulation of transcripts did not result in an accumulation of protein indicating a transcriptional or post-translational regulation of PR-1. Recently, the wheat PR-1 protein was identified as a target of necrotrophic pathogen effector proteins, which led to the assumption that the expression of PR-1, or other PR proteins, may be actively regulated by pathogens (Breen et al., 2016). In the second study, PR protein levels were determined in 'Arkula' and 'Granola'. An up-regulation of *PR* genes due to pathogen treatment could not be confirmed on protein level. However, a significantly higher transcript level of *PR*-2 in 'Granola' compared to 'Arkula' was consistent with results of the protein analysis. Discordances between transcript and protein levels can arise from different reasons. Firstly, on the way to the completed protein, mRNAs are subjected to various post-transcriptional processes so that protein levels are greatly affected (Greenbaum et al., 2003; Hajduch et al., 2010). Moreover, the rate of translation not only depends on the level of transcripts but also on the number of ribosomes present on the transcript and their speed of translation (Piques et al., 2009). Thus, the rate of translation is highly variable and directly affects mRNA-protein correlations (Maier et al., 2009). Secondly, the life time of a specific protein can vary distinctly depending on intrinsic protein stability, posttranslational processing, and localisation of the protein (Maier et al., 2009). Considering these factors, discordances between measured transcript and protein abundance might be explained.

## 5.4 Concentrations of plant secondary metabolites in potato tissue differ dependent on cultivar resistance to black scurf disease

In addition to well-known PR proteins, a variety of plant secondary metabolites, functioning either as phytoanticipins or phytoalexins, constitutes an essential part of the plant's defence. It was hypothesised that the degree of field resistance of potato to *R. solani* AG3PT is related to the concentration of shoot and root plant secondary metabolites which function as such

phytoanticipins or phytoalexins. To get a first impression, the metabolite diversity was investigated in highly susceptible 'Arkula' and less susceptible 'Granola', prior to (constitutive) and after inoculation with *R. solani* AG3PT using a non-targeted metabolomic approach. This non-targeted analysis revealed cultivar-dependent differences in the concentration of several compounds. These compounds, like phenylpropanoids and glycoalkaloids, belonged mainly to the secondary plant metabolism. Significant differences between resistant and susceptible cultivars regarding the abundance of phenylpropanoid and terpenoid-derived compounds have been described before for the pathosystem *P. infestans*-potato (Pushpa et al., 2014). Therefore, phenylpropanoids, carotenoids, and glycoalkaloids were subsequently analysed using a targeted approach. Furthermore, transcript levels of selected biosynthetic genes were investigated.

*Carotenoids*

Although carotenoids are not specifically attributed to defence responses against pathogens in general, their analysis was included as well. Besides quenching damaging singlet oxygen species during photosynthesis, they are protecting phospholipids in biomembranes from free radicals under different kinds of stresses (Boba et al., 2009). Additionally, carotenoid catabolism leads to formation of hormones like abscisic acid (ABA), a hormone which also has an important role in response to abiotic and biotic stresses (Asselbergh et al., 2008; Cazzonelli and Pogson, 2010). Therefore, plant carotenoids might play a role during pathogen attack.

Carotenoids are derived, like steroidal potato alkaloids and sesquiterpenoids (phytoalexins in potato), from the isoprenoid pathway (Krits et al., 2007; Cazzonelli and Pogson, 2010). Via UHPLC-DAD-ToF-MS analysis the carotenoids α-carotene, β-carotene, lutein, zeaxanthin, neoxanthin, and violaxanthin were identified and quantified in roots and shoots of both cultivars. A study investigating carotenoids in potato showed that violaxanthin and lutein were the most abundant carotenoids in tubers (Payyavula et al., 2013). In our study, these compounds were the most prominent in shoots and roots as well. In roots of *Lycopersicon*, β-carotene, lutein, antheraxanthin, neoxanthin, and violaxanthin had already been found (Parry and Horgan, 1992). Due to the health beneficial properties of carotenoids, studies on their contents are mainly focused on the edible part of the plant, in case of potato the tuber (Andre et al., 2007; Campbell et al. 2010; Cazzonelli and Pogson, 2010; Payyavula et al., 2013). However, it was shown that, in roots, ABA is derived from the carotenoid pathway, specifically the 9'-*cis*-neoxanthin (Parry and Horgan, 1992). ABA has been shown to either promote or suppress disease resistance dependent on the stage of the infection and the pathogen. For instance, in *A. thaliana* ABA mediates stomata closure leading to increased resistance to *P. syringae* prior to the infection (Melotto et al. 2006). In contrast, tomato

mutants, which had reduced ABA levels, showed increased resistance to *B. cinerea* compared to the wildtype indicating that ABA promotes susceptibility in this pathosystem (Audenaert et al., 2002). It has been shown that ABA suppresses PAL activity at transcript level which influences the biosynthesis of phenylpropanoids, important compounds with antimicrobial properties (Asselbergh et al., 2008). In sum, ABA has an impact on the outcome of plant-pathogen interactions, so that it should be considered in studies on plant responses to pathogens (Asselbergh et al., 2008; Robert-Seilaniantz et al., 2011). In turn, this implies the investigation of carotenoid contents.

In our study, non-inoculated 'Arkula' had a significantly higher concentration of carotenoids in roots and shoots compared to 'Granola'. Cultivar-dependent differences in carotenoid concentrations of potato tubers have been described before (Andre et al., 2007; Payyavula et al., 2013). Since the catabolism of carotenoids results *inter alia* in ABA, the transcript level of a gene encoding a 9'-*cis*-epoxycarotenoid dioxygenase (*NCED*), a key enzyme involved in its biosynthesis, was also investigated in our study (Campbell et al., 2010). In roots, the gene encoding NCED was significantly higher expressed in 'Arkula' than in 'Granola'. This indicates a higher biosynthesis of ABA. Since increased susceptibility of plants due to higher levels of ABA has been reported before, it might explain a higher susceptibility of 'Arkula' to *R. solani* AG3PT compared to 'Granola' (Jiang et al., 2010). It has to be considered though that the NCED concentration is presumably correlated to the endogenous ABA concentration which is regulated by the balance between biosynthesis and catabolism of ABA (Nambara and Marion-Poll, 2005). Additionally, enzymes involved in upstream metabolic processes of ABA biosynthesis affect the ABA level. It has been shown that besides plants phytopathogenic fungi like *R. solani* are also able to produce ABA (Dörffling and Petersen, 1984). During plant-pathogen interactions, a production of hormones by phytopathogens is often related to disease (Robert-Seilaniantz et al., 2011). We did not determine ABA concentrations in the plants, so that further interpretations would be premature.

Unexpectedly, the gene coding for phytoene synthase (*PSY*), a key enzyme initiating the carotenoid biosynthesis (Diretto et al., 2010), was significantly higher expressed in 'Granola'. In contrast to our results, recent work has shown that enhanced expression levels of phytoene synthase lead to an increased accumulation of carotenoids (Diretto et al., 2007; Goo et al., 2009). However, phytoene synthase is acting at very early steps of the carotenoid biosynthesis and a possible degradation of carotenoids has to be considered as well. Moreover, many plants possess different *PSY* genes. In maize endosperm it has been shown for instance that changes in the transcript level of *PSY1* correlated with carotenoid concentrations, while the transcript levels of *PSY2* and *PSY3* were not correlated with carotenoid accumulation (Walter and Strack, 2011). In tomato, only the recently identified putative *PSY3* gene was responding to nutrient stress and mycorrhizal colonisation, while

*PSY1* and *PSY2* were not affected (Walter et al., 2015). The *PSY3* gene has not been described for potato, so that we only determined the expression of the chloroplastic phytoene synthase 2.

*Glycoalkaloids*

The most prominent phytoanticipins of potato are the steroidal glycoalkaloids α-chaconine and α-solanine (Ginzberg et al., 2009). These glycoalkaloids are known for their antifungal activities (Fewell and Roddick, 1993). Steroidal glycoalkaloids of potato are generally found in all parts of the potato plant (Ginzberg et al., 2009). Based on their toxicity for humans, the recommended threshold value for consumption is 20 mg per 100 g fresh weight of potato tubers (Ginzberg et al., 2009). Considering these health-impairing effects, the reduction of glycoalkaloid concentrations in potato tubers is one aspect of potato breeding. On the other hand, glycoalkaloids possess antimicrobial properties, so that decreased concentrations in potato might increase their susceptibility to phytopathogens. The content of glycoalkaloids is dependent on genotype, plant tissue, developmental stage, and abiotic factors like climate (Friedman, 2006). Due to a poor correlation between tuber content and content in aboveground tissue, it is presumed that their biosynthesis occurs tissue-specific (Ginzberg et al., 2009). Highest concentrations of glycoalkaloids were found in shoots and leaves, followed by roots (Friedman, 2004). In our investigations, the concentrations of α-chaconine and α-solanine measured in roots and shoots were significantly higher in 'Granola' than in 'Arkula'. A recent study investigating quantitative resistance of potato against *P. infestans* also revealed significantly higher constitutive concentrations of α-chaconine and α-solanine in leaves of a resistant compared to a susceptible cultivar (Pushpa et al., 2014). Based on the fact that α-chaconine is more toxic than α-solanine, the ratio (α-chaconine/α-solanine) might be even more relevant in determining the antimicrobial potential of different cultivars. 'Granola' did also show a significantly higher ratio (α-chaconine/α-solanine) than 'Arkula'.

Potato glycoalkaloids are synthesised via the mevalonate pathway from acetate over mevalonate, isopentenyl, pyrophosphate, squalene, cholesterol to the aglycone solanidine (Friedmann, 2006; Krits et al., 2007; Milner et al., 2011). Solanidine is the precursor of γ-solanine and γ-chaconine. The formation of their α-forms is catalysed by rhamnosyltransferase (SGT3) (McCue et al., 2007). 'Granola' revealed a significantly higher transcript level of genes encoding a squalene synthase (*SQS*) as well as rhamnosyltransferase (*SGT3*) in roots and shoots compared to 'Arkula'. This is in accordance with the higher content of α-chaconine and α-solanine in 'Granola'. A positive relationship between increased transcript levels of *SQS* and *SGT3* and increased contents of α-chaconine and α-solanine has been repeatedly described (Manjulatha et al., 2014; Mariot et al., 2016; Nahar et al., 2017).

The significantly higher concentration of α-chaconine and α-solanine detected in roots of 'Granola' might contribute to a higher quantitative resistance to *R. solani* AG3PT. Yet, previous studies reported only low growth inhibiting effects of these two glycoalkaloids on *R. solani* (Fewell and Roddick, 1993). However, the authors did not mention the anastomosis group (AG) of the isolate they tested, but the sensitivity to glycoalkaloids can considerably vary between different isolates. Therefore, we tested the growth impairing effect of α-chaconine and α-solanine on *R. solani* AG3PT using an *in vitro* culture system. Thus, strong growth inhibiting effects were revealed for both glycoalkaloids at concentrations of 200 and 100 µM but also at 50 µM. Yet, a higher resistance of the plant to this fungus is probably not distinctly dependent on these two glycoalkaloids, although they may have a supporting effect in combination with other defence-associated compounds. A synergistic effect of α-chaconine and α-solanine was observed in our experiments and in a previous study (Fewell and Roddick, 1993).

Most studies focus mainly on α-chaconine and α-solanine, although other glycoalkaloids present in *Solanum* species possess antifungal properties as well. *In vitro* experiments showed that 50 µM of the glycoalkaloid solamargine applied in combination with 50 µM of solasonine inhibited the growth of *R .solani* up to 41 % dependent on the pH (Fewell et al., 1994). Solamargine possesses membrane-disrupting properties and was most effective in combination with either solasonine or chaconine (Roddick et al., 1990). In addition, β-solamarine showed liposome-disrupting activities *in vitro* (Roddick et al., 2001). A disruption of membranes is supposedly caused due to the binding of glycoalkaloids with sterols of the cell membrane of other organisms (Sucha and Tomsik, 2016). Using a non-targeted metabolomic approach, we were able to identify several additional glycoalkaloid components in both cultivars. Thus, the glycoalkaloids β-solamarine, solasonine, teinemine, and additionally the aglycone β-tomatidine were found in roots and shoots of both cultivars. In shoots, the glycoalkaloid hydrolysis product γ-chaconine was also detected. Tai et al. (2014) found β-solamarine and solasonine in *Solanum tuberosum* cv. Shepody, whereas tomatidine was only found in wild species like *S. chomatophilum*, *S. paucissectum,* and *S. piurae*. Teinemine is a precursor of solanidine, which is converted into γ-chaconine and γ-solanine respectively (Kaneko et al., 1977, Ginzberg et al., 2009). Tomatidine, an aglycone of tomatine and sisunine, has been shown to impair the biosynthesis of ergosterol in yeast which is probably causing its antifungal activity (Simons et al., 2006; Milner et al., 2011).

*Phenylpropanoids*

Phenylpropanoid-derived metabolites like flavonoids/isoflavonoids, lignin or salicylic acid are known for their important role in protecting the plant from various abiotic e.g. UV-light and biotic stresses (Hahlbrock and Scheel, 1989). Flavonoids can be involved in preformed as

well as induced plant defence. Phenolic compositions of potato tubers and parameters which affect their concentration have been extensively studied (Lewis et al., 1998; Navarre et al., 2011; Payyavula et al., 2013). The phenolic content has been shown to be strongly correlated to the antioxidant activity (Navarre et al., 2011). In this study, the phenolic compounds 1-caffeoylquinic acid, 3-caffeoylquinic acid (neochlorogenic acid), quercetin-3-rutinoside (rutin), and kaempferol-3-rutinoside (nicotiflorin) were identified in roots and shoots of potato. Additionally, quercetin-3-rutinoside-7-glucoside, quercetin-diglucoside, kaempferol-3-rutinoside-7-glucoside, and kaempferol-3-diglucoside were detected in shoots. These compounds have also been detected in tubers of different potato cultivars in previous studies (Navarre et al., 2011; Kröner et al., 2012). In potato, chlorogenic acids are the most abundant phenolics, while rutin is one of the most abundant flavonols (Navarre et al., 2011). Navarre et al. (2011) detected concentrations of rutin up to 0.14 mg per gram dry weight which is similar to amounts determined in roots and shoots of our study. 'Granola' had significantly higher concentrations of 3-caffeoylquinic acid, rutin, and nicotiflorin than 'Arkula'. In contrast, 'Arkula' revealed higher concentrations of quercetin-diglucoside and kaempferol-3-diglucoside. Since the phenolic content is not only influenced by abiotic and biotic factors, but also dependent on the plant genotype, differences between cultivars are not surprising (Shaked-Sachray et al., 2002; Navarre et al., 2011). Phenylpropanoids are known for their antimicrobial properties which make them interesting agents of non-specific resistance (Kröner et al., 2012). Therefore, a higher constitutive level of phenylpropanoid compounds determined in ‘Granola’ indicates a higher quantitative resistance against *R. solani* AG3PT.

Interestingly, Kröner et al. (2012) revealed that the total phenolic content in potato tubers was negatively correlated with disease severity against *Pectobacterium atrosepticum*, but positively correlated with severity in the case of *P. infestans*. This leads to the assumption that, although associated with unspecific defence, the role of phenolics in defence may be dependent on specific plant-pathogen interactions. Additionally, it has to be considered that *P. infestans* is a biotroph, whereas *P. atrosepticum* is a necrotroph, so that contrasting impacts of phenolics might be ascribed to the different lifestyles of the pathogens. All in all, our results indicate a negative correlation between the phenolic content and disease susceptibility during the interaction between the necrotroph *R. solani* AG3PT and potato.

### 5.4.1 Concentrations of glycoalkaloids, carotenoids, and phenylpropanoids in ‘Arkula’ and ‘Granola’ are differently affected by *Rhizoctonia solani* AG3PT inoculation

*Carotenoids*

Several carotenoids (lutein, neoxanthin, violaxanthin) were significantly decreased in *R. solani* AG3PT-treated 'Arkula', whereas 'Granola' showed increased contents upon

inoculation with the pathogen. This effect was more pronounced in roots compared to shoots. Supposedly, the cleavage of carotenoids, which leads to the formation of apocarotenoids, is enhanced by abiotic and biotic stresses (Walter and Strack, 2011). Thus, apocarotenoids might contribute to defence responses of plants against pathogens. Plant hormones like strigolactones for instance, which are derived from carotenoids by carotenoid cleavage dioxygenases, have been shown to regulate other plant hormones like ABA, SA, and JA (Torres-Vera et al., 2014). An accumulation of strigolactones has been found in roots colonised by arbuscular mycorrhizal fungi and their synthesis was also induced in tomato plants parasitised by *Phelipanche ramosa* (Strack and Fester, 2006; Torres-Vera et al., 2016). Moreover, strigolactone inhibited growth of root pathogens like *Fusarium oxysporum* f. sp. *melonis*, *Fusarium solani* f. sp. *mango*, *Sclerotinia sclerotiorum* and *Macrophomina phaseolina in vitro* (Dor et al., 2011). In tobacco, Salt et al. (1986) detected an increased content of the apocarotenoid β-ionone in uninfected cells close to cells infected with *Peronospora tabacina* Adam. Thus, the observed decrease in the content of carotenoids in inoculated roots of 'Arkula' may be caused by an elevated cleavage of carotenoids. In an additional RNA-Seq experiment, we detected a significantly increased transcript level of a 9-*cis*-beta-carotene 9',10'-cleaving dioxygenase (CCD7) in *R. solani* AG3PT-inoculated sprouts of 'Arkula' compared to the respective control at 3 days post inoculation. The enzyme CCD7 catalyses the formation of the above-mentioned strigolactones. This is in favour of an elevated carotenoid cleavage induced by *R. solani* AG3PT infection. However, it is interesting to note that the reverse trend regarding changes in contents of carotenoids was made for 'Granola'. An induced activation of carotenoid biosynthesis has also been described in roots colonised by mycorrhizal fungi (Fester et al., 2005). Studies investigating the influence of pathogen infection on carotenoid content in roots are rarely found. Yet, an increased concentration of carotenoids has been detected in wheat leaves and spikes inoculated with *Fusarium culmorum* and *F. graminearum* compared to respective controls (Khaledi et al., 2016). Furthermore, the partially resistant cultivar had a significantly higher content of carotenoids compared to the susceptible cultivar after inoculation with the pathogens. Besides, Galindo-Gonzalez and Deyholos (2016) determined an increased transcript abundance of a phytoene synthase, key enzyme in early steps of carotenoid biosynthesis, in moderately resistant flax (*Linum usitatissimum* L.) after inoculation with *Fusarium oxysporum* f. sp. *lini*. This indicates an activation of carotenoid biosynthesis due to the pathogen infection. Although not significant, we also detected a slightly increased transcript level of a gene encoding a phytoene synthase after inoculation with *R. solani* AG3PT in roots of 'Arkula' and 'Granola'.

It has been suggested that an increased antioxidant potential in plants, e. g. based on elevated carotenoid concentrations, might improve plant resistance to pathogens (Boba et

al., 2011). The authors reported that mutants overexpressing carotenoid biosynthetic genes were more resistant to *F. oxysporum* and *F. culmorum* compared to wildtype plants. The production of reactive oxygen species (ROS) by plants during plant-pathogen interactions is generally associated with disease resistance against biotrophic pathogens, whereas against necrotrophic pathogens ROS formation is assumed to enhance plant susceptibility (Govrin and Levine, 2000). This is explained by their different lifestyles. Biotrophs are dependent on a living host, while necrotrophs can feed on dead tissue (Glazebrook, 2005). Moreover, it has been shown that the necrotroph *Botrytis cinerea* actually induces ROS formation during interaction with the host plant (Schouten et al., 2002). In addition, cell death causing proteins, secreted by *R. solani* AG1 IA during interaction with rice, have been identified as well (Zheng et al., 2013). Carotenoids quench free radicals and toxins (Bouvier et al., 1998, Boba et al., 2011). Accordingly, higher carotenoid concentrations in *R. solani* AG3PT-infected roots of 'Granola' might contribute to a lower degree of susceptibility.

*Glycoalkaloids*

After inoculation with *R. solani* AG3PT, a significant up-regulation of a squalene synthase gene was detected in roots and shoots of both cultivars. Similarly, inoculation with *F. oxysporum* resulted in increased transcript levels of squalene synthase in roots and leaves of *S. nigrum* at 7 dpi (Sun et al., 2012). The biosynthesis of steroidal glycoalkaloids has not been fully elucidated, but it is known that squalene synthase catalyses the formation of squalene (Fig. 4.1) which is a precursor of sterols and steroidal glycoalkaloids (Yoshioka et al., 1999). Besides, it has been previously shown that squalene synthase plays an important role in non-host resistance of tobacco against *Pseudomonas syringae* and *Xanthomonas campestris*. The authors suggest that the activity of enzymes involved in sterol biosynthesis is altered after pathogen attack in order to prevent a nutrient efflux into the apoplast (Wang et al., 2012). Plant sterols have various functions and are mainly compounds of cell membranes where they regulate permeability and fluidity (Schaller, 2003). Thus, a change in the composition of sterols would affect membrane properties which might be an important factor in disease resistance. A part of plant sterols functions as precursor of brassinosteroids. Brassinosteroids have been recently shown to be involved in plant immune responses to pathogens (Belkhadir et al., 2012). Therefore, an up-regulation of squalene synthase might be an indicator for an increased biosynthesis of phytosterols or brassinosteroids upon pathogen infection. An up-regulation of squalene synthase in inoculated treatments also indicates an involvement of potato steroidal glycoalkaloids in the defence response against *R. solani* AG3PT. However, the content of α-chaconine and α-solanine decreased in roots of 'Arkula', whereas there was a significant increase detectable in case of 'Granola'. A decrease of α-chaconine and α-solanine, caused by *R. solani* AG3PT, has been described before in

inoculated sprouts of the potato variant ‘Kennebek’ (Aliferis and Jabaji, 2012). The authors suggest that *R. solani* AG3PT might be able to metabolise the α-forms into their less toxic β- and γ-forms, because they revealed an increase of β- and γ-chaconine in inoculated sprouts. Several filamentous fungi like *Plectosphaerella cucumerina*, *Cladosporium cladosporioides,* and *Penicillum* sp. have been shown to convert α-chaconine into its less toxic form β-chaconine (Oda et al., 2002). It is known that phytopathogenic fungi produce glycosidases which hydrolise α-glycoalkaloids to their β- and γ-forms (Sandrock and van Etten, 1998; Milner et al., 2011). However, a study conducted by Fewell and Roddick (1997) rather contradicts a possible hydrolysis of α-chaconine and α-solanine by *R. solani*. The authors found no reduction in α-chaconine and only a slow decrease of α-solanine at 10 days post inoculation. Moreover, the transcript level of *SGT3*, which catalyses the formation of β-solanine and β-chaconine into their α-forms, was reduced in *R. solani* AG3PT-treated roots of 'Arkula' and 'Granola', although only significantly in ‘Granola’. McCue et al. (2005) suggested a feedback inhibition of enzymes catalysing the primary step of the glycosylation of solanidine into solanine and chaconine. They observed that adding α-chaconine or α-solanine reduced the activity of galactose:solanidine galactosyltransferase (SGT1) which catalyses the formation of γ-solanine. Accordingly, high contents of α-solanine and α-chaconine determined in ‘Granola’ might have led to a decreased transcript level of *SGT3* via negative feedback regulation. In addition, *cis*-elements, which are related to disease response, have been recently identified in the promotor region of *SGT3* (Mariot et al., 2016). This supports expression changes of *SGT3* observed after inoculation with *R. solani* AG3PT.

Besides α-chaconine and α-solanine, we detected the glycoalkaloid components β-solamarine, solasonine, β-tomatidine, teinemine, and γ-chaconine in shoots of 'Arkula' and 'Granola'. Aliferis and Jabaji (2012) observed an increase of several glycoalkaloids like solasonine, solanaviol, solasodenone, solasodiene, and solaspiralidine in *R. solani* AG3PT-inoculated shoots of potato. An increase of the aglycones solanidine and solasodine concomitant with a decrease of α-chaconine and α-solanine has also been observed in potatoes at 8 dpi with *Pythium ultimum* (Tata et al., 2015). Solanidine leads to the formation of solanine and chaconine, whereas solasodine results in the formation of solamargine and solasonine. In addition, the authors reported an increase of the glycoalkaloid components solanidine, solasodenone, solanaviol, solasodiene, solaspiralidine, γ-solanine/γ-chaconine, β-solanine, and β-chaconine. In contrast to results of Aliferis and Jabaji (2012), they observed no increase of solasonine in sprouts infected with *P. ultimum*. Furthermore, Sun et al. (2012) detected an increase of the glycoalkaloids γ-solamargine and solasodine in roots and leaves of *S. nigrum* due to an infection with *F. oxysporum*. A growth-inhibiting effect of these glycoalkaloids on *F. oxysporum* has been observed as well. This suggests that a

decreased susceptibility of potato might be based on the synergistic action of various glycoalkaloids and not exclusively on α-chaconine and α-solanine.

In our study, the comparison of both cultivars revealed significant differences regarding the glycoalkaloids α-chaconine and α-solanine as well as transcript levels of genes encoding enzymes involved in their biosynthesis. 'Granola' had higher concentrations of α-chaconine and α-solanine than 'Arkula'. In addition, the expression of the gene *SGT3* was significantly higher in 'Granola'. Using a non-targeted metabolomic approach, significant differences between both cultivars regarding the content of alkaloids like β-solamarine, solasonine, β-tomatidine, teinemine, and γ-chaconine were detected. Since β-solamarine and solasonine show antimicrobial activities, they might contribute synergistically with α-chaconine and α-solanine to a higher resistance of 'Granola' against *R. solani* AG3PT. To test this hypothesis, further experiments studying the effect of different glycoalkaloid compounds and the effect of their combinations on *R. solani* AG3PT should be included in further studies.

*Phenylpropanoids*

Several studies revealed an induction of the phenylpropanoid pathway after recognition of the pathogen by the plant underlining the importance of this pathway during plant defence (Matsuda et al., 2005; Kröner et al., 2012). For instance, chlorogenic acids and caffeic acids are accumulated in potato tuber tissue due to wounding or infection with pathogenic and non-pathogenic microorganisms. Wounding leads to oxidation of these compounds resulting in oxidation products which are toxic to microorganisms (Kuć, 1973). In order to study the relevance of phenylpropanoid metabolites for quantitative resistance of potato against *R. solani* AG3PT, we determined the content of several phenylpropanoids using HPLC. Interestingly, inoculation with *R. solani* AG3PT led to a significant decrease of rutin and 1-caffeoylquinic acid in roots and quercetin-3-rutinoside-7-glucoside as well as quercetin-diglucoside in shoots of 'Arkula'. This is in contrast to findings made by Hadrami et al. (2011), where inoculation of a moderately susceptible potato cultivar with *V. dahlia*e led to a significant increase of rutin. However, Azevedo et al. (2010) also determined a decreased content of caffeoylquinic acids and quercetins in cells of *Pinus pinaster* treated with *Botrytis cinerea*. Caffeoylquinic acids are precursors of lignin and thus important compounds supporting the plant cell wall stability (Pandino et al., 2011). Since the deposition of lignin is part of the plant's defence, a decrease of caffeoylquinic acids could have been the result of an increased biosynthesis of lignin and thus an increased consumption of caffeoylquinic acids (Nicholson and Hammerschmidt, 1992). However, contrasting results regarding the induction of lignin biosynthesis after pathogen infection are described in the literature. Azevedo et al. (2010) observed a decreased lignin content, while Xu et al. (2011) (cotton-*Verticillium dahliae*) and Singh et al. (2014) (sunflower-*R. solani*) detected an increased

accumulation of lignin in plants challenged with a pathogen. The analysis of transcriptome changes in sprouts of 'Arkula' after inoculation with *R. solani* AG3PT revealed a significant up-regulation of a gene encoding a lignin-forming anionic peroxidase (*unpublished data*). This was an individual experiment, which had been conducted additionally to the experiments described in this study. Nevertheless, the elevated transcript level of this gene supports the assumption of lignin accumulation in *R. solani* AG3PT-challenged shoots of 'Arkula'. An increased biosynthesis of lignin entails a higher consumption of p-coumaroyl-CoA, which is also a precursor of flavones and flavanols like rutin (Ferrer et al., 2008). This is in accordance with a decreased content of rutin detected in pathogen-treated roots of ‘Arkula’.

In contrast to ‘Arkula’, no significant changes in contents of phenylpropanoids were detected in inoculated roots and shoots of 'Granola' in comparison to the non-inoculated treatments. In accordance with this observation, the transcript level of a gene coding for PAL*,* a key enzyme of the phenylpropanoid pathway*,* was not increased due to the pathogen treatment in 'Granola' as well (chapter 4.3.5). Consistent with this, it has been shown before that the PAL activity and the content of phenylpropanoids are related (Kröner et al., 2011). Similarly, Mittelstraß et al. (2006) detected no effect of an infection with *P. infestans* on the content of chlorogenic acids and rutin in potato leaves. Although the content of detected phenylpropanoids was not significantly affected due to the pathogen treatment in ‘Granola’, these compounds might play a crucial role in quantitative resistance against *R. solani* AG3PT.

After all, ‘Granola’ contains significantly higher amounts of phenolic acids, nicotiflorin, and rutin which have been shown to possess antimicrobial properties (Bàidez et al., 2007; Kröner et al., 2011; Soberón et al., 2014). Using *in vitro* culture tests, we could show for the first time that nicotiflorin significantly reduces the growth of *R. solani* AG3PT. Previously, the growth of *Pseudomas syringae* and *Erwinia carotovora* had been shown to be impaired by nicotiflorin as well (Soberón et al., 2014). In contrast to these results, Kröner et al. (2012) did not observe any toxic effect of nicotflorin on *P. infestans* or *P. atrosepticum*. This suggests that the effect of nicotiflorin is pathogen-specific. However, quercetin, rutin, and 3-caffeoylquinic acid (neochlorogenic acid) had no growth-reducing effect on *R. solani* AG3PT. Likewise, Padmavati et al., (1997) detected no inhibitory effect of quercetin on rice-infecting *R. solani*. Furthermore, the growth of *V. dahliae* was not significantly reduced when medium was supplemented with rutin or quercetin (Hadrami et al., 2011). Moreover, through supplementation of nutrient-poor medium with rutin or quercetin the growth of *V. dahliae* was promoted (Hadrami et al., 2011). The authors suggested that *V. dahliae* uses them as carbon sources by degrading these compounds. Induction of flavonol cleaving quercetinases in the presence of rutin, kaempferol or quercetin has already been revealed for several filamentous

fungi (Tranchimand et al., 2008). The enhanced growth of *R. solani* AG3PT, determined on medium containing rutin, quercetin or kaempferol in the current study, indicates a similar mechanism. Weidenbörner et al. (1990) observed that isoflavonoids can have a growth inhibiting or stimulating effect on *R. solani* dependent on the concentration. A very recent study indicates that *R. solani* is able to hydrolase chlorogenic acids (Nieter et al., 2017). It seems reasonable to presume that *R. solani* AG3PT is able to degrade neochlorogenic acid as well.

Since most of the phenolic compounds tested did not inhibit growth of *R. solani* AG3PT, they might have other functions during plant defence. Kröner et al. (2012) suggested that phenolic compounds do not always have antimicrobial activities but rather contribute in other ways to resistance. Consistent with this view, an early study by Gayed and Rosa (1975) indicated that higher levels of chlorogenic acid in tobacco plants seem to be related with higher resistance against *Thielaviopsis basicola*, although the growth of *T. basicola* was not affected by chlorogenic acid. The authors suggested that high levels of phenolics indicate higher lignin biosynthesis. Thus, phenolic acids might rather contribute to resistance by cell wall stabilisation and not by direct growth inhibition of the fungus. Moreover, chlorogenic acid and flavonoids possess antioxidant activity (Papp et al., 2004; Niggeweg et al., 2004). The phenolic content of potatoes has been shown to be strongly correlated to the antioxidant activity (Navarre et al., 2011). Presumably, a higher antioxidant activity in roots of ‘Granola’, which results from a higher phenolic content, contributes to a higher degree of resistance against *R. solani* AG3PT. Consistent with this, relatively high antioxidant activities have been found in tubers of 'Granola' (Al-Saikhan et al., 1995).

In summary, higher contents of phenylpropanoids in roots and shoots of potato seem to be related to higher resistance against *R. solani* AG3PT. However, the specific roles of rutin and neochlorogenic acid within the interaction between potato and this pathogen await further investigation. Nicotiflorin might contribute to a higher resistance to *R. solani* AG3PT through its direct growth-reducing effects.

### 5.4.2 Plant secondary metabolites - short summary

Since qualitative resistance within the pathosystem of *R. solani* AG3PT and potato has not been described until now, our investigation was focused on quantitative resistance. Quantitative resistance is polygenic and thus built on additive effects of phytoanticipins, phytoalexins, and defence-related proteins (Kushalappa and Gunnaiah, 2013). In our studies, two potato cultivars with different degrees of field resistance to *R. solani* AG3PT showed significant differences in the content of these potentially antimicrobial and defence-associated compounds. A higher content of flavonol gylcosides, hydroxycinnamic acid derivatives, and glycoalkaloids was determined in more resistant ‘Granola’. Moreover, *in vitro*

culture tests confirmed growth-reducing effects of the glycoalkaloids α-chaconine and α-solanine as well as of the flavonol nicotiflorin on *R. solani* AG3PT. Furthermore, the content of carotenoids, effective free radical scavengers, was higher in the more resistant cultivar during infection with the pathogen. Our results suggest that a higher degree of resistance of potato to *R. solani* AG3PT is, in addition to glycoalkaloids, also related to higher contents of phenylpropanoids and carotenoids. To unravel specific functions of these compounds within this pathosystem further investigations are required. However, the group of sesquiterpenoids, important phytoalexins in potato, was not included in this study. Due to their high relevance during plant-pathogen interactions they ought to be included in future studies.

# 5. CONCLUSION AND OUTLOOK

Potato, one of the most important food crops worldwide, is also threatened by many pests and microbial pathogens (Fiers et al., 2012). The potato-infecting fungus *Rhizoctonia solani* Kühn can lead to considerable economic losses in almost all potato growing areas (Tsror, 2010). Effective alternative control measures are strongly demanded. The use of resistant cultivars represents the most favourable alternative. Although cultivars showing complete resistance to *R. solani* infection have not been found, differences in the degree of susceptibility have been repeatedly observed (Thangavel et al., 2014). However, underlying mechanisms involved in the manifestation of resistance levels await clarification. Genes, which are crucial for the inheritance of resistance against *R. solani* AG3PT in different potato cultivars, are unknown (Sedláková et al. 2013). Presumably, differences in susceptibility to *R. solani* are based on quantitative resistance which is built on additive effects of various mechanisms involving the biosynthesis of secondary plant metabolites and the expression of pathogenesis-related (PR) proteins. It has been previously shown that the expression of *PR* genes and the content of secondary metabolites are positively related to a higher resistance of plants (Vleeshouwers et al., 2000; Mazid et al., 2011). Pathogenesis-related proteins like PR-2 (β-1,3-glucanase) and PR-3 (chitinase) directly attack fungal hyphae leading to inhibited or delayed plant colonisation (Collinge et al., 1993). Consequently, low amounts of pathogen DNA in plant tissue indicate an activation of plant mechanisms which reduce pathogen spreading, and thus contributing to resistance (Vandemark and Barker 2003).

To address these aspects, the studies of this thesis focused on two potato cultivars which had shown distinct differences in field resistance to *R. solani* AG3PT. Molecular and biochemical analyses were conducted on these selected cultivars. Quantitative real-time PCR confirmed a higher pathogen density in the less resistant cultivar compared to the cultivar with higher resistance to *R. solani* AG3PT. In the following it was hypothesised that the expression of *PR* genes would be higher in the potato cultivar with higher level of resistance. Since plant secondary metabolites constitute an essential part of the plant's defence, the more resistant cultivar was assumed to contain higher amounts of plant secondary metabolites compared to the less resistant cultivar.

Indeed, a higher expression of *PR* genes and higher contents of several plant secondary metabolites were determined in roots of the more resistant cultivar compared to the less resistant cultivar. These metabolites included the glycoalkaloids α-chaconine and α-solanine and the phenylpropanoids rutin, nicotiflorin, as well as caffeoylquinic acids. Surprisingly, no increased expression of *PR* genes was detected after inoculation with *R. solani* AG3PT in the case of the more resistant cultivar. Generally, *PR* genes are known to be induced upon pathogen infection. Moreover, cultivars expressing a higher level of resistance are expected

to respond earlier and to a higher extent to an infection than susceptible ones (Tonón et al. 2002). Although no up-regulation was observed in the case of the more resistant cultivar, the *PR* gene expression level was still higher compared to the less resistant cultivar. This indicates that preformed defence mechanisms are more relevant for resistance of potato against *R. solani* AG3PT than induced defence. Using *in vitro* culture tests, a growth-reducing effect of the glycoalkaloids α-chaconine and α-solanine and of the flavonoid nicotiflorin on *R. solani* AG3PT was confirmed. This could be the causal principle for the reduced pathogen density determined in the more resistant cultivar.

Concluding, a higher resistance of potato cultivars to *R. solani* AG3PT seems to be related to a higher preformed level of defence-associated compounds which includes *inter alia* a higher *PR* gene expression level as well as higher contents of plant secondary metabolites. Although these findings await completion e.g. confirmation of these results testing additional potato cultivars and *R. solani* isolates, they are very useful for potato breeding companies. If findings of this study can be confirmed for other potato cultivars, the expression level of *PR* genes might be an indicator for the degree of resistance which can be useful in breeding processes. It has to be considered though, that genes and compounds analysed in this thesis only represent a small proportion of candidates involved plant defence. Various additional metabolites like the sesquiterpenoids are most definitely crucial in this context and need to be considered in future studies.

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## 8. APPENDIX

**Table 17:** Assortment of potato cultivars which were screened regarding their susceptibility to *Rhizoctonia solani* AG3PT during field experiments conducted in cooperation with potato breeders. Maturity groups according to Saatguterzeugergemeinschaft Niedersachsen e. V. (2010)

| **Year** | **2013** | **2014** | **2015** | **Breeder** | **Maturity group** |
|---|---|---|---|---|---|
| **Cultivar** | **Arkula** | **Arkula** | **Arkula** | Norika GmbH | Very early maturing |
| | Belana | Belana | Belana | Böhm-Nordkartoffel Agrarproduktion GmbH & Co. OHG | Early maturing |
| | Birgit | | | Saatzucht Firlbeck GmbH & Co.KG | Medium early maturing |
| | Bonza | | | Saatzucht Firlbeck GmbH & Co.KG | Late maturing |
| | | | Cascada | Norika GmbH | Medium late / late maturing |
| | Cilena | Cilena | Cilena | Böhm-Nordkartoffel Agrarproduktion GmbH & Co. OHG | Early maturing |
| | Gala | | | Norika GmbH | Early maturing |
| | | **Granola** | **Granola** | SaKa Pflanzenzucht GmbH & Co. KG | Medium early maturing |
| | Jasia | | | Zuchtstation Niehoff | Late maturing |
| | | | Jelly | Böhm-Nordkartoffel Agrarproduktion GmbH & Co. OHG | Medium late / late maturing |
| | Lolita | Lolita | Lolita | Saatzucht Firlbeck GmbH & Co.KG | Medium early maturing |
| | Marabel | | | Böhm-Nordkartoffel Agrarproduktion GmbH & Co. OHG | Early maturing |
| | Panda | Panda | Panda | SaKa Pflanzenzucht GmbH & Co. KG | Medium late / late maturing |
| | Prima-donna | | | Solana GmbH & Co. KG | Early maturing |
| | Queen Anne | | | Solana GmbH & Co. KG | Early maturing |
| | Salute | Salute | Salute | Norika GmbH | Medium early maturing |
| | Skawa | | | Zuchtstation Niehoff | Late maturing |
| | Skonto | Skonto | Skonto | Zuchtstation Niehoff | Medium early maturing |
| | Solara | Solara | Solara | Böhm-Nordkartoffel Agrarproduktion GmbH & Co. OHG | Medium early maturing |
| | | | Troja | Norika GmbH | Medium late / late maturing |

**Table 18:** Spray rate of applied plant protection products during field experiments.

| Product | Type | Cause | 2015 | 2016 |
|---|---|---|---|---|
| **Dantop (Spiess-Urania)** | insecticide | *Leptinotarsa decemlineata* | 150 g/ha | 150 g/ha |
| **FASTAC (BASF)** | insecticide | *Leptinotarsa decemlineata* | 0 | |
| **FORUM (BASF)** | fungicide | *Phytophthora infestans* | 3 L/ha | 3 L/ha |
| **KARATE ZEON (Syngenta)** | insecticide | *Leptinotarsa decemlineata* | 0 | |
| **ORTIVA (Syngenta)** | fungicide | *Phytophthora infestans* | 0.72 L/ha | |
| **Ran Man A (ISK Biosciences)** | fungicide | *Phytophthora infestans* | 0 | |
| **Ran Man B (ISK Biosciences)** | fungicide | *Phytophthora infestans* | 0 | |
| **Ridomil Gold (Syngenta)** | fungicide | *Phytophthora infestans* | 3 kg/ha | 2 kg/ha |
| **TAMARON (Bayer CropScience)** | insecticide | *Leptinotarsa decemlineata* | 0 | |

**Table 19:** Monthly precipitation [mm] during the vegetation period.

| Month | 2015 | 2016 |
|---|---|---|
| **May** | 10.1 | 24.2 |
| **June** | 61.0 | 74.3 |
| **July** | 65.5 | 45.1 |
| **August** | 34.4 | 23.2 |
| **September** | 35.1 | 11.7 |
| **Sum of precipitation during vegetation** | **206.1** | **178.5** |

**Table 20:** Influence of cultivar and pathogen treatment on disease symptoms, assessed by Mann-Whitney U Test ($p \leq 0.05$). In 2015, disease symptoms were evaluated under low (control) and high pathogen pressure (inoculated with *Rhizoctonia solani* AG3PT).

| | | p-values for | | | |
|---|---|---|---|---|---|
| **Disease symptoms** | **Year** | **Cultivar** | | **Pathogen treatment** | |
| | | **control** | **inoculated** | **Arkula** | **Granola** |
| **Black scurf incidence** | 2015 | 0.0411 | 0.0022 | 0.025974 | 0.008658 |
| | 2016 | 0.0159 | - | - | - |
| **Black scurf severity** | 2015 | 0.044952 | 0.015568 | 0.041126 | 0.002165 |
| | 2016 | 0.012186 | - | - | - |
| **% deformed tubers** | 2015 | 0.040492 | 0.023471 | 0.326306 | 0.740301 |
| | 2016 | 0.015651 | - | - | - |
| **% tubers with drycore** | 2015 | 0.235198 | 0.089270 | 0.029193 | 0.155796 |
| | 2016 | 0.043438 | - | - | - |

## ERKLÄRUNG ZUR SELBSTSTÄNDIGKEIT

Hiermit versichere ich, Franziska Genzel, dass ich die vorliegende Dissertation mit dem Titel „The molecular basis of the plant-pathogen interaction of potato and *Rhizoctonia solani*" selbstständig angefertigt habe und keine anderen als die aufgeführten Hilfsmittel benutzt habe. Die Stellen der Arbeit, einschließlich Tabellen und Abbildungen, die anderen Werken im Wortlaut oder Sinn entnommen wurden, sind in jedem Fall durch Angabe der Quelle kenntlich gemacht. Die vorliegende Arbeit ist teilweise in wissenschaftlichen Fachzeitschriften veröffentlicht und entsprechende Daten sind in der Publikationsliste aufgeführt.

Die Dissertation wurde noch nicht (auch nicht anderer Form) an einer anderen Fakultät oder Universität zur Prüfung vorgelegt. Ich habe mich zu keinem früheren Zeitpunkt um einen Doktorgrad beworben.

Die dem angestrebten Verfahren zugrundeliegende Promotionsordnung ist mir bekannt.

Berlin, 27. Juli 2017