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Minor thesis

Finding candidate genes for the regulation of potato tuber protein content by a reverse genetics approach

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Abstract

The main potato tuber proteins are patatin, protease inhibitors and a 'high molecular weight' fraction which represents 22.9, 53.3 and 23.7 % of the potato fruit juice (PFJ) protein content respectively. Besides a storage function, these proteins are known to possess enzymatic properties which are important for the plants resistance against biotic and abiotic stresses.

Potato tuber proteins are of high quality and might therefore serve as substitute for animal proteins is several industrial products. Since about ten years it is possible to extract the proteins from the potato tuber at commercial scale. Breeding for potato cultivars with a high tuber protein content became therefore a major goal. For breeding it is crucial to understand the regulation of tuber protein synthesis. Information about this is currently limited. Genome wide association studies (GWAS) are an effective tool to gain insight in the regulation of complex traits like potato tuber protein content. QTL regions for potato tuber proteins were previously found by GWAS, but candidate genes for the regulation of protein synthesis are not determined yet. The current study aims to find such candidate genes by a reverse genetics approach.

Six previously found candidate genes for soybean protein content were used to search by BLAST for candidate genes in potato. Sixteen candidate genes were found to lie within the QTLs for potato tuber protein content. These include five putative transcription factors, one putative ammonium transporter, two putative sulphate transporters, putative xylogen, a cluster of six carbohydrate transporters and one gene of unknown function. For most genes no literature information was found. Based on information about closely related genes in other crops like Arabidopsis, rice and tomato, hypothesis are done about the relation of the candidate genes with potato tuber protein content. Most candidate genes are proposed to be related to tuber protein content due to their putative involvement in potato (tuber) development and/or resistance against (a)biotic stresses. Further research is needed to proof the function of the candidate genes in potato and their relation with potato tuber protein content.

1. Introduction

1.1. Potato

Potato (*Solanum tuberosum* L.) is an important food crop of the *Solanaceae* family. Other well-known crops of this family are tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), peppers (*Capsicum*) and tobacco (*Nicotiana tobacum*). Cultivated potato is tetraploid with 2n=4x=48, but also diploid, triploid, pentaploid and hexaploid (wild) species are known (Hawkes, 1990). After sugar cane, maize, rice and wheat, potato is the fifth most produced food crop with a worldwide production of about 382 million tonnes in 2014 (FAOSTAT, 2017). In the Netherlands in 2014 a total amount of about 7.1 million tonnes of potatoes was produced on approximately 156 thousand hectares (CBS, 2015). These potatoes can be roughly divided in human-consumption potatoes, starch potatoes and seed potatoes, with a production of 3.87, 1.47 and 1.75 million tonnes in the Netherlands in 2014 respectively (CBS, 2015).

1.2. Potato tuber proteins

About 10 % of the dry matter (DM) of potato tubers consist of crude protein, of which about half of it is pure[1] protein (Bártová et al., 2013; Kolbe and Stephan-Beckmann, 1997). Tuber protein content increases with increasing tuber weight (Kolbe and Stephan-Beckmann, 1997). Differences in protein content between cultivars however exists. Bártová et al. (2013) found in five potato cultivars crude protein ranging from 8.0 till 11.1 % of DM and pure protein from 3.4 till 4.7 %. Bárta et al. (2012) found in 20 potato cultivars the pure protein content ranging from 3.85 till 7.39 %. The ten processing cultivars (mostly starch cultivars) had on average a significantly higher pure protein content (5.36 %) than the ten human-consumption cultivars (4.79 %).
Potato proteins belong to three main groups: patatin, protease inhibitors (PIs) and a 'high molecular weight' (or 'other') fraction which represents 22.9, 53.3 and 23.7 % of the potato fruit juice (PFJ) protein content of cultivar 'Russet Burbank' respectively (Waglay et al., 2014). Pouvreau (2004) found patatin, PIs and other proteins to contribute 37.5, 50.6 and 11.9 % to total protein content of PFJ of cultivar Elkana respectively. Differences might among others be caused by cultivar, tuber development, tuber storage and extraction method of PFJ and proteins (Pouvreau et al., 2001).

Patatin

That patatin relative abundance (PRA; % of total tuber protein) greatly differed between cultivars was confirmed by Bárta et al. (2012), Bártová et al. (2013) and Bárta and Bártová (2008). Over several years in more than 40 cultivars these authors found PRA to range from 0.33[2] till 36.8 %. Patatin content (% patatin of total DM) was found to range from 0.01[3] till 2.19 % (Bárta and Bártová, 2008). PRA of processing cultivars was on average, depending on the year of growing, 6 till 35 % higher than of human-consumption cultivars (Bárta et al., 2012; Bárta and Bártová, 2008). Patatin content of processing cultivars was, depending on the year of growing, 9 till 35 % higher than of human-consumption cultivars (Bárta and Bártová, 2008). Patatin as percentage of tuber dry weight and as percentage of total soluble protein remains constant during the growth of tubers grow from 2 gram till 200 gram at harvest (Racusen, 1983).

Many genes encoding patatin have been sequenced already (Mignery et al., 1984; Stiekema et al., 1988; Twell and Ooms, 1988). Twell and Ooms (1988) estimated that patatin is regulated by 16-18 genes per haplotype. Patatin proteins are divided in two subgroups based on the presence (class II) or absence (class I) of sequence of 22 bp in the 5' untranslated region. Class I proteins are mainly present in the tubers, class II protein mainly in the roots (Aminedi and Das, 2014; Jefferson et al., 1990; Liu et al., 1991; Mignery et al., 1988; Wenzler et al., 1989). Patatin is expressed in the vacuoles

[1] Crude protein is just an estimate of the protein content, by multiplying total nitrogen content with 6.25. Pure protein is a measurement of protein itself by a two-step colorization method (BCA Protein Assay, Pierce, USA).
[2] Two cultivars stably showed very low PRA (about 10 and 14 %), but 0.33 is an exception.
[3] Two cultivars stably showed very low patatin content (about 0.45 and 0.78 %), but 0.01 is an exception.

(and to a much lesser extent also in the cytosol) of parenchyma cells of the potato tuber (Aminedi and Das, 2014; Liu et al., 1991; Rocha-Sosa et al., 1989; Sonnewald et al., 1989a). Rosahl et al. (1986) found that patatin is regulated at the level of transcription. Class I patatin however is strongly induced in tubers after tuberization, but can also be induced in other plant parts by the addition of sucrose. Therefore it is concluded that transcription of class I patatin is regulated both by developmental and metabolic processes (Rocha-Sosa et al., 1989; Wenzler et al., 1989).
After removal of tubers and axillary buds, patatin class I also accumulates in other plant parts like stems and petioles without showing tuber-like swelling. This accumulation of patatin was always accompanied by accumulation of starch (Paiva et al., 1983). Jefferson et al. (1990) suggest that the organ specific expression of patatin class I is possibly determined by source-sink relationships of sugars. Class II patatin is only developmentally regulated and did not respond to sugars (Köster-Töpfer et al., 1989).
Bárta et al. (2012) found three different patatin mass isoforms of 40.6, 41.8 and 42.9 kDa. Differences in weight were probably caused by one, two or three glycosylations of the patatin protein chain (Bárta et al., 2012; Pots et al., 1999). All of the three isoforms were present in most of the cultivars, but the first two isoforms were the most abundant (Bárta et al., 2012). No differences in biochemical and structural properties or conformational stability was found (Pots et al., 1999). These latter authors therefore concluded that patatin can be studied as group without separating between the different isoforms.

Patatin is a major storage protein, but also enzymatic properties are known. Probably the most investigated enzymatic function is its lipid acyl hydrolase activity (LAH), which functions on a broad range of lipids e.g. mono- and diglycerides (also called mono- and diacylglycerols), galactolipids and phospholipids (Anderson et al., 2002; Andrews et al., 1988; Galliard, 1971; Galliard and Dennis, 1974; Racusen, 1984). Other enzymatic functions of patatin are acyl transferase activity (Galliard, 1970 and 1971; Galliard and Dennis, 1974), wax ester formation (Dennis and Galliard, 1974), esterase activity (Racusen, 1986; Rosahl et al., 1987; Sonnewald et al., 1989a), β-1,3-glucanase activity (Tonón et al., 2001) and β1,2-xylosidase activity (Peyer et al., 2004).
The enzymatic functions of patatin might be important for the defence of the potato plants against biotic and abiotic stresses. LAH of phospholipids (also called phospholipase activity) is thought to be involved in the resistance reaction against *Phytophthora infestans* (Kawakita et al., 1993; Senda et al., 1996). The LAH and esterification functions of patatin might be used for the formation cytotoxic fatty acid derivatives and wax esters which inhibit microbial invasion (Racusen, 1984). β-1,3-glucanase is supposed to be involved in resistance against *P. infestans*, possibly by degrading glucans in the cell wall of this pathogen (Andreu et al., 1998; Tonón et al., 2001). Patatin might also be involved in resistance against insects. Strickland et al. (1995) found that patatin has an inhibitory effect on Southern- (*Diabrotica undecimpunctata howardi)* and Western Corn Rootworm (*Diabrotica virgifera virgifera*) larvae growth when fed in an artificial diet. Growth of Colorado potato beetles (*Leptinotarsa decemlineata*) fed with potato leaves coated with patatin was also inhibited. Despite aforementioned results, Logemann et al. (1988) found that most patatin steady state mRNA disappeared within 30 minutes after wounding of the tuber. This might suggests that the involvement of patatin in resistance is post transcriptionally regulated. A possibility is that patatin is inactively stored in the vacuol beacause of the low pH and is released from the vacuole after wounding and then becomes active (Racusen, 1984; Sonnewald et al., 1989a).
Besides resistance against biotic stresses patatin is also affected by abiotic stresses. Wegener et al. (2014 and 2015) found soluble protein content and LAH activity significantly increased in tubers of potato plants grown under drought stressed compared to control conditions. Gong et al., 2015 found the expression of four patatin genes significantly downregulated in potato stolons under drought stress compared to control conditions.
In conclusion patatin is thought to be a major storage protein and to be involved in defence against biotic and abiotic stresses. Nevertheless its precise function needs to be determined.

Protease inhibitors

The second group of potato tuber proteins are the PIs. Pouvreau et al. (2001) classified the protease inhibitors in seven groups: potato inhibitor I, potato inhibitor II, potato cysteine protease inhibitor, potato aspartate protease inhibitor, potato kunitz-type protease inhibitor, potato carboxypeptidase inhibitor and 'other serine protease inhibitors'. The most abundant in cultivar Elkana were potato inhibitor II family (approximately 20.5 kDa) and potato cysteine protease inhibitor family (20.1-22.8 kDa) with 22 and 12 % of PFJ protein content respectively (Pouvreau et al., 2001). Many genes encoding PIs are already sequenced (Bauw et al., 2006; Cleveland et al., 1987; Heibges et al., 2003; Pouvreau et al., 2003; Stiekema et al., 1988; Valueva et al., 2008; Waldron et al., 1993). PIs are predominantly expressed in the tubers, but some members are to a much lower level also expressed in other tissues (Hannapel, 1991; Stiekema et al., 1988; Suh et al., 1990; Waldron et al., 1993).

Because of the large abundance in the potato tuber PIs are supposed to function as storage proteins, but also enzymatic properties are known. PIs are able to inhibit the activity of several proteases like trypsin, chymotrypsin and subtilisin. The substrate specificity however differs between PIs (Hermosa et al., 2006; Melville and Ryan, 1972; Suh et al., 1991; Valueva et al., 2008). In leaves PIs are environmentally regulated, in tubers both developmentally and environmentally (Peña-Cortés et al., 1991; Suh et al., 1991). Expression of two PIs was found to be upregulated after wounding of the leaves (Hildmann et al., 1992). In the wound-inducible upregulation of PIs, abscisic acid (ABA) and jasmonic acid (JA) plays an important role (Hildmann et al., 1992; Peña-Cortés et al., 1995; Peña-Cortés et al., 1989). Chaves et al. (2009) cut potato tubers in slices and extracted proteins from different slices over a period of eight days. They found 22 PIs significantly affected by this wounding, which were divided in three groups based on their response to wounding. The amount of the first group increased sharply the first day after wounding, but decreased soon. The second group remained high for all days, while the third group increased slowly and reached the highest abundance at the end of the period.

In potato tubers PIs were shown to inhibit up to about 40 % of the protease activity of *Botrytis cinerea*. Spore germination and germ tube elongation of this fungus was about 50 % inhibited (Hermosa et al., 2006). Inoculation of resting potato tubers (stored for five months before inoculation) with *P. infestans* caused an induction of accumulation of PIs (Valueva et al., 2003). The same PIs were found to accumulate after wounding the tuber. *In vitro* these PIs inhibited the length of hyphae and the germination of zoospores of *P. infestans* up to 100 % depending on the on the concentration of PIs (Valueva et al., 2003). Outchkourov et al. (2004) showed in *in vitro* experiments that potato PIs were able to inhibit up to 95 % of the proteolytic activity of western flower thrips (*Frankliniella occidentalis* Pergande). These authors found also with choice experiments with leaf discs, that the western flower thrips preferred the leaf discs with low levels of PIs.

Also some evidence is present in literature that PIs are involved in resistance against abiotic stresses. Ledoigt et al. (2006) found that drought stressed potato tuber slices secreted two types of PIs, which were not secreted by non-drought stressed slices. Gong et al., 2015 found the expression of three PIs significantly downregulated in potato stolons under drought stress compared to control conditions. In potato leaves PIs were found to be upregulated under drought (Kang et al., 2002) and osmotic stress (sucrose, sorbitol and salt; Aghaei et al., 2008; Folgado et al., 2013). Johnson and Ryan (1990) found the accumulation of a wound inducible PI in leaves more than threefold enhanced under sucrose levels of 3 - 6 %. Under cold stress some PIs in leaves were upregulated, while another was downregulated (Folgado et al., 2013). Whether the expression of PIs not only in potato leaves, but also in the tubers are affected by osmotic and cold stress needs to be investigated in future.

Potato multicystatin (PMC; a cysteine PI) is supposed to play a key role in regulation of accumulation of other PIs and patatin during tuberization (Weeda et al., 2009). Non tuberizing stolons contain high amounts of proteases which are proposed to inhibit patatin and PI accumulation. At the beginning of tuberization, PMC starts accumulating and inhibits the proteases. When protease activity is inhibited about 60 %, other PIs and patatin start accumulating.

In conclusion evidence is present that PIs are involved in the regulation of protein content and in resistance against biotic and abiotic stresses. A lot of research however is done to the function of PIs

in the potato leaves, while the interest of the current research is in the tubers. Moreover a limited number of PIs is currently investigated, while the PIs are a large group of proteins. The potato Kunitz-type protease inhibitors consists already out of at least 21 members (Heibges et al., 2003). So more research is needed to clarify the precise role of individual PI members in the potato tubers.

High molecular weight proteins

All proteins which do not belong to the patatin family, neither show protease inhibitor activity are called 'high molecular weight proteins' or 'other proteins'. These group of proteins is less intensively studied than the other two groups. Some, among potato variants highly conserved proteins of this group are: annexin (35.8 kDa), glyoxalase I (32.8 kDa) and enolase (47.8 kDa) (Bauw et al., 2006). These authors also sequenced some high molecular weight proteins.

1.3. GWAS approach

A useful tool to gain insight in the regulation of complex traits like protein content is a genome-wide association study (GWAS). Some advantages of GWAS over QTL mapping are that random mating populations can be used and that many alleles at one locus can be tested (Griffiths et al., 2012). For GWAS mostly a large number of accessions is used with a large number of markers (mostly SNPs) which are spread over the genome. By association mapping someone is searching for correlation between SNPs and certain traits. Once a correlation between a SNP and a certain trait is found, someone has to determine the size the QTL interval. The QTL interval can be determined by estimation of the LD. The basic statistic for determination of LD is calculated by means of the following formula (Griffiths et al., 2012):

$$D = P_{AB} - P_A P_B$$

Where P_{AB} is the frequency of haplotypes with allele A and B at two loci and $P_A P_B$ is the product of the frequency of allele A and B. With D different statistics can be calculated. For association studies the most favoured is r^2 because it gives information about the correlation between markers and QTLs (Flint-Garcia et al., 2003). r^2 can be calculated by the following formula (Flint-Garcia et al., 2003):

$$r^2 = D^2 / P_A P_a P_B P_b$$

In rice r^2 was found to be 0.25 and 0.28 for *Oryza sativa indica* and *O. sativa japonica* respectively (Huang et al., 2010). For their association mapping with 950 rice varieties, Huang et al. (2012) therefore used all genes within approximately 200 kb of peak SNPs. r^2 however is known to differ between genomic regions (Kwon et al., 2012; Hwang et al., 2014) and is also known to decrease with increasing genetic distance between loci (e.g. Jun et al., 2008; Hwang et al., 2014; Fernandez, 2010). r^2 needs therefore to be determined for every QTL separately.

1.4. Problem statement and research questions

Potato tuber proteins are of high quality and can among others be used for foams and emulsions (Koningsveld, 2001). Potato tuber proteins might therefore serve as substitute for animal proteins in these products (Brink, 2008). Until recently however, it was not possible to extract proteins from the potato tuber at a large scale. In 2007 Solanic (a subsidiary company unit of AVEBE) started with the commercial extraction of potato tuber proteins (Brink, 2008). Potato tuber proteins became therefore more interesting and breeding for potato cultivars with a high tuber protein content became a major goal. For breeding it is crucial to understand the regulation of tuber protein synthesis. Information about the regulation of tuber protein synthesis is currently limited. GWAS are an effective tool to gain insight in the regulation of complex traits like the regulation of tuber proteins synthesis (paragraph 1.3). QTL regions for potato tuber proteins are already found by GWAS (Fernandez, 2010; Peter Vos, unpublished results). Candidate genes for the regulation of protein synthesis however are not determined. The current study aims to find candidate genes for regulation and synthesis of potato tuber proteins by a reverse genetics approach. To reach this goal the following questions needs to be answered:

1. Which possible candidate genes for the regulation of potato tuber protein content can be found by a reverse genetics approach?
2. What is the possible relation between these genes and potato tuber protein content based on literature information?

2. Materials and methods

Selection of GWAS papers

For many crops GWAS were performed for protein content (Table 1). Out of these investigations, the markers associated with seed protein content and the physical position of these markers were needed for the current study. When searching for this information, it appeared that this in most studies was lacking. Some studies for example performed GWAS for protein content, but did not showed that results, others showed the results, but did not gave the physical position of the markers.

Table 1. GWAS references for tuber/seed protein content for several crops. For every reference the unit in which the protein content is expressed, the way in which protein content was determined and the r^2 (unit of linkage disequilibrium) were shown. % of seed/tuber FW/DW means protein as percentage of seed/tuber fresh weight/dry weight. NIR spectroscopy means near-infrared spectroscopy. A stripe is placed when the information was not provided in the concerning paper.

Crop	Protein (unit)	Protein (method)	r^2	Reference
Potato	% tuber FW	[1]	< 0.5[2]	Fernandez, 2010
	% tuber FW	-	-	Peter Vos, unpublished results
Oilseed rape	% seed DW	NIR spectroscopy	0.037[3]	Gajardo et al., 2015
Pea	% seed DW	[4]	0.047-0.301[5]	Kwon et al., 2012
	% seed DW	-	0.0169	Cheng et al., 2015
Soybean	% seed DW	[6]	0.033	Jun et al., 2008
	% seed DW	Total N (LECO CHN 2000 analyser) * 6.25	[7]	Hwang et al., 2014
	% seed DW	NIR spectroscopy	-	Zhang et al., 2014
	% seed DW	[6]	-	Vaughn et al., 2014
	% seed DW	NIR spectroscopy	< 0.2	Sonah et al., 2015
	% seed DW	[6]	0.23 and < 0.5[8]	Bandillo et al., 2015
Sesame	% seed FW	Kjeltec 8400 Analyzer	-	Wei et al., 2013
	% seed FW	NIR spectroscopy	0.0173	Li et al., 2014
Maize	% seed DW	NIR spectroscopy	-	Cook et al., 2012
Rice	-	Total N (Kjeldahl) * 5.95	0.25 and 0.28[9]	Huang et al., 2012
Wheat	% seed DW	Total N (Dumas combustion method) * 5.62	-	Plessis et al., 2013
Barley	% seed FW	Total N (Kjeldahl) * 6.25	~ 0.1	Cai et al., 2013

[1] Measurements performed by companies involved. Methodology not described. [2] For SNPs on the same chromosome. [3] r^2 was estimated at 0.037, 0.057 and 0.017 in the whole, the spring and the winter oilseed rape collections, respectively. [4] Phenotypic data was obtained from USDA core collection. [5] Report only about the r^2 of a small part of the genome of 68.6 cM, in which the r^2 differs between 0.047 and 0.301. [6] Obtained from Germplasm Resource Information Network (GRIN). [7] r^2 was determined for every 200 Kbp separately. [8] r^2 of two significant regions on chromosome 15 and 20 respectively. [9] r^2 estimate of Huang et al., 2010 was used. r^2 was 0.25 and 0.28 for for *Oryza sativa indica* and *O. sativa japonica* respectively.

Only five GWAS studies for soybean (Hwang et al., 2014; Zhang et al., 2014; Vaughn et al., 2014; Sonah et al., 2015 and Bandillo et al., 2015), one for potato (Peter Vos, unpublished results), one for rice (Huang et al., 2012) and one for maize (Cook et al., 2012) provided the needed information. Of these five studies of soybean, Bandillo et al. (2015) used the most markers (ca. 36.000) and accessions (ca. 12.000). Therefore this study, together with the three mentioned studies of potato, rice and maize, was chosen for the current investigation.

BLAST strategy

For an effective search for candidate genes, r^2 needs to be determined for every QTL interval separately (paragraph 1.3). Such a detailed information however, is mostly missing in the GWAS studies that were used for the current study. Therefore a standard QTL interval of two Mbp upstream and two Mbp downstream the most significant marker will be used in this study for all crops (Appendices I-IV).

Table 2. BLAST strategy.

Phase 1		**Phase 2**	
Crop	**BLAST against**	**Crop**	**BLAST against**
Glycine max	*O. sativa indica*	*O. sativa indica*	*Glycine max*
	O. sativa japonica		*O. sativa japonica*
	Solanum tuberosum		*Solanum tuberosum*
	Zea mays		*Zea mays*
		O. sativa japonica	*Glycine max*
			O. sativa indica
			Solanum tuberosum
			Zea mays
		Solanum tuberosum	*Glycine max*
			O. sativa indica
			O. sativa japonica
			Zea mays
		Zea mays	*Glycine max*
			O. sativa indica
			O. sativa japonica
			Solanum tuberosum

Initially the approach was to make an overview of all genes within the QTL regions. In appeared however soon that this strategy would result in several thousands of genes. That is practically not feasible. Therefore the approach was changed. Because of the high number of markers and accessions used, Bandillo et al. (2015) was the best GWAS of all crops. They moreover proposed already candidate genes on chromosome 15 and 20 (Table 3). The coding sequences of most of these genes were adapted from EnsemblPlants (2017), the others from SoyBase (2017). These sequences were used to search by BLAST (Basic Local Alignment Search Tool) for candidate genes in the other three crops (Table 2, phase 1). As BLAST a sequence to protein (BLASTx) procedure[4] of EnsemblPlants (2017) was used, because in that way also genes with a different sequence, but the same protein could be found. Two criteria were used for selection of genes out of the BLAST results: 1. A score of more than 100[5], 2. Genes lie within the QTL interval of two Mbp upstream and two Mbp downstream the most significant marker. Genes at the borders of these criteria are also used. Thereafter the same

[4] For all other options, the default settings were used.

[5] The score is calculated based on the identity of the genes and is indicative for the 'quality' of the BLAST result.

procedure was repeated, but in the reverse way, so potato versus soybean, rice and maize etc. (Table 2, phase 2). Sequences of all candidate genes of these crops were adapted from EnsemblPlants (2017).

Function of the candidate genes in potato

A literature study was performed for the candidate genes which were found in potato. If no, or limited information about these genes was found, a literature study was performed for closely related genes in other crops, mainly Arabidopsis, rice and tomato because in general more research was done in these crops. Moreover are potato and tomato both members of the Solanaceae family. Based on this literature information hypothesis about the function of the candidate genes and their relation to potato tuber protein content were done.

Table 3. Candidate genes as result of GWAS by Bandillo et al. (2015). Descriptive information originates from Soybase (2017), EnsemblPlants (2017) and UniProt (2017). A stripe means that that information was unknown. Bp means base pairs.

Candidate genes	Chromosome	Start (bp)	End (bp)	Molecular function	Biological process
GLYMA15G05470	15	3.856.854	3.858.704	sugar transmembrane transporter activity	carbohydrate transmembrane transport; carbohydrate transport
GLYMA15G05760	15	4.082.251	4.087.146	secondary active sulphate transmembrane transporter activity; sulphate transmembrane transporter activity	sulphate transmembrane transport
GLYMA15G05770	15	4.094.166	4.097.741	lipid binding	lipid transport
GLYMA20G21030[1]	20	29.984.895	29.986.397	ammonium transmembrane transporter activity	ammonium transmembrane transport; cellular response to nitrogen starvation; nitrogen utilization; organic cation transport
GLYMA20G21080[2]	20	30.044.891	30.045.091	-	-
GLYMA20G21361[3]	20	30.607.866	30.610.932	-	intra-Golgi vesicle-mediated transport
GLYMA20G21535[1]	20	30.873.110	30.873.943	-	-
GLYMA20G21780	20	31.385.164	31.389.333	phosphorelay sensor kinase activity	-

[1] Bandillo et al. (2015) used GLYMA20G21040 instead of GLYMA20G21030 and GLYMA20G21540 instead of GLYMA20G21535. This are the names of genome sequence Glyma v1.0. EnsemblPlants (2017) in opposite to that uses: Glyma v1.1. In this report therefore the new annotation of Glyma v1.1 will be used.

[2] This gene does not exist anymore in newer versions than Glyma v1.0. BLAST with this gene does also not resulted in any hits in the other crops. This gene will therefore be excluded from further analysis.

[3] BLAST with this gene also does not resulted in any hits in the other crops. This gene will therefore be excluded from further analysis.

3. Results and discussion

3.1. BLAST

In total there were 7 QTLs in soybean (0), 6 in rice (Appendix II), 11 in potato (Appendix III) and 31 in maize (Appendix IV). In rice, in fact only 4 QTLs were found; 1 on chromosome 7 in the japonica population and 3 in the 'total' (indica + japonica) population. In the indica population no QTL was found (Huang et al., 2012). The used BLAST program (EnsemblPlants, 2017) only had the genome sequence for the indica and japonica population separately, not of the total population. Therefore the three QTLs of the total population were also used for the indica population and the QTLs of chromosome 6 and 11 were also used for the japonica population. The QTL on chromosome 7 of the total population was not used in the japonica population because in this population already an QTL at an almost similar position was found.

The BLAST strategy resulted finally in eleven candidate genes in soybean (Appendix V), sixteen in potato (Table 4), nine in rice (Appendix VI) and thirteen in maize (Appendix VII). The sixteen candidate genes of potato consists out of five putative transcription factors, one putative ammonium transporter, two putative sulphate transporters, a cluster of six carbohydrate transporters, putative xylogen and one gene of unknown function (Table 4). For the most candidate genes limited literature information was found. Therefore in the literature reviews also information of closely related genes in other crops was included. These literature reviews can be found in the Appendices VIII till XV. In the paragraphs 3.2 till 3.4 the most relevant literature information with respect to potato tuber protein content will be discussed. In these paragraphs also hypotheses about the functions of the genes in potato will be done, especially about their relation with potato tuber protein content.
PGSC0003DMG400030518 is a conserved gene of unknown function (EnsemblPlants, 2017). It was the only BLAST hit for GLYMA20G21535 (data not shown), of which the function also is unknown. Reverse BLAST with PGSC0003DMG400030518 resulted also only in a hit with GLYMA20G21535 (data not shown). Further BLAST of PGSC0003DMG400030518 with total genomes of potato, tomato, Arabidopsis, rice, soybean, maize, wheat, barley, cauliflower, *Populus trichocarpa* and *Prunus persica* resulted in many genes (data not shown), but of none of them the function was known. PGSC0003DMG400030518 was therefore excluded from further analysis.

Table 4. Candidate genes of potato found by BLAST. Chr. means chromosome. Bp means base pairs. Name and function of the genes are based on the literature review and discussion as indicated in the table. PGSC0003DMG400030518 is excluded from analysis (see text).

Candidate gene	Chr.	Start (bp)	End (bp)	Name	Function	Discussion	Literature review
PGSC0003DMG400012567	1	81.769.460	81.772.177	Putative xylogen	Xylem development	Paragraph 3.4	Appendix XV
PGSC0003DMG400033576	1	82.686.049	82.692.942	Putative ZIM	Transcription factor	Paragraph 3.2.1	Appendix VIII
PGSC0003DMG400010684	1	82.700.202	82.705.631	Putative ZML	Transcription factor	Paragraph 3.2.1	Appendix VIII
PGSC0003DMG400018761	3	10.325.657	10.330.408	Putative AMT1;3	Ammonium transport	Paragraph 3.3.1	Appendix XII
PGSC0003DMG400033693	3	45.373.076	45.375.604	SWEET11b	carbohydrate transport	Paragraph 3.3.3	Appendix XIV
PGSC0003DMG400004337	3	45.837.419	45.839.266	SWEET12d	carbohydrate transport	Paragraph 3.3.3	Appendix XIV
PGSC0003DMG400004335	3	45.874.219	45.875.598	SWEET12c	carbohydrate transport	Paragraph 3.3.3	Appendix XIV
PGSC0003DMG400031742	3	45.971.763	45.973.950	SWEET10d	carbohydrate transport	Paragraph 3.3.3	Appendix XIV
PGSC0003DMG402031741	3	45.993.740	45.995.580	SWEET10c	carbohydrate transport	Paragraph 3.3.3	Appendix XIV
PGSC0003DMG400031738	3	46.050.896	46.052.880	SWEET12a	carbohydrate transport	Paragraph 3.3.3	Appendix XIV
PGSC0003DMG400000584	3	47.434.904	47.440.043	Putative PRR5	Transcription factor	Paragraph 3.2.2.2	Appendix X
PGSC0003DMG400030518	5	3.710.910	3.715.061	Unknown	Unknown	-	-
PGSC0003DMG400018422	5	4.417.126	4.421.492	Putative SULTR3;3	Sulphate transport	Paragraph 3.3.2	Appendix XIII
PGSC0003DMG400023534	5	50.112.914	50.120.114	Putative type B-I RR	Transcription factor	Paragraph 3.2.2.1	Appendix IX
PGSC0003DMG400023515	5	50.492.402	50.501.214	Putative SULTR4	Sulphate transport	Paragraph 3.3.2	Appendix XIII
PGSC0003DMG400023402	5	50.783.752	50.787.902	Putative ETR	Ethylene receptor (transcription factor)	Paragraph 3.2.2.3	Appendix XI

3.2. Transcription factors

3.2.1. ZML

Except the discovery of the potato genome (The Potato Genome Sequencing Consortium, 2011), no information about PGSC0003DMG400033576 and PGSC0003DMG400010684 was found in literature. BLAST searches however resulted in high scores (data not shown) with members of group I of the tify gene family (Vanholme et al, 2007). Bai et al. (2011) divided the tify family in four groups and gave group I of Vanholme et al. the name ZML. PGSC0003DMG400033576 had the highest BLAST score (data not shown) with Arabidopsis ZIM[6] (also named TIFY1) and PGSC0003DMG400010684 with Arabidopsis ZML1[7] (also named TIFY2b) and will therefore in the rest of this report be annotated as putative ZIM and putative ZML1 respectively.

The ZML gene family is involved in plant development as well as resistance against biotic and abiotic stresses, however many differences between separate genes and crops exists. Salt stress for example strongly induced the expression of ZIM in rice (Ye et al., 2009), while it hardly affected the expression of ZIM in *Brachypodium distachyon* (Zhang et al., 2015b). Drought stress for instance suppressed the expression of ZML1 in both investigated maize lines, while it increased the expression of ZML2 and ZML3 in only one line and hardly affected them in the other (Zhang et al., 2015a). Potato tuber proteins were also found to be affected by among others drought and salt stress (Aghaei et al., 2008; Gong et al., 2015; Kang et al., 2002). The large variation between separate crops and ZML members makes it however very difficult to do reliable hypotheses about the role of putative ZIM and putative ZML1 in potato defence and especially their relation with potato tuber proteins. Resistance to biotic and abiotic stresses are moreover very complex. Mostly several thousands of genes are affected in separate and/or interrelated pathways. Trinidad Ascencio-Ibáñez et al. (2008) for example found the expression of more than 5000 genes significantly affected after inoculation of Arabidopsis with cabbage leaf curl virus compared to control plants. Gong et al. (2015) found the expression of more than 3000 genes in potato stolons significantly affected by drought stress. It can be concluded that even if potato tuber proteins and ZML members both respond to certain biotic and abiotic stresses it is therefore not possible to conclude a causative correlation between proteins and putative ZIM and putative ZML1 just based on that responsiveness.

Overexpression of ZIM in Arabidopsis resulted among others in the upregulation of some β-1,3-glucanases (Shikata et al., 2004). Patatin possess also β-1,3-glucanase activity (Tonón et al., 2001). BLAST searches with the genes found by Shikata et al. (2004) against the potato genome resulted however in no hits with patatin genes (data not shown), suggesting that the β-1,3-glucanases regulated by ZIM belong to another gene family than the patatin genes.
In conclusion currently no evidence is available that suggests that ZIM and ZML1 directly regulate the transcription of any of the potato tuber proteins. More research is needed to clarify the function of putative ZIM and putative ZML1 in potato and their relation with potato tuber proteins.

3.2.2. Phosphorelay signal transduction system

PGSC0003DMG400023534 (putative type B-I RR), PGSC0003DMG400000584 (putative PRR5) and PGSC0003DMG400023402 (putative ETR) are all genes involved in the phosphorelay signal transduction system. Therefore first some general information about this system will be given. In the paragraphs 3.2.2.1. till 3.2.2.3. the function of the three mentioned genes and their relation with potato tuber protein content will be discussed.
In prokaryotes the simple two-component system is the predominant signal system (Figure 1a). This system consists out of a transmembrane histidine kinase (HK) and response regulator protein (RR). The HK is activated by an (environmental) signal outside the cell. Secondly a signal is send to the

[6] This gene was called ZIM because of its Zinc-finger protein expressed in Inflorescence Meristem (Nishii et al., 2000).
[7] ZML means ZIM like protein.

histidine kinase (transmitter) domain which catalyses autophosphorylation. The phosphoryl residue is then transferred to the receiver of the RR. After phosphorylation of the RR, the output domain is activated (for more detailed review see Stock et al., 2000).
In eukaryotes a more complicated signal system is common: the phosphorelay signal system (Figure 1b). In this system the HK is replaced by a hybrid kinase, which beside the histidine kinase also contains a receiver domain. In addition to the simple two component system, the phosphorelay system also contains a phosphotransfer protein (HPt). In the phosphorelay system, after activation by a intracellular signal the phosphoryl residue is transferred from the histidine kinase to the receiver domain and then transferred by the HPt protein to the RR which regulates the output. In both, the simple two-component and the phosphorelay system, the level of RR phosphorylation determines the output response (for more detailed review see West and Stock, 2001).
Above is a brief and simple description of both the two-component and phosporelay signal system, for more detailed reviews see among others Schaller et al. (2011), Stock et al., 2000 and West and Stock (2001).

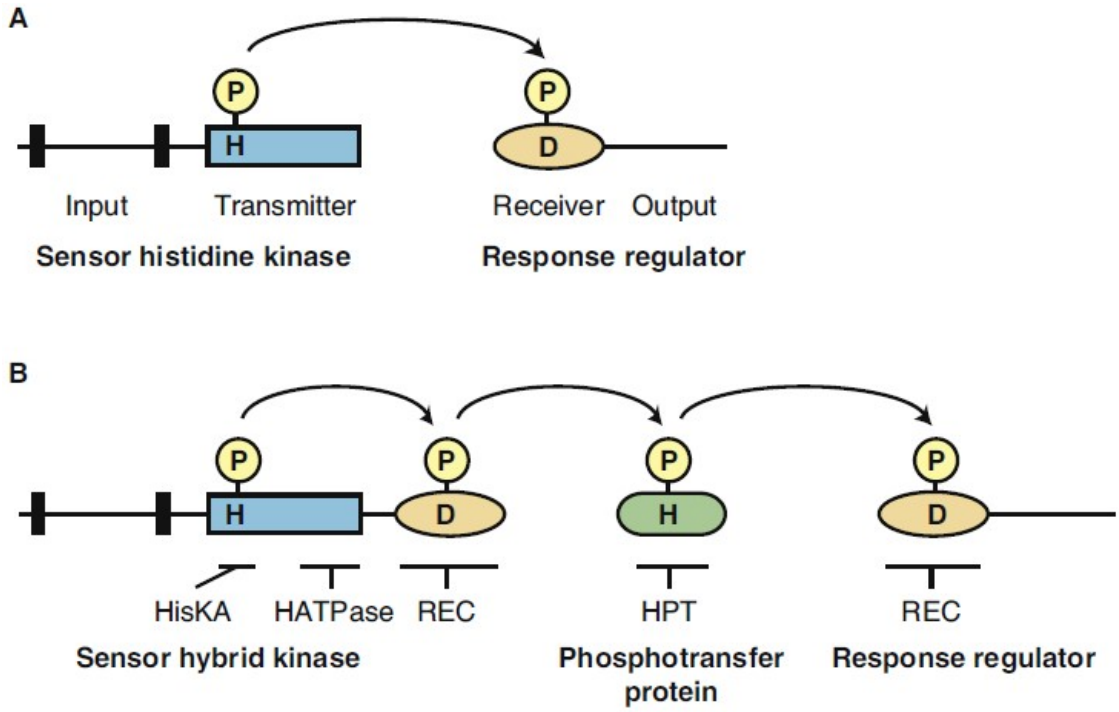

Figure 1. Schematic presentation of A) simple two-component and B) phosphorelay signal transduction system. In the simple two-component system, after an intracellular signal a phosphoryl residue is transferred from the histidine kinase transmitter to the receiver domain of the response regulator. In the phosphorelay system the phosphoryl residue is first transferred to a receiver domain of the hybrid kinase and then by a phosphotransfer protein to the response regulator (Schaller et al., 2011).

3.2.2.1. Type B-I response regulator

According to EnsemblPlants (2017) PGSC0003DMG400023534 is a type B RR, but no literature information was provided to support that. BLAST searches however resulted in high scores (data not shown) with subgroup I or B-I of the type B RRs of Arabidopsis and rice (Du et al., 2007; Hwang et al., 2002; Mason et al., 2004; Pareek et al., 2006; Tsai et al., 2012). PGSC0003DMG400023534 will therefore in the rest of this report annotated as putative type B-I RR. Subgroup I members play an important role in cytokinin signalling in roots and shoots of both monocots and dicots (Kim et al., 2006; Sakai et al., 2001; Tsai et al., 2012; for review see Appendix IX). No information was available that supports a possible direct role of cytokinin in potato tuber protein synthesis. Cytokinin, however is involved in many growth and developmental processes. *In vitro* tuberization of potato is not influenced by cytokinin alone (Raspor et al., 2012), but in combination with sucrose it stimulates

induction and development of micro tubers (Aslam and Iqbal, 2010). Jefferson et al. (1990) found that cytokinin stimulates *in vitro* tuberization of potato cuttings. Tuber protein content increases with increasing tuber weight (Kolbe and Stephan-Beckmann, 1997). So by stimulating tuberization, cytokinin might also indirectly influence potato tuber protein content. Further research is needed to prove this hypothesis. More research is also needed to investigate whether subgroup I RRs are involved in tuberization. Finally not only research is needed to clarify the potential role of subgroup I genes in tuberization, but also if they directly regulate potato tuber protein content.

3.2.2.2. Putative PRR5

PGSC0003DMG400000584 shows high BLAST scores (data not shown) with Arabidopsis and rice pseudo response regulators (PRR) belonging to the 'C' or 'clock' subgroup (Hwang et al., 2002; Mason et al., 2004; Tsai et al., 2012). These PRRs have essential roles in regulating the circadian clock (e.g. Matsushika et al., 2000; Makino et al., 2001; for review see Appendix X). The circadian clock is proposed to be the main regulator of plant growth, development and physiology and allows the plant to cope with diurnal and seasonal variations and biotic and abiotic stresses (for review about the circadian clock see Sanchez and Kay, 2016). PGSC0003DMG400000584 has the highest BLAST score with Arabidopsis PRR5 (data not shown) and will therefore in the rest of this report be annotated as putative PRR5.

StSP6A (flowering locus T) is the main inducer of tuberization (Kloosterman and Bachem, 2014; Navarro et al., 2011). This gene is first expressed in the leaves after which the protein is transported to the stolons where it induces the expression of the same gene resulting in tuberization (Navarro et al., 2011). StSP6A in the leaves is repressed by StSP5G (flowering locus T-like) which in turn is induced by StCOL1 (CONSTANS-like). StCOL1 is induced by StphyB (phytochrome B), but repressed by StCDFs (cycling DOF factors) (Abelenda et al., 2016; Kloosterman et al., 2013). All these genes are under the control of the circadian clock (for review see Kloosterman and Bachem, 2014). By regulating tuberization the circadian clock might also indirectly influence potato tuber protein content because tuber protein content increases with increasing tuber weight (Kolbe and Stephan-Beckmann, 1997). Further research is needed to prove this hypothesis.

Little evidence is present about the role of the circadian clock in the resistance of potato against abiotic stresses. Singh et al. (2015) found among others the clock controlled genes StCDF, StCOL, StSP5G and StSP6A differentially expressed in a heat tolerant than in a susceptible potato cultivar under a night temperature of 24 °C compared 20 °C. This resulted in tuberization in the tolerant cultivar, but no tuber formation at all in the susceptible one. Additionally Hancock et al. (2014) found a reduction of tuber yield of potato cultivar Desirée under moderately elevated temperature (day/night: 30/20°C) compared to control temperature (day/night: 22/16°C). This decreased tuber yield was accompanied by increased expression of StSP5G and decreased expression of StSP6A suggesting an important role for the circadian clock in temperature response. The expression of some PIs in potato leaves was also found to be affected by cold stress (Folgado et al., 2013) and might therefore also be regulated by the circadian clock. The effect of temperature stress on potato tuber protein content needs still to be investigated.

The circadian clock plays an important role in tuberization and abiotic stress resistance of potato. Nevertheless the role of the PRRs in potato is unknown and needs therefore to be investigated. With respect to potato tuber protein content putative PRR5 is of special interest because this gene was found by the current BLAST approach. It is remarkable that only this gene was found because it functions in a cascade with PRR1, 3, 7 and 9 in Arabidopsis (Appendix X; Ito et al., 2003; Makino et al., 2001). It is not known whether these PRRs also functions in a cascade in potato too, or that potato putative PRR5 has other or additional functions compared to PRR5 ofArabidopsis. Further research to all aforementioned PRRs is needed to clarify their functions in general and especially their possible role in regulating potato tuber protein.

3.2.2.3. Putative ETR

PGSC0003DMG400023402 is a putative ETR (ethylene response or ethylene resistant) gene of subfamily II of the ethylene receptors (Appendix XI). Ethylene receptors are negative regulators of the ethylene response pathway (Hall et al., 2007). When the ethylene receptors bind to ethylene their activity is inhibited and the response pathway is activated (Hua and Meyerowitz, 1998).
Little evidence is present supporting that ethylene directly regulates potato protein content. Taghizadeh and Ehsanpour (2013) had grown potato plants on MS medium containing different levels of $CoCl_2$ (an ethylene synthesis inhibitor). When $CoCl_2$ was increased till at least 20 mg L^{-1}, protein content of the leafs and stems was significantly higher than that of the control plants. They made however not clear whether this was due to the inhibition of ethylene synthesis, or due to the possible negative effect of the heavy metal cobalt. Kang et al. (2002) found one leaf specific PI induced by ethylene[8]. For patatin such a direct relation with ethylene is not known. Moreover it needs to be investigated whether ethylene also has a role in regulating the protein content in the tubers and not only in the leaves. Finally the role of putative ETR in this regulatory process in unknown.

The subfamily II ethylene receptors of Arabidopsis, rice, tomato and tobacco were mainly expressed in the reproductive organs (for review see Appendix XI). Wuriyanghan et al. (2009) found for example that ETR2 and ETR3 (both subfamily II) affect flowering time in rice. The major interest for potato (proteins) is not in its reproductive tissues, but in its tubers. Nevertheless, Hannapel (2007) found that the expression of several genes that were involved in flowering time, was strongly affected the first sixteen[9] days of tuberization. If putative ETR also affect flowering time in potato, there might be a link with tuber protein content because tuber protein content increases with increasing tuber weight (Kolbe and Stephan-Beckmann, 1997). Further research should clarify if this is a causal relation and what the physiological relevance of this link is for potato tuber protein content.
In Arabidopsis, subfamily II members ETR2, EIN4 and ERS2 were also found to have a role in root development. Loss of function mutants of these genes resulted in reduced size of both shoots and roots (Hua and Meyerowitz, 1998). The role of ethylene itself in potato tuber development is currently not clear (Kloosterman and Bachem, 2014). If ethylene in general and subfamily II ethylene receptors in special are involved in potato tuber development, a relation with patatin and PIs is also possible because tuber protein content increases with increasing tuber weight (Kolbe and Stephan-Beckmann, 1997). Further research is needed to clarify a possible relation of subfamily II ethylene receptors and tuberization and if putative ETR in that way is involved in the regulation of potato tuber protein synthesis.

Ethylene receptor subfamily II members are involved in resistances against biotic and abiotic stresses, but differences between crops and genes exist (Appendix XI). Salt stress for example induced HK1 in tobacco (Cao et al., 2006), but had no significant effect on HK2 in the same crop (Zhang et al., 2004), while both belong to subfamily II. Patatin and PI's are both known to be involved in resistance against biotic and abiotic stresses (for review see paragraph 1.2). Aghaei et al. (2008) for example found a PI in potato shoots upregulated by salt stress. As discussed in paragraph 3.2 resistances are very complex processes for what reason it is not possible to conclude a causative correlation between ethylene receptors and potato tuber proteins just based on the fact that they both responded to a certain stress.
Based on results of other crops putative ETR and the potato tuber proteins might be linked in several ways. Further research should clarify if this subfamily II ethylene receptor is involved in the regulation of protein content in potato and in which way.

[8] The effect of ethylene was investigated by the application of exogenous ethephon to the plants.
[9] This author investigated only the expression of these genes the first sixteen days of tuberization.

3.3. Transporters

3.3.1. Putative AMT1;3

PGSC0003DMG400018761 had about 97 % sequence identity (data not shown) with member 3 of ammonium transporter family 1 of tomato (LeAMT1;3) and will therefore be annotated as putative AMT1;3. LeAMT1;3 has only 62.8 % sequence identity with the two other tomato AMT1 members LeAMT1;1 and LeAMT1;2 (Von Wirén et al., 2000a) and is phylogenetically distantly related from all currently known AMT1 members in plants (Loque and Von Wirén, 2004; Ludewig et al., 2007; Von Wirén et al., 2000b; Wu, 2004). The currently found putative potato AMT1;3 is the only gene which is closely related to LeAMT1;3. LeAMT1;3 is a functional ammonium transporter which is expressed during the dark in the leaves (Von Wirén et al., 2000a).

Currently no research has been done to the physiological function of LeAMT1;3. Von Wirén et al. (2000a) propose that LeAMT1;3 might be linked to the deamination of glutamate by glutamate dehydrogenase (GDH) which also occurs during the dark (Melo-Oliveira et al., 1996) and from which ammonium is released (Stewart et al., 1995). One would expect that the ammonium concentration in the leaves is increased because of this deamination process. Matt et al. (2001) however found that the ammonium concentration of tobacco leaves is slightly decreased during the night. Unknown is if this is also the case in tomato and potato, but if this is true AMT1;3 is might play a role in this decrease of ammonium concentration because its expression is increased during the darkness (Von Wirén et al., 2000a). This hypothesis however lets arise the question how the released ammonium is transported in other crops because this deamination of glutamate by GDH is common among plants, while potato AMT1;3 is a unique gene. Moreover, GDH is mainly expressed in the shoots, but to a lesser extent also in the roots (Melo-Oliveira et al., 1996), while LeAMT1;3 is solely expressed in the leaves (Von Wirén et al., 2000b). But even if AMT1;3 of potato is involved in the decrease of the ammonium concentration in leaves, the question arises what the physiological relevance would be of that for potato tuber protein.

Another possibility is that AMT1;3 possess a totally new and currently unknown function in tomato and potato. This is supported by the uniqueness of these two genes.

Finally it cannot be excluded that the putative potato AMT1;3 has no clear role in potato tuber protein synthesis. This might be supported by the fact that this gene lie around the border of 2 Mbp distance of the closest marker (Table 4 and Appendix III). Further research should clarify the function of this gene and its possible relation the potato tuber protein content.

3.3.2. SULTRs

Two sulphate transporters were found, PGSC0003DMG400018422 (putative SULTR3;3) and PGSC0003DMG400023515 (putative SULTR4; see Appendix XIII for literature review). Evidence for a direct relation between both SULTRs and potato tuber proteins was not found. Nevertheless they are both assumed to play a role in plants defence against biotic and abiotic stresses (for reviews see paragraph 1.2 and Appendix XIII). Patatin is for example involved in resistance against *P. infestans* by degrading glucans in the cell wall of this pathogen (Andreu et al., 1998; Tonón et al., 2001), while PIs *in vitro* were able to inhibit the length of hyphae and the germination of zoospores (Valueva et al., 2003). Potato SULTR4 and putative SULTR3;3 were upregulated by among others *P. infestans* (Vatansever et al., 2016), but currently no evidence was found that SULTRs or sulphate is also involved in the direct defence of the plant against *P. infestans*. Klikocka et al. (2005) however found that the percentage of infected potato tubers by *Rhizoctonia solani* in general significantly decreased with increasing levels of sulphate fertilization. Infection severity was in one of the two years not significantly affected, while it was significantly increased in the other year. Sulphate fertilization had in general no, or an increasing effect on infection rate and severity of potato tubers by *Streptomyces scabies* (Klikocka et al., 2005).

Sulphur is known to be involved in plant defence by sulphur-containing defence compounds (SCDs). Synthesis of SCDs is upregulated after exposure of plants to biotic or abiotic stresses. In this stress response pathway JA and possibly other signals play a crucial role. During this stress response pathway among others expression of sulphate transporters is induced (for review about the role of

sulphur in plant defence see Rausch and Wachter, 2005). Potato putative SULTR3;3 and SULTR4 were both also upregulated by several stresses (Vatansever et al., 2016). Further research should clarify if these two genes could be part of the aforementioned sulphur stress response pathway. Similar to the sulphur stress response pathway, JA also plays an important role in the upregulation of PIs under certain stress conditions (Hildmann et al., 1992; Peña-Cortés et al., 1995; Peña-Cortés et al., 1989). It is not known if induction of the PIs and of the sulphur stress response pathway functions parallel to each other or are interrelated. Further research is needed to clarify the relation of tuber proteins, the both SULTRs and SCDs.

Information about the role of sulphate in tuber development and tuber protein synthesis is currently limited. Puzina (2004) immersed tubers prior to planting in a zinc-sulphate solution for six hours. Control tubers were immersed in water. The zinc-sulphate treatment resulted in a more than 60 % increase in tuber yield compared to the control plants. It is however not clear if this was due to the zinc or due to the sulphate. Further research should clarify the importance of sulphate for tuberization and tuber protein content.
It is remarkable that the tissue expression profile of putative SULTR3;3 and SULTR4 in rice show high similarity, while they in Arabidopsis show clear difference (Kataoka et al., 2004; Kumar et al., 2011 Takahashi et al., 2000; Zuber et al., 2010a; Zuber et al., 2010b). Tissue expression profile and cellular localization of putative SULTR3;3 and SULTR4 in potato still needs to be investigated. Together with the role of sulphate in tuberization and tuber protein synthesis this might shed light on the possible relation of putative SULTR3;3, putative SULTR4 and potato tuber protein content.

3.3.3. SWEETs

All six currently found carbohydrate transporters belong to clade III of the SWEET sugar transporter family (Manck-Götzenberger and Requena, 2016). Arabidopsis clade III SWEETs preferentially transport sucrose and to a lesser extend also glucose and fructose, but not maltose (Chen et al., 2012; Hir et al., 2015). Clade III transporters perform the first step in phloem loading; they transport sucrose from the phloem parenchyma cells into the apoplast after which sugar transporters of the SUC/SUT family transport it into the sieve elements and companion cells (Chen et al., 2012). A clade I SWEET in Arabidopsis however was found to function as a bidirectional sugar transporter (Chen et al., 2010). For clade III members a bidirectional transport function is unknown yet. However because at least some clade III SWEETs are expressed in both potato leaves and tubers it might be possible that they are involved in phloem loading in the leaves (sucrose sources) and phloem unloading in the tubers (sucrose sinks).
Timmermans (2016) found that SWEET11b was only expressed when first swelling of the stolon was visible, while SWEET12d showed strong expression from the stage that the stolon gets a tuber shape. They hypothesize therefore that SWEET11b might fulfil an important role in signalling at the start of tuberization. This is in agreement with Xu et al. (1998a) which found that sucrose had an important role in regulating tuberization. Similar to SWEET12d sucrose is also strongly increased when tuberization starts (Ross et al., 1994). A causative correlation between this SWEET and sucrose content might be possible because clade III SWEETs in Arabidopsis preferentially transports sucrose (Chen et al., 2012; Hir et al., 2015). It can however not be excluded that more clade III SWEETs are involved in the increase in sucrose content at early stages of tuberization. The major tuber protein, patatin is also strongly induced in tubers after tuberization starts, but can also be induced in other tissues after the addition of sucrose (Liu et al., 1991; Wenzler et al., 1989). Because sucrose content also increases in early stages of tuberization (Ross et al., 1994), the accumulation of patatin in tubers might also be regulated by sucrose. In that way an indirect relation of patatin with SWEET clade III members might be possible.
Patatin is a glycoprotein with one, two or three glycans which make up about 3, 6 or 9 % of patatins total weight respectively (Pots et al., 1999; Sonnewald et al., 1989b). The glycans consist out of fucose, xylose, mannose and N-acetylglucosamine in a 1:1:3:2 ratio (Sonnewald et al., 1989b). Clade III SWEETs preferentially transports sucrose (Chen et al., 2012; Hir et al., 2015), but currently no

evidence is present that they are able to transport any of the sugars of the patatin glycans. It is therefore not likely that the currently found SWEETs directly contribute to the glycosylation of patatin.
Potato tuber proteins were also found to be involved in resistances against biotic and abiotic stresses (for review see paragraph 1.2). In several crops SWEET clade III members were also found to be involved in resistances against biotic and abiotic stresses (for review Appendix XIV), but for potato clade III SWEETs this still needs to be investigated. However as mentioned before resistance reactions are very complex because mostly several thousands of genes are affected (paragraph 3.2.1). It is therefore not possible to conclude a causative correlation just based on the fact that SWEETs and tuber proteins both are affected by certain stresses.
Many clade III genes in potato tubers were found to be affected after infection with the arbuscular mycorrizal (AM) fungi *Rhizophagus irregularis* (Manck-Götzenberger and Requena, 2016). Infection with a AM fungi positively influences yield and therefore also indirectly potato tuber protein content, because tuber protein content increases with increasing tuber weight (Kolbe and Stephan-Beckmann, 1997). Currently no evidence was found for a direct relation of *R. irregularis* with potato tuber proteins.

3.4. Putative xylogen

According to UniProt (2017), PGSC0003DMG400012567 is a lipid binding protein involved in lipid transport, but no literature was found to support that. BLAST searches (data not shown) resulted in high scores with genes of the xylogen family of both Arabidopsis and rice. PGSC0003DMG400012567 will therefore be annotated in this report as putative xylogen. The highest BLAST score (data not shown) was obtained with Arabidopsis AtXYP1 and AtXYP2 and rice OsXYLP5, all belonging to clade A of the xylogen gene family (Ma et al., 2014). These genes have a coordinating function in xylem, and maybe also in phloem, development (Motose et al.,2004; Zhao et al., 2005). Xylem is important for tuberization. Stolon and tuber-roots transport water by the xylem to the tubers (Kratzke and Palta, 1985). These authors only measured water, but with the water of course also nutrients are transported by the xylem. Xu et al. (1998b) found that when tubers reached a diameter of 0.8 cm, increasing thickness of the perimedullary region including xylem and phloem is the main reason for tuber growth. During this stage the xylem and phloem elements were scattered in the entire perimedullary region. Since xylogen in Arabidopsis is involved in coordinating vascular development (Motose et al., 2004), putative xylogen might have a coordinating function in this scattering during tuber development. Potato tuber protein content increases when tuberization starts (Kolbe and Stephan-Beckmann, 1997). Therefore an indirect relation of putative xylogen with potato tuber proteins through xylem development and water and nutrient supply is possible. Currently however no evidence is available for a direct coordinating influence of putative xylogen on potato tuber protein content.

Provart et al. (2003) found AtXYP2 in Arabidopsis shoots upregulated under cold stress, compared to control plants. These authors suggest a role for AtXYP2 as lipid transfer protein in changing cellular membranes to increase tolerance against low temperatures. Folgado et al. (2013) found that under cold stress some PIs in leaves were upregulated, while another was downregulated. They did not investigate if the cell membranes were changed during cold stress. The effect of cold stress on PIs in the tubers is also not investigated yet. Gong et al. (2015) found the expression of four patatins, three PIs and putative xylogen significantly downregulated in potato stolons under drought stress. However as discussed in paragraph 3.2 resistances are very complex processes for what reason it is not possible to conclude a causative correlation between putative xylogen and potato tuber proteins just based on the fact that they both responded to a certain stresses.

Arabidopsis AtXYP1 and AtXYP2 has the ability to bind to stigmasterol and also weakly to brassicasterol (Motose et al., 2004). In potato these two sterols together make up only 6.4 % of total tuber sterol content (Ramadan and Elsanhoty, 2012). The main potato tuber sterols are campesterol,

β-sitosterol and Δ5-avenasterol, which constitute about 27, 43 and 20 % of total potato tuber sterol content respectively (Ramadan and Elsanhoty, 2012). Arabidopsis AtXYP1 and AtXYP2 were not able to bind campesterol and β-sitosterol, while the ability to bind to Δ5-avenasterol was not investigated (Motose et al., 2004). It can however not be excluded that potato xylogen protein is able to bind other sterols than its Arabidopsis counterparts. Further research is needed to clarify the sterol binding characteristics of putative xylogen in potato.
Mucharromah et al. (1995) found that sterols, mainly β-sitosterol and stigmasterol, showed reduced accumulation after inoculation of potato tuber slices with incompatible *P. infestans* races. Pre-treatment of the slices with β-sitosterol or stigmasterol suppressed this effect and allowed colonization by the incompatible *P. infestans*. Infection with a compatible *P. infestans* race showed no reduction in accumulation of the sterols (Mucharromah et al., 1995). These results suggests that these sterols are involved in resistance of the potato tuber against this oomycete, but their precise role is not clear yet. It needs to be investigated if putative potato xylogen is involved in the regulation of the accumulation of these sterols and if it in that way contributes to the plants defence against *P. infestans*. Kidd et al. (2011) found the expression of AtXYP2 in Arabidopsis leaves reduced after inoculation with *Fusarium oxysporum* compared to control plants. Additionally Arabidopsis AtXYP1 and AtXYP2 has the ability to bind to stigmasterol (Motose et al., 2004), which both might support the hypothesis that putative xylogen plays a role in the defence potato of against *P. infestans*.
Patatin's LAH activity and β-1,3 glucanase activity are involved in resistance against *P. infestans* (Andreu et al., 1998; Kawakita et al., 1993; Senda et al., 1996; Tonón et al., 2001). Patatin possess LAH activity on several lipids, but was inactive on sterol esters (Anderson et al., 2002; Andrews et al., 1988; Galliard, 1971; Galliard and Dennis, 1974; Racusen, 1984). The ability of patatin to hydrolyse others sterols is not investigated yet. It is also unknown if patatin in that way could contribute to the resistance reaction as described by Mucharromah et al. (1995). Further research is needed to clarify the role of putative xylogen in the resistance of potato against *P. infestans* and its relation with patatin and possibly with other tuber proteins.

3.5. Concluding remarks

General

By using the current reverse genetics approach, fifteen candidate genes were found (Table 4). Most of these genes are part of a large signalling pathway or cycle; putative type B-I RR of the cytokinin signalling pathway, putative PRR5 of the circadian clock, putative ETR of the ethylene signalling pathway, putative AMT1;3 of the nitrogen cycle, putative SULTR3;3 and SULTR4 of sulphur cycle and clade III SWEETs of the carbohydrate cycle. Only for putative xylogen, putative ZIM and putative ZML1 is not clear to which signalling pathway they belong. In the current literature review it was only possible to briefly describe some characteristics and functions of the candidate genes. However, for a better understanding of these genes and their relation with potato tuber protein synthesis, it is crucial to understand the total pathways or cycles and localize the particular candidate genes in these pathways or cycles. Moreover these pathways might be interrelated as will be discussed further in this paragraph. Further literature and practical studies are needed to clarify these issues.

Remarkable is also that by the current research a RR and PRR were found (Table 4), but no corresponding hybrid kinases or HPts (Figure 1). For putative ETR in opposite no corresponding (P)RR was found. A possibility is that parts of the phosphorelay signalling pathways functions (partly) redundantly resulting in crosstalk, meaning that a single hybrid kinase can regulate more than one specific (P)RR or that a single (P)RR can be regulated by more than one hybrid kinase. Further (literature) research is needed to clarify that.

Relation candidate genes

Interestingly several candidate genes seemed to be linked to each other, either by their targets or by the signalling pathway they belong to. Putative xylogen, clade III SWEETs and putative type B-I RR all are involved in xylem development, but their function differs. Xylogen has a coordinating function in

xylem development in Arabidopsis (Motose et al., 2004). They found that double knockout mutants of AtXYP1 and AtXYP2 resulted in defects in the vascular system like thicker veins and improper connection of the tracheary elements in Arabidopsis. SWEET11 and 12 were supposed to supply sugars to developing xylem cells to support the formation of secondary cell walls in Arabidopsis floral stems (Hir et al., 2015). These authors found that single and especially double knock out mutants of SWEET11 and 12 had a smaller xylem and phloem pole area, less xylem and phloem cells per pole area and a smaller diameter of the xylem cells. Yokoyama et al. (2007) found that RR10 and RR12 (both type B-I) negatively regulate the development from procambium into protoxylem. Further research is needed to make clear if these genes were really linked or that they function parallel to each other.

In rice, ETR2 was shown to be involved in starch accumulation and sugar transport (Wuriyanghan et al.,2009). With the current reverse genetic approach also six sugar transporters of the SWEET family clade III were found (paragraph 3.3.3; Appendix XIV). Starch (build-up of sugars) and patatin content are positively correlated in potato tubers (Bárta and Bártová, 2008). Putative ETR and clade III SWEETs could therefore also be indirectly related to potato tuber protein content by regulating starch accumulation. It is however not clear if the correlation of Bárta and Bártová (2008) implies a causal relationship. It is also unknown if putative ETR and clade III SWEETs functions in the same pathway, or parallel to each other. Further research should clarify the relation of putative ETR and clade III SWEETs and if they influence tuber protein content by regulating starch accumulation.

Putative PRR5 is assumed to play a central role in the circadian clock (e.g. Matsushika et al., 2000; Makino et al., 2001; for review see Appendix X). StSP6A, the main inducer of tuberization, is under the control of the circadian clock (for review see Kloosterman and Bachem, 2014). Interestingly Timmermans (2016) found that StSP6A induces some clade III SWEET sugar transporters. One of these SWEETs was also found with the current BLAST searches (see paragraph 3.3.3 and Appendix XIV), suggesting an indirect relation of putative PRR5 and these SWEETs. Ammonium transporter LeAMT1;3 is mainly expressed during the dark in tomato leaves (Von Wirén et al., 2000a). Therefore this gene might also be regulated by the circadian clock. Further research should clarify the hypothetical relation of putative PRR5 with putative AMT1;3 and clade III SWEETs.

Implications and limitations

The currently used reverse genetics approach has the clear advantage that easily and without practical experiments, candidate genes can be found. Disadvantageous, however, is that therefore is started with a limited number of genes (only the candidate genes on chromosome 15 and 20 of soybean, see chapter 2). Bandillo et al. (2015) namely only defined candidate genes for the QTLs which they found on chromosome 15 and 20 and not for the QTLs they found on chromosome 6 and 13. Moreover it is possible that there are genes involved in potato tuber protein synthesis which are not related to genes involved in the regulation of soybean protein content and which therefore cannot be found by the current reverse genetics approach. Additionally Fernandez (2010) found a QTL in potato associated with tuber protein content which was not found by Peter Vos (unpublished results). Together this suggests that the currently found list of candidate genes contributing to potato tuber protein content is not complete.

It is also possible that there are genes involved in the regulation of soybean protein which are not involved in the regulation of potato tuber protein content. Therefore it is possible that (some of) the currently found candidate genes do not have a direct relation with tuber protein content.

The characteristics and functions of the candidate genes were in most cases only described for other crops and not for potato, neither for tuber crops nor *Solanaceae* members. Tubers differs physiologically greatly from other storage organs like seeds. Additionally the main tuber protein, patatin class I, is mainly expressed in the tubers (Aminedi and Das, 2014; Jefferson et al., 1990; Liu et al., 1991; Mignery et al., 1988; Wenzler et al., 1989). Caution has therefore to be taken by

transferring these results to potato. Further research should clarify the function and characteristics of the candidate genes in potato.

Because EnsemblPlants (2017) only had the genome sequence for the *O. sativa indica* and *O. sativa japonica* separately, QTLs of the total population (indica + japonica) are therefore used in the both subpopulations (for more details see chapter 2). The two subpopulations are however not identical. It can therefore not be excluded that the QTL intervals in rice were placed on a (slightly) wrong position. Therefore wrong candidate genes in rice might be found, which in turn could have resulted in wrong candidate genes in potato.

In conclusion fifteen candidate genes were found which all might be related to tuber protein content due to their proposed involvement in potato (tuber) development and/or resistance against (a)biotic stresses. Further research is needed to prove if the currently found candidate genes in potato are really involved in potato tuber protein synthesis and to investigate whether other genes, not related to genes in the other crops, are involved. Potato mutants overexpressing or not expressing (knock out) the candidate genes will be a useful tool to gain insight into the function of the candidate genes in potato. A GWAS with more accessions and SNPs might be useful to investigate whether other or more genes are involved in the regulation of potato tuber protein synthesis.

References

Abelenda, J.A., Cruz-Oró, E., Franco-Zorrilla, J.M., Prat, S., 2016. Potato StCONSTANS-like1 suppresses storage organ formation by directly activating the FT-like StSP5G repressor. Current Biology 26: 872–881.

Aghaei, K., Ehsanpour, A.A. and Komatsu, S., 2008. Proteome analysis of potato under salt stress. Journal of Proteome Research 7: 4858–4868.

Aminedi, R. and Das, N., 2014. Class I patatin genes from potato (*Solanum tuberosum* L.) cultivars: molecular cloning, sequence comparison, prediction of diverse cis-regulatory motifs, and assessment of the promoter activities under field and *in vitro* conditions. In Vitro Cellular & Developmental Biology – Plant 50: 673‑687.

Anderson, C., Pinsirodom, P. and Parkin, K.I., 2002. Hydrolytic selectivity of patatin (lipid acyl hydrolase) from potato (*Solanum tuberosum* L.) tubers toward various lipids. Journal of Food Biochemistry 26: 63-74.

Andreu, A., Tonón, C., Van Damme, M., Huarte, M. and Daleo, G., 1998. Effect of glucans from different races of *Phytophthora infestans* on defense reactions in potato tuber. European Journal of Plant Pathology 104: 777–783.

Andrews, D.L., Beames, B., Summers, M.D. and Park, W.D., 1988. Characterization of the lipid acyl hydrolase activity of the major potato (*Solanum tuberosum*) tuber protein, patatin, by cloning and abundant expression in a baculovirus vector. Biochemical Journal 252: 199-206.

Aslam, A. and Iqbal, J., 2010. Combined effect of cytokinin and sucrose on *in vitro* tuberization parameters of two cultivars i.e., Diamant and Red Norland of potato (*Solanum tuberosum*). Pakistan Journal of Botany 42 (2): 1093-1102.

Bai, Y., Meng, Y., Huang, D., Qi, Y. and Chen, M., 2011. Origin and evolutionary analysis of the plant-specific TIFY transcription family. Genomics 98: 128-136.

Bandillo, N., Jarquin, D., Song, Q., Nelson, R., Cregan, P., Specht, J., and Lorenz, A., 2015. A population structure and genome-wide association analysis on the USDA soybean germplasm collection. The Plant Genome 8 (3): doi: 10.3835/plantgenome2015.04.0024

Bárta, J. and Bártová, V., 2008. Patatin, the major protein of potato (*Solanum tuberosum* L.) tubers, and its occurrence as genotype effect: processing versus table potatoes. Czech Journal of Food Sciences 26: 347-359.

Bárta, J., Bártová, V., Zdráhal, Z. and Šedo, O., 2012. Cultivar variability of patatin biochemical characteristics: table versus processing potatoes (*Solanum tuberosum* L.). Journal of Agricultural and Food Chemistry 60: 4369-4378.

Bártova, V., Diviš, J., Bárta, J., Brabcová, A. and Švajnerová, M., 2013. Variation of nitrogenous components in potato (*Solanum tuberosum* L.) tubers produced under organic and conventional crop management. European Journal of Agronomy 49: 20-31.

Bauw, G., Nielsen, H.V., Emmersen, J., Nielsen, K.L., Jørgensen, M. and Welinder, K.G., 2006. Patatins, Kunitz protease inhibitors and other major proteins in tuber of potato cv. Kuras. FEBS Journal 273: 3569-3584.

Binder, B.M. and Schaller, G.E., 2015. The Role of Protein–Protein Interactions in Signaling by the Ethylene Receptors. In: Ethylene in plants (ed. Wen, C.K.). Springer Science+Business Media Dordrecht, 61-72.

Borner, G.H.H., Lilley, K.S., Stevens, T.J. and Dupree, P., 2003. Identification of glycosylphosphatidylinositol-anchored proteins in Arabidopsis. A proteomic and genomic analysis. Plant Physiology 132: 568-577.

Brink, E. van de, 2008. Vissen naar aardappeleiwitten. BIOCHEM februari 2008.

Cai, S., Yu, G., Chen, X., Huang, Y., Jiang, X., Zhang, G. and Jin, X., 2013. Grain protein content variation and its association analysis in barley. BMC Plant Biology 13 (35): http://www.biomedcentral.com/1471-2229/13/35

Cao, W., Dong, Y., Zhang, J. and Chen, S., 2003. Characterization of an ethylene receptor homolog gene from rice. Science in China 46 (4): 370-378.

Cao, W.H., Liu, J., Zhou, Q.Y., Cao, Y.R., Zheng, S.F., Du, B.X., Zhang, J.S. and Chen, S.Y., 2006. Expression of tobacco ethylene receptor NTHK1 alters plant responses to salt stress. Plant, Cell and Environment 29: 1210–1219.

CBS, visited on September 1, 2015. http://statline.cbs.nl/StatWeb/publication/?VW=T&DM=SLNL&PA=7100OOGS&LA=NL

Chang, C., Kwok, S.F., Bleecker, A.B. and Meyerowitz, E.M., 1993. Arabidopsis ethylene-response gene ETR1: similarity of product to two-component regulators. Science 262 (5133): 539-544.

Chaves, I., Pinheiro, C., Paiva, J.A.P., Planchon, S., Sergant, K., Renaut, J., Graça, J.A., Costa, G., Coelho, A.V. and Pinto Ricardo, C.P., 2009. Proteomic evaluation of wound-healing processes in potato (*Solanum tuberosum* L.) tuber tissue. Proteomics 9: 4154–4175.

Chen, L.Q., Hou, B.H., Lalonde, S., Takanaga, H., Hartung, M.L., Qu, X.Q., Guo, W.J., Kim, J.G., Underwood, W., Chaudhuri, B., Chermak, D., Antony. G., White, F.F., Somerville, S.C., Mudgett, M.B. and Frommer, W.B., 2010. Sugar transporters for intercellular exchange and nutrition of pathogens. Nature 468: 527-532.

Chen, L.Q., Lin, I.W., Qu, X.Q., Sosso, D., McFarlane, H.E., Londoño, A., Samuels, A.L. and Frommer, W.B., 2015. A cascade of sequentially expressed sucrose transporters in the seed coat and endosperm provides nutrition for the Arabidopsis embryo. The Plant Cell 27: 607-619.

Chen, L.Q., Qu, X.Q., Hou, B.H., Sosso, D., Osorio, S., Fernie, A.R. and Frommer, W.B., 2012. Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science 335: 207-211.

Cheng, P., Holdsworth, W., Ma, Y., Coyne, C.J., Mazourek, M., Grusak, M.A., Fuchs, S. and McGee, R.J., 2015. Association mapping of agronomic and quality traits in USDA pea single-plant collection. Molecular Breeding 35 (75): doi: 10.1007/s11032-015-0277-6

Ciardi, J.A., Tieman, D.M., Jones, J.B. and Klee, H.J., 2001. Reduced expression of the tomato ethylene receptor gene LeETR4 enhances the hypersensitive response to X*anthomonas campestris pv. Vesicatoria*. Molecular Plant-Microbe Interactions14 (4): 487–495.

Cleveland, T.E., Thornburg, R.W. and Ryan, C.A., 1987. Molecular characterization of a wound-inducible inhibitor I gene from potato and the processing of its mRNA and protein. Plant Molecular Biology 8: 199-207.

Cook, J.P., McMullen, M.D., Holland, J.B., Tian, F., Bradbury, P., Ross-Ibarra, J., Buckler, E.S. and Flint-Garcia, S.A., 2012. Genetic architecture of maize kernel composition in the nested association mapping and inbred association panels. Plant Physiology 158: 824–834.

Dello Ioio, R., Nakamura, K., Moubayidin, L., Perilli, S., Taniguchi, M., Morita, M.T., Aoyama, T., Constantino, P. and Sabatini, S., 2008. A genetic framework for the control of cell division and differentiation in the root meristem. Science 322: 1380-1384.

Dello Ioio, R., Scaglia Linhares, F., Scacchi, E., Casamitjana-Martinez, E., Heidstra, R., Constantino, P. and Sabatini, S., 2007. Cytokinins determine Arabidopsis root-meristem size by controlling cell differentiation. Current Biology 17: 678–682.

Dennis, S. and Galliard, T., 1974. Wax ester formation catalysed by isoenzymes of lipolytic acyl hydrolase. Phytochemistry 13: 2469-2473.

Du, L., Jiao, F., Chu, J., Jin, G., Chen, M. and Wu, P., 2007. The two-component signal system in rice (*Oryza sativa* L.): A genome-wide study of cytokinin signal perception and transduction. Genomics 89: 697–707.

Edstam, M.M., Blomqvist, K., Eklöf, A., Wennergren, U. and Edqvist, 2013. Coexpression patterns indicate that GPI-anchored non-specific lipid transfer proteins are involved in accumulation of cuticular wax, suberin and sporopollenin. Plant Molecular Biology 83: 625-649.

Edstam, M.M., Viitanen, L., Salminen, T.A. and Edqvist, J., 2011. Evolutionary history of the non-specific lipid transfer proteins. Molecular Plant 4 (6): 947-964.

EnsemblPlants, visited may 10, 2017. http://plants.ensembl.org/index.html

Fabro, G., Rienzo, J.A. Di, Voigt, C.A., Savchenko, T., Dehesh, K., Somerville, S. and Alvarez, M.E., 2008. Genome-wide expression profiling Arabidopsis at the stage of *Golovinomyces cichoracearum* haustorium formation. Plant Physiology 146: 1421-1439.

FAOSTAT, visited on May 2, 2017. http://faostat3.fao.org/browse/Q/QC/E

Farré, E.M., Harmer, S.L., Harmon, F.G., Yanovsky, M.J. and Kay, S.A., 2005. Overlapping and distinct roles of PRR7 and PRR9 in the Arabidopsis circadian clock. Current Biology 15: 47–54.

Feng, C.Y., Han, J.X., Han, X.X. and Jiang, J., 2015. Genome-wide identification, phylogeny, and expression analysis of the SWEET gene family in tomato. Gene 573: 261-272.

Fernandez, A.L.A., 2010. Association mapping in tetraploid potato (*Solanum tuberosum* L.) using AFLPs and fully genotyped SNPs. MSc thesis Wageningen UR. 51 pp.

Flint-Garcia, S.A., Thornsberry, J.M. & Bukler, E.S., 2003. Structure of linkage disequilibrium in plants. Annual Review Plant Biology 54: 357-374.

Folgado, R., Panis, B., Sergeant, K., Renaut, J., Swennen, R. and Hausman, J.F., 2013. Differential protein expression in response to abiotic stress in two potato species: *Solanum commersonii* Dun and *Solanum tuberosum* L. International Journal of Molecular Sciences 14, 4912-4933.

Fujiwara, S., Wang, L., Han, L., Suh, S.S., Salomé, P.A., McClung, C.R. and Somers, D.E., 2008. Post-translational regulation of the Arabidopsis circadian clock through selective proteolysis and phosphorylation of pseudo-response regulator proteins. The Journal of Biological Chemistry 283 (34): 23073–23083.

Gajardo, H.A., Wittkop, B., Soto-Cerda, B., Higgins, E.E., Parkin, I.A.P., Snowdon, R.J., Federico, M.L. and Iniguez-Luy, F.L., 2015. Association mapping of seed quality traits in Brassica napus L. using GWAS and candidate QTL approaches. Molecular Breeding 35: 143.

Galliard, T., 1970. The enzymic breakdown of lipids in potato tuber by phospholipid- and galactolipid-acyl hydrolase activities and by lipoxygenase. Phytochemistry 9: 1725-1734.

Galliard, T., 1971. The enzymic deacylation of phospholipids and galactolipids in plants; purification and properties of a lipolytic acyl-hydrolase from potato tubers. Biochemical Journal 121: 379-390.

Galliard, T. and Dennis, S., 1974. Phospholipase, galactolipase and acyl transferase activities of a lipolytic enzyme from potato. Phytochemistry 13: 1731-1735.

Gong, L., Zhang, H., Gan, X., Zhang, L., Chen,Y., Nie, F., Shi, S., Li, M., Guo, Z., Zhang, G. and Song, Y., 2015. Transcriptome profiling of the potato (*Solanum tuberosum* L.) plant under drought stress and water-stimulus conditions. PLoS ONE 10 (5): doi: 10.1371/journal.pone.0128041

Graff, L., Obrdlik, P., Yuan, L., Loqué, D., Frommer, W.B. and Von Wirén, N., 2011. N-terminal cysteines affect oligomer stability of the allosterically regulated ammonium transporter LeAMT1;1. Journal of Experimental Botany 62 (4): 1361–1373.

Griffiths, A.J.F., Wessler, S.R., Carrol, S.B. and Doebley, J., 2012. Introduction to genetic analysis. International tenth edition. W.H. Freeman and company, New York. 802 pp.

Hall, B.P., Shakeel, S.N. and Schaller, G.E.,2007. Ethylene receptors: ethylene perception and signal transduction. Journal of plant growth regulation 26: 118–130.

Hancock, R.D., Morris, W.L., Ducreux, L.J.M., Morris, J.A., Usman, M., Verrall, S.R., Fuller, J., Simpson, C.G., Zhang, R., Hedley, P.E. and Taylor, M.A., 2014. Physiological, biochemical and molecular responses of the potato (*Solanum tuberosum* L.) plant to moderately elevated temperature. Plant, Cell and Environment 37: 439–450.

Hannapel, D.J., 1991. Distribution of potato tuber proteins during development. American Potato Journal 68: 179-190.

Hannapel, D.J., 2007. Signalling the induction of tuber formation. In: Potato Biology and Biotechnology: Advances and Perspectives (ed. Vreugdenhil, D., Bradshaw, J., Gebhardt, C., Govers, F., Taylor, M., MacKerron, D. and Ross, H.). Elsevier Science, 237-256.

Hass, C., Lohrmann, J., Albrecht, V., Sweere, U., Hummel, F., Yoo, S.D., Hwang, I., Zhu, T., Schäfer, E., Kudla, J. and Harter, K., 2004. The response regulator 2 mediates ethylene signalling and hormone signal integration in Arabidopsis. The EMBO Journal 23, 3290–3302.

Hawkes, J.G., 1990. The potato. Evolution, biodiversity and genetic resources. Belhaven Press, London. 259 pp.

He, D.H., Lei, Z.P., Tang, B.S., Xing, H.Y., Zhao, J.X. and Jing, Y.L., 2015. Identification and analysis of the TIFY gene family in Gossypium raimondii. Genetics and Molecular Research 14 (3): 10119-10138.

Heibges, A., Glaczinski, H., Ballvora, A., Salamini, F. and Gebhardt, C., 2003. Structural diversity and organization of three gene families for Kunitz-type enzyme inhibitors from potato tubers (*Solanum tuberosum* L.). Molecular Genetics and Genomics 269: 526-534.

Hermosa, M.R., Turrà, D., Fogliano, V., Monte, E. and Lorito, M., 2006. Identification and characterization of potato protease inhibitors able to inhibit pathogenicity and growth of *Botrytis cinerea*. Physiological and Molecular Plant Pathology 68: 138–148.

Hildmann, T., Ebneth, M., Peña-Cortés, H.,Sánchez-Serrano, Willmitzer, L. and Prat, S., 1992. General roles of abscisic and jasmonic acids in gene activation as a result of mechanical wounding. The Plant Cell 4: 1157-1170.

Hir, R. Le, Spinner, L., Klemens, P.A.W., Chakraborti, D., Marco, F. de, Vilaine, F., Wolff, N., Lemoine, R., Porcheron, B., Géry, C., Téoulé, E., Chabout, S., Mouille, G., Neuhaus, H.E., Dinant, S. and Belline, C., 2015. Disruption of the sugar transporters AtSWEET11 and AtSWEET12 affects vascular development and freezing tolerance in Arabidopsis. Molecular Plant 8: 1687-1690.

Hopkins, L., Parmar, S., Blaszczyk, A., Hesse, H., Hoefgen, R. and Hawkesford, M.J., 2005. O-acetylserine and the regulation of expression of genes encoding components for sulfate uptake and assimilation in potato. Plant Physiology 138: 433–440.

Hu, W., Wang, Y., Bowers, C. and Ma, H., 2003. Isolation, sequence analysis, and expression studies of florally expressed cDNAs in Arabidopsis. Plant Molecular Biology 53: 545-563.

Hua, J. and Meyerowitz, E.M., 1998. Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. Cell volume 94: 261–271.

Hua, J., Chang, C., Sun, Q. and Meyerowitz, E.M., 1995. Ethylene insensitivity conferred by Arabidopsis ERS gene. Science 269: 1712-1714.

Hua, J., Sakai, H., Nourizadeh, S., Chen, Q.G., Bleecker, A.B., Ecker, J.R. and Meyerowitz, E.M., 1998. EIN4 and ERS2 are members of the putative ethylene receptor gene family in Arabidopsis. The Plant Cell 10: 1321–1332.

Huang, X., Wei, X., Sang, T., Zhao, Q., Feng, Q., Zhao, Y., Li, C., Zhu, C., Lu, T., Zhang, Z., Li, M., Fan, D., Guo, Y., Wang, A., Wang, L., Deng, L., Li, W., Lu, Y., Weng, Q., Liu, K., Huang, T., Zhou, T., Jing, Y., Li, W., Lin, Z., Buckler, E.S., Qian, Q., Zhang, Q.F., Li, J. and Han, B., 2010. Genome-wide association studies of 14 agronomic traits in rice landraces. Nature Genetics 42 (11): 961-967.

Huang, X., Zhao, Y., Wei, X., Li, C., Wang, A., Zhao, Q., Li, W., Guo, Y., Deng, L., Zhu, C., Fan, D., Lu, Y., Weng, Q., Liu, K., Zhou, T., Jing, Y., Si, L., Dong, G., Huang, T., Lu, T., Feng, Q., Qian, Q., Li, J. and Han, B., 2012. Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. Nature Genetics 44 (1): 32 41.

Hwang, I., Chen, H.C. and Sheen, J., 2002. Two-component signal transduction pathways in Arabidopsis. Plant Physiology 129: 500-515.

Hwang, E.Y., Song, Q., Jia, G., Specht, J.E., Hyten, D.L., Costa, J. and Cregan, P.B., 2014. A genome-wide association study of seed protein and oil content in soybean. BMC Genomics 15 (1): http://www.biomedcentral.com/1471-2164/15/1

Imamura, A., Kiba, T., Tajima, Y., Yamashino, T., Mizuno, T., 2003. In vivo and in vitro characterization of the ARR11 response regulator implicated in the His-to-Asp phosphorelay signal transduction in *Arabidopsis thaliana*. Plant Cell Physiol. 44 (2): 122–131.

Ishida, K., Yamashino, T., Yokoyama, A. and Mizuno, T., 2008. Three type-B response regulators, ARR1, ARR10 and ARR12, play essential but redundant roles in cytokinin signal transduction throughout the life cycle of *Arabidopsis thaliana*. Plant and Cell Physiology 49 (1): 47–57.

Ito, Y. and Kurata, N., 2006. Identification and characterization of cytokinin-signalling gene families in rice. Gene 382: 57–65.

Ito, S., Matsushika, A., Yamada, H., Sato, S., Kato, T., Tabata, S., Yamashino, T. and Mizuno, T., 2003. Characterization of the APRR9 pseudo-response regulator belonging to the APRR1/TOC1 quintet in *Arabidopsis thaliana*. Plant and Cell Physiology 44 (11): 1237–1245.

Jefferson, R., Goldsbrough, A. and Bevan, M., 1990. Transcriptional regulation of a patatin-1 gene in potato. Plant Molecular Biology 14: 995-1006.

Johnson, R. and Ryan, C.A., 1990. Wound-inducible potato inhibitor II genes: enhancement of expression by sucrose. Plant Molecular Biology 14: 527-536.

Jun, T.H., Van, K., Kim, M.Y., Lee, S.H. and Walker, D.R., 2008. Association analysis using SSR markers to find QTL for seed protein content in soybean. Euphytica 162: 179-191.

Kang, S.G., Choi, J.H. and Suh, S.G., 2002. A leaf-specific 27 kDa protein of potato kunitz-type proteinase inhibitor is induced in response to abscisic acid, ethylene, methyl jasmonate, and water deficit. Molecules and cells 13 (1): 144-147.

Kataoka, T., Watanabe-Takahashi, A., Hayashi, N., Ohnishi, M., Mimura, T., Buchner, P., Hawkesford, M.J., Yamaya, T. and Takahashi, H., 2004. Vacuolar sulfate transporters are essential determinants controlling internal distribution of sulfate in Arabidopsis. The Plant Cell 16: 2693-2704.

Kawakita, K., Senda, K. and Doke, N., 1993. Factors, affecting in vitro activation of potato phospholipase A_2. Plant Science 92 183-190.

Kevany, B.M., Taylor, M.G. and Klee, H.J., 2008. Fruit-specific suppression of the ethylene receptor LeETR4 results in early-ripening tomato fruit. Plant Biotechnology Journal 6: 295–300.

Kevany, B.M., Tieman, D.M., Taylor, M.G., Dal Cin, V. and Klee, H.J., 2007. Ethylene receptor degradation controls the timing of ripening in tomato fruit. The Plant Journal 51: 458–467.

Kiba, T., Taniguchi, M., Imamura, A., Ueguchi, C., Mizuno, T., Sugiyama, T., 1999. Differential expression of genes for response regulators in response to cytokinins and nitrate in *Arabidopsis thaliana*. Plant and Cell Physiology 40 (7): 767-771.

Kidd, B.N., Kadoo, N.Y., Dombrecht, B., Tekeoğlu, M., Gardiner, D.M., Thatcher, L.F., Aitken, E.A.B., Schenk, P.M., Manners, J.M. and Kazan, K., 2011. Auxin signaling and transport promote susceptibility to the root-infecting fungal pathogen *Fusarium oxysporum* in Arabidopsis. Molecular Plant-Microbe Interactions 24 (6): 733-748.

Kim, H.J., Ryu, H., Hong, S.H., Woo, H.R., Lim, P.O., Lee, I.C., Sheen, J., Nam, H.G. and Hwang, I., 2006. Cytokinin-mediated control of leaf longevity by AHK3 through phosphorylation of ARR2 in Arabidopsis. Proceedings of the National Academy of Sciences 103 (3): 814-819.

Klee, H. and Tieman, D., 2002. The tomato ethylene receptor gene family: Form and function. Physiologia Plantarum 115: 336–341.

Klikocka, H., Haneklaus, S., Bloem, E., Schnug, E., 2005. Influence of sulfur fertilization on infection of potato tubers with *Rhizoctonia solani* and *Streptomyces scabies*. Journal of Plant Nutrition 28 (5): 819-833.

Kloosterman, B., Abelenda, J.A., Mar Carretero Gomez, M. del, Oortwijn, M., Boer, J.M. de, Kowitwanich, K., Horvath, B.M., Eck, H.J. van, Smaczniak, C., Prat, S., Visser, R.G.F. and Bachem, C.W.B., 2013. Naturally occurring allele diversity allows potato cultivation in northern latitudes. Nature 495: 246-250.

Kloosterman, B. and Bachem, C., 2014. Tuber development. In: the potato: botany, production and uses (ed. Navarre, R. and Pavek, M.J.).CABI, Wallingford, 45-63.

Kobayashi, Y., Motose, H., Iwamoto, K. and Fukuda, H., 2011. Expression and genome-wide analysis of the xylogen-type gene family. Plant and Cell Physiology 52 (6): 1095–1106.

Kolbe, H. and Stephan-Beckmann, S., 1997. Development, growth and chemical composition of the potato crop (*Solanum tuberosum* L.). II. Tuber and whole plant. Potato Research 40: 135-153.

Koningsveld, G.A. van, 2001. Physico-chemical and functional properties of potato proteins. PhD Thesis Wageningen UR. 150 pp.

Köster-Töpfer, M., Frommer, W.B., Rocha-Sosa, M., Rosahl, S., Schell, J. and Willmitzer, L., 1989. A class II patatin promoter is under developmental control in both transgenic potato and tobacco plants. Molecular Genetics and Genomics 219: 390-396.

Kratzke, M.G. and Palta, J.P., 1985. Evidence for the existence of functional roots on potato tubers and stolons: significance in water transport to the tuber. American Potato Journal 62: 227-236.

Kumar, S., Asif, M.H., Chakrabarty, D., Tripathi, R.D. and Trivedi, P.K., 2011. Differential expression and alternative splicing of rice sulphate transporter family members regulate sulphur status during plant growth, development and stress conditions. Functional and Integrative Genomics 11: 259-273.

Kwon, S.J., Brown, A.F., Hu, J., McGee, R., Watt, C., Kisha, T., Timmerman-Vaughan, G., Grusak, M., McPhee, K.E. and Coyne, C.J., 2012. Genetic diversity, population structure and genome-wide marker-trait association analysis emphasizing seed nutrients of the USDA pea (*Pisum sativum* L.) core collection. Genes and Genomics 34: 305-320.

Lauter, F.R., Ninneman, O., Bucher, M., Riesmeier, J.W. and Frommer, W.B., 1996. Preferential expression of an ammonium transporter and of two putative nitrate transporters in root hairs of tomato. Proceedings of the National Academy of Sciences 93: 8139-8144.

Ledoigt, G., Griffaut, B., Debiton, E., Vian, C., Mustel, A., Evray, G., Maurizis, J.C. and Madelmont, J.C., 2006. Analysis of secreted protease inhibitors after water stress in potato tubers. International Journal of Biological Macromolecules 38: 268–271.

Li, C., Mioa, H., Wei, L., Zhang, T., Han, X. and Zhang, H., 2014. Association mapping of seed oil and protein content in *Sesamum indicum* L. using SSR markers. PLoS ONE 9 (8): doi: 10.1371/journal.pone.0105757

Li, X., Yin, X., Wang, H., Li, J., Guo, C., Gao, H., Zheng, Y., Fan, C. and Wang, X., 2015. Genome-wide identification and analysis of the apple (Malus × domestica Borkh.) TIFY gene family. Tree Genetics & Genomes 11: 808.

Lin, I.W., Sosso, D. Chen, L.Q., Gase, K., Kim, S.G., Kessler, D., Klinkenberg, P.M., Gorder, M.K., Hou, B.H., Qu, X.Q., Carter, C.J., Baldwin, I.T. and Frommer, W.B., 2014. Nectar secretion requires sucrose phosphate synthases and the sugar transporter SWEET9. Nature 208: 546-549.

Liu, T., Carlsson, J., Takeuchi, T., Newton, L. and Farré, E.M., 2013. Direct regulation of abiotic responses by the Arabidopsis circadian clock component PRR7. The Plant Journal 76, 101–114.

Liu, X.Y., Rocha-Sosa, M., Hummel, S., Willmitzer, L. and Frommer, W.B., 1991. A detailed study of the regulation and evolution of the two classes of patatin genes in *Solanum tuberosum* L. Plant Molecular Biology 17: 1139-1154.

Logemann, J., Mayer, J.E., Schell, J. and Willmitzer, L., 1988. Differential expression of genes in potato tubers after wounding. Proceedings of the National Academy of Sciences 85: 1136-1140.

Lohrman, J., Sweere, U., Zabaleta, E., Bäurle, I., Keitel, C., Kozma-Bognar, L., Brennicke, A., Schäfer, E., Kudla, J. and Harter, K., 2001. The response regulator ARR2: a pollen-specific transcription factor involved in the expression of nuclear genes for components of mitochondrial Complex I in Arabidopsis. Molecular Genetics and Genomics 265: 2-13.

Loqué, D. and Von Wirén, N., 2004. Regulatory levels for the transport of ammonium in plant roots. Journal of Experimental Botany 55 (401): 1293-1305.

Ludewig, U., Neuhäuser, B. and Dynowski, M., 2007. Molecular mechanisms of ammonium transport and accumulation in plants. FEBS Letters 581: 2301–2308.

Ma, T., Ma, H., Zhao, H., Qi, H. and Zhao, J., 2014. Identification, characterization, and transcription analysis of xylogen-like arabinogalactan proteins in rice (*Oryza sativa* L.). BMC Plant Biology 14 (299): http://www.biomedcentral.com/1471-2229/14/299

Manaa, A., Mimouni, H., Wasti, S., Gharbi, E., Terras, A. and Ahmed, H.B., 2014. Characterization of transgenic Arabidopsis and tomato plants antisensed for the ethylene receptor gene CcEIN4 under NaCl stress. Journal of Plant Interactions 9 (1): 539–549.

Manck-Götzenberger, J. and Requena, N., 2016. Arbuscular mycorrhiza symbiosis induces a major transcriptional reprogramming of the potato SWEET sugar transporter family. Frontiers in Plant Science 7 (487): doi: 10.3389/fpls.2016.00487

Makino, S., Kiba, T., Imamura, A., Hanaki, N., Nakamura, A., Suzuki, T., Taniguchi, M., Ueguchi, C., Sugiyama, T. and Mizuno, T., 2000. Genes encoding pseudo-response regulators: insight into His-to-Asp phosphorelay and circadian rhythm in *Arabidopsis thaliana*. Plant and Cell Physiology 41 (6): 791-803.

Makino, S., Matsushika, A., Kojima, M., Oda, Y., Mizuno, T., 2001. Light response of the circadian waves of the APRR1/TOC1 quintet: when does the quintet start singing rhythmically in Arabidopsis? Plant and Cell Physiology 42 (3): 334–339.

Mason, M.G., Jha, D., Salt, D.E., Tester, M., Hill, K., Kiever. J.J., and Schaller, G.E., 2010. Type-B response regulators ARR1 and ARR12 regulate expression of AtHKT1;1 and accumulation of sodium in Arabidopsis shoots. The Plant Journal 64: 753–763.

Mason, M.G., Li, J., Mathews, D.E., Kieber, J.J. and Schaller, G.E., 2004. Type-B response regulators display overlapping expression patterns in Arabidopsis. Plant Physiology 135: 927–937.

Mason, M.G., Mathews, D.E., Argyros, D.A., Maxwell, B.B., Kieber, J.J., Alonso, J.M., Ecker, J.R., and Schaller, G.E., 2005. Multiple type-B response regulators mediate cytokinin signal transduction in Arabidopsis. The Plant Cell 17: 3007–3018.

Matsushika, A., Makino, S., Kojima, M. and Mizuno, T., 2000. Circadian waves of expression of the APRR1/TOC1 family of pseudo-response regulators in *Arabidopsis thaliana*: insight into the plant circadian clock. Plant and Cell Physiology 41 (9): 1002–1012.

Matt, P., Geiger, M., Walch-Liu, P., Engels, C. and Stitt, M., 2001. The immediate cause of the diurnal changes of nitrogen metabolism in leaves of nitrate-replete tobacco: a major imbalance between the rate of nitrate reduction and the rates of nitrate uptake and ammonium metabolism during the first part of the light period. Plant, Cell and Environment 24, 177–190.

Melo-Oliveira, R., Oliveira, I.C. and Coruzzi, G.M., 1996. Arabidopsis mutant analysis and gene regulation define a nonredundant role for glutamate dehydrogenase in nitrogen assimilation. Proceedings of the National Academy of Sciences 93: 4718-4723.

Melville, J.C. and Ryan, C.A., 1972. Chymotrypsin Inhibitor I from Potatoes; large scale preparation and characterization of its subunit components. The Journal of Biological Chemistry 247 (11) 3445-3453.

Mignery, G.A., Pikaard, C.S., Hannapel, D.J. and Park, W.D., 1984. Isolation and sequence analysis of cDNAs for the major potato protein, patatin. Nucleic Acids Research 12: 7987-8000.

Mignery, A., Pikaard, C.S. and Park, W.D., 1988. Molecular characterization of the patatin multigene family of potato. Gene 62: 27-44.

Motose, H., Sugiyama, M. and Fukuda, H., 2004. A proteoglycan mediates inductive interaction during plant vascular development. Nature 429: 873-878.

Moubayidin, L., Perilli, S., Dello loio, R., Di Mambro, R., Costantino, P. and Sabatini, S., 2010. The rate of cell differentiation controls the Arabidopsis root meristem growth phase. Current Biology 20: 1138–1143.

Moussatche, P., 2004. The ethylene receptor multigene family: insights on expression, localization and function in Arabidopsis and tomato. PhD thesis University of Florida. 136 pp.

Moussatche, P. and Klee, H.J., 2004. Autophosphorylation activity of the Arabidopsis ethylene receptor multigene family. The Journal of Biological Chemistry 279 (47): 48734-48741.

Mucharromah, H., Burton, R. and Kuć, J., 1995. The effect of sterols on phytoalexin, steroid glycoalkaloid, and sterol accumulation in potato tuber discs inoculated with *Phytophthora infestans* or treated with arachidonic acid. Physiological and Molecular Plant Pathology 47: 13-27.

Murakami-Kojima, M., Nakamichi, N., Yamashino, T. and Mizuno, T., 2002. The APRR3 component of the clock-associated APRR1/TOC1 quintet is phosphorylated by a novel protein kinase belonging to the WNK family, the gene for which is also transcribed rhythmically in *Arabidopsis thaliana*. Plant and Cell Physiology 43 (6): 675-683.

Murakami, M., Tago, Y., Yamashino, T. and Mizuno, T., 2007. Characterization of the rice circadian clock-associated pseudo-response regulators in *Arabidopsis thaliana*. Bioscience, Biotechnology, and Biochemistry 71 (4): 1107-1110

Murakami, M., Ashikari, M., Miura, K., Yamashino, T. and Mizuno, T., 2003. The evolutionarily conserved OsPRR quintet: rice pseudo-response regulators implicated in circadian rhythm. Plant and Cell Physiology 44 (11): 1229–1236.

Nakamichi, N., Kiba, T., Henriques, R., Mizuno. T., Chua, N.H. and Sakakibara, H., 2010. Pseudo-response regulators 9, 7, and 5 are transcriptional repressors in the Arabidopsis circadian clock. The Plant Cell 22: 594–605.

Nakamichi, N., Kiba, T., Kamioka, T., Suzuki, T., Yamashino, T., Higashiyama, T., Sakakibara, H. and Mizuno, T., 2012. Transcriptional repressor PRR5 directly regulates clock-output pathways. PNAS 109 (42): 17123–17128.

Nakamichi, N., Kita, M., Ito, S., Yamashino, T. and Mizuno, T., 2005. Pseudo-response regulators, PRR9, PRR7 and PRR5, together play essential roles close to the circadian clock of *Arabidopsis thaliana*. Plant and Cell Physiology 46 (5): 686–698.

Nakamichi, N., Kusano, M., Fukushima, A., Kita, M., Ito, S., Yamashino, T., Saito, K., Sakakibara, H. and Mizuno, T., 2009. Transcript profiling of an Arabidopsis pseudo response regulator arrhythmic triple mutant reveals a role for the circadian clock in cold stress response. Plant and Cell Physiology 50 (3): 447–462.

Navarro, C., Abelenda, J.A., Cruz-Oró, E., Cuéllar, C.A., Tamaki, S., Silva, J., Shimamoto, K. and Prat, S., 2011. Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Nature 478: 119-122.

Nishii, A., Takemura, M., Fujita, H., Shikata, M., Yokota, A. and Kohchi, T., 2000. Characterization of a novel gene encoding a putative single zinc-finger protein, ZIM, expressed during the reproductive phase in *Arabidopsis thaliana*. Bioscience, Biotechnology, and Biochemistry 64 (7): 1402-1409.

O'Donnell, P.J., Schmelz, E.A., Moussatche, P., Lunda, S.T., Jones, J.B. and Klee, H.J., 2003. Susceptible to intolerance – a range of hormonal actions in a susceptible *Arabidopsis* pathogen response. The Plant Journal 33: 245–257.

O'Malley, R.C., Rodriguez, F.I., Esch, J.J., Binder, B.M., O'Donnell, P., Klee, H.J. and Bleecker, A.B., 2005. Ethylene-binding activity, gene expression levels, and receptor system output for ethylene receptor family members from Arabidopsis and tomato. The Plant Journal 41: 651-659.

Outchkourov, N.S., Kogel, W.J. de, Schuurman-de Bruin, A., Abrahamson, M. and Jongsma, M.A., 2004. Specific cysteine protease inhibitors act as deterrents of western flower thrips, Frankliniella occidentalis (Pergande), in transgenic potato. Plant Biotechnology Journal 2: 439–448.

Paiva, E., Lister, R.M. and Park, W.D., 1983. Induction and accumulation of major tuber proteins of potato in stems and petioles. Plant Physiology 71: 161-168.

Pandiyan, M.J., 2010. A bioinformatics approach to investigate the function of non specific lipid transfer proteins in *Arabidopsis thaliana*. Msc thesis Linköping University. 24 pp.

Para, A., Farré, E.M., Imaizumi, T., Pruneda-Paz, J.L., Harmon, F.G. and Kay, S.A., 2007. PRR3 is a vascular regulator of TOC1 stability in the Arabidopsis circadian clock. The Plant Cell 19: 3462–3473.

Pareek, A., Singh, A., Kumar, M., Kushwaha, H.R., Lynn, A.M. and Singla-Pareek, S.L., 2006. Whole-genome analysis of *Oryza sativa* reveals similar architecture of two-component signaling machinery with Arabidopsis. Plant Physiology 142: 380-397.

Peña-Cortés, H., Fisahn, J. and Willmitzer, L., 1995. Signals involved in wound-induced proteinase inhibitor II gene expression in tomato and potato plants. Proceedings of the National Academy of Sciences 92: 4106-4113.

Peña-Cortés, H., Sánchez-Serrano, J.J., Mertens, R., Willmitzer, L. and Prat, S., 1989. Abscisic acid is involved in the wound-induced expression of the proteinase inhibitor II gene in potato and tomato. Proceedings of the National Academy of Sciences 86: 9851-9855.

Peña-Cortés, H., Willmitzer, L. and Sánchez-Serrano, J.J., 1991. Abscisic acid mediates wound induction but not developmental-specific expression of the proteinase inhibitor II gene family The Plant Cell 3: 963-972.

Peyer, C., Bonay, P. and Staudacher, E., 2004. Purification and characterization of a β-xylosidase from potatoes (*Solanum tuberosum*). Biochimica et Biophysica Acta 1672: 27-35.

Plessis, A., Ravel, C., Bordes, J., Balfourier, F. and Martre, P., 2013. Association study of wheat grain protein composition reveals that gliadin and glutenin composition are trans-regulated by different chromosome regions. Journal of Experimental Botany 64 (12): 3627–3644.

Pots, A.M., Gruppen, H., Hessing, M., Boekel, M.A.J.S. van and Voragen, A.G.J., 1999. Isolation and characterization of patatin isoforms. Journal of Agricultural and Food Chemistry 47: 4587-4592.

Pouvreau, L., 2004. Occurrence and physico-chemical properties of protease inhibitors from potato tuber (*Solanum tuberosum*). PhD thesis Wageningen UR. 157 pp.

Pouvreau, L., Gruppen, H., Koningsveld, G.A. van, Broek, L.A.M. van and Voragen, A.G.J., 2003. The most abundant protease inhibitor in potato tuber (cv. Elkana) is a serine protease inhibitor from the Kunitz family. Journal of Agricultural and Food Chemistry 51: 5001-5005.

Pouvreau, L., Gruppen, H., Piersma, S.R., Broek, L.A.M. van, Koningsveld, G.A. van, Voragen, A.G.J., 2001. Relative abundance and inhibitory distribution of protease inhibitors in potato juice from cv. *Elkana*. Journal of Agricultural and Food Chemistry 49: 2864-2874.

Provart, N.J., Gil, P., Chen, W., Han, B., Chang, H.S., Wang, X. and Zhu, T., 2003. Gene expression phenotypes of Arabidopsis associated with sensitivity to low temperatures. Plant Physiology 132: 893-906.

Puzina, T.I., 2004. Effect of zinc sulfate and boric acid on the hormonal status of potato plants in relation to tuberization. Russian Journal of Plant Physiology 51 (2): 209–214.

Racusen, D., 1983. Occurrence of patatin during growth and storage of potato tuber. Canadian Journal of Botany 61: 370-373.

Racusen, D., 1984. Lipid acyl hydrolase of patatin. Canadian Journal of Botany 62: 1640- 1644.

Racusen, D., 1986. Esterase specificity of patatin from two potato cultivars. Canadian Journal of Botany 64: 2104-2106.

Ramadan, M.F. and Elsanhoty, R.M., 2012. Lipid classes, fatty acids and bioactive lipids of genetically modified potato Spunta with Cry V gene. Food Chemistry 133: 1169-1176.

Raspor, M., Motyka, V., Žižková, E., Dobrev, P.I., Trávníčková, T., Zdravković-Korać, S., Simonović, A., Ninković, S., Dragićevic, I.C., 2012. Cytokinin profiles of AtCKX2-overexpressing potato plants and the impact of altered cytokinin homeostasis on tuberization in vitro. Journal of Plant Growth Regulation 31: 460–470.

Rausch, T. and Wachter, A., 2005. Sulfur metabolism: a versatile platform for launching defence operations. TRENDS in Plant Science 10 (10): 503-509.

Reyes, J.C., Muro-Pastor, M.I. and Florencio, F.J., 2004. The GATA family of transcription factors in Arabidopsis and rice. Plant Physiology 134: 1718-1732.

Rocha-Sosa, M., Sonnewald, U., Frommer, W., Stratmann, M., Schell, J. and Willmitzer, L., 1989. Both developmental and metabolic signals activate the promoter of a class I patatin gene. The EMBO Journal 8 (1): 23-29.

Rosahl, S., Eckes, P., Schell, J. and Willmitzer, L., 1986. Organ-specific gene expression in potato: isolation and characterization of tuber-specific cDNA sequences. Molecular Genetics and Genomics 202: 368-373.

Rosahl, S., Schell, J. and Willmitzer, L., 1987. Expression of a tuber-specific storage protein in transgenic tobacco plants: demonstration of an esterase activity. The EMBO Journal 6 (5): 1155- 1159.

Ross, H.A., Davies, H.V., Burch, L.R., Viola, R. and McRae, D., 1994. Developmental changes in carbohydrate content and sucrose degrading enzymes in tuberising stolons of potato (*Solanum tuberosum*). Physiologia Plantarum 90: 748-756.

Sakai, H., Aoyama, T., Bono, H. and Oka, A., 1998b. Two-component response regulators from *Arabidopsis thaliana* contain a putative DNA-binding motif. Plant and Cell Physiology 39 (11): 1232-1239.

Sakai, H., Aoyama, T. and Oka, A., 2000. Arabidopsis ARR1 and ARR2 response regulators operate as transcriptional activators. The Plant Journal 24 (6): 703-711.

Sakai, H., Honma, T., Aoyama, T., Sato, S., Kato, T., Tabata, S. and Oka, A., 2001. ARR1, a transcription factor for genes immediately Responsive to cytokinins. Science 294: 1519-1521.

Sakai, H., Hua, J., Chen, Q.G., Chang, C., Medrano, L.J., Bleecker, A.B., Meyerowitz, E.M., 1998a. ETR2 is an ETR1-like gene involved in ethylene signaling in Arabidopsis. Proceedings of the National Academy of Sciences 95: 5812–5817.

Salomé, P.A. and McClung, C.R., 2005. Pseudo-response regulator 7 and 9 are partially redundant genes essential for the temperature responsiveness of the Arabidopsis circadian clock. The Plant Cell 17: 791–803.

Sanchez, S.E. and Kay, S.A., 2016. The plant circadian clock: from a simple timekeeper to a complex developmental manager. Cold Spring Harbor Perspectives in Biology: doi: 10.1101/cshperspect.a027748

Sato, E., Nakamichi, N., Yamashino, T. and Mizuno, T., 2002. Aberrant expression of the Arabidopsis circadian-regulated APRR5 gene belonging to the APRR1/TOC1 quintet results in early flowering and hypersensitiveness to light in early photomorphogenesis. Plant and Cell Physiology 43 (11): 1374–1385.

Schaller, G.E., Doi, K., Hwang, I., Kieber, J.J., Khurana, J.P., Kurata, N., Mizuno, T., Pareek, A., Shiu, S.H., Wu, P. and Yip, W.K., 2007. Nomenclature for Two-Component Signaling Elements of Rice. Plant Physiology 143: 555–557.

Schaller, G.E., Shiu, S.H. and Armitage, J.P., 2011. Two-component systems and their co-option review for eukaryotic signal transduction. Current Biology 21: 320–330.

Senda, K., Yoshioka, H., Doke, N. and Kawakita, K., 1996. A cytosolic phospholipase A2 from potato tissues appears to be patatin. Plant and Cell Physiology 37(3): 347-353.

Seo, P.J., Park, J.M., Kang, S.K., Kim, S.G. and Park, C.M., 2011. An Arabidopsis senescence-associated protein SAG29 regulates cell viability under high salinity. Planta 233: 189-200.

Shi, Y., Tian, S., Hou, L., Huang, X., Zhang, X., Guo, H. and Yang, S., 2012. Ethylene signaling negatively regulates freezing tolerance by repressing expression of CBF and type-A ARR genes in *Arabidopsis*. The Plant Cell 24: 2578–2595.

Shikata, M., Takemura, M., Yokota, A. and Kohchi, T., 2003. Arabidopsis ZIM, a plant-specific GATA factor, can function as a transcriptional activator. Bioscience, Biotechnology, and Biochemistry 67 (11): 2495-2497.

Shikata, M., Matsuda, Y., Ando, K., Nishii, A., Takemura, M., Yokota, A. and Kohchi, T., 2004. Characterization of Arabidopsis ZIM, a novel plant-specific GATA factor gene family. Journal of Experimental Botany 55 (397): 631-639.

Singh, A., Siddappa, S., Bhardway, V., Singh, B., Kumar, D. and Singh, B.P., 2015. Expression profiling of potato cultivars with contrasting tuberization at elevated temperature using microarray analysis. Plant Physiology and Biochemistry 97: 108-116.

Sirhindi, G., Sharma, P., Arya, P., Goel, P., Kumar, G., Acharya, V. and Singh, A.K., 2016. Genome-wide characterization and expression profiling of TIFY gene family in pigeonpea (Cajanus cajan (L.) Millsp.) under copper stress. Journal of Plant Biochemistry and Biotechnology 25 (3): 301-310.

Sonah, H., O'Donoughue, L., Cober, E., Rajcan, I. and Belzile, F., 2015. Identification of loci governing eight agronomic traits using a GBS-GWAS approach and validation by QTL mapping in soya bean. Plant Biotechnology Journal 13: 211-221.

Sonnewald, U., Studer, D., Rocha-Sosa, M. Willmitzer, L., 1989a. Immunocytochemical localization of patatin, the major glycoprotein in potato (*Solanum tuberosum* L.) tubers. Planta 178: 176-183.

Sonnewald, U., Sturm, A., Chrispeels, M.J. and Willmitzer, L., 1989b. Targeting and glycosylation of patatin the major potato tuber protein in leaves of transgenic tobacco. Planta 179: 171 180.

SoyBase, visited may 10, 2017. https://soybase.org/

Stewart, G.R., Shatilov, V.R., Turnbull, M.H., Robinson, S.A. and Goodall, R., 1995. Evidence that glutamate dehydrogenase plays a role in the oxidative deamination of glutamate in seedlings of *Zea mays*. Australian Journal of Plant Physiology 22: 805-809.

Stiekema, W.J., Heidekamp, F., Dirkse, W.G., Beckum, J. van, Haan, P. de, Bosch, C. ten and Louwerse, J.D., 1988. Molecular cloning and analysis of four potato tuber mRNAs. Plant Molecular Biology 11: 255-269.

Stock, A.M., Robinson, V.L. and Goudreau, P.N., 2000. Two-component signal transduction. Annual Review of Biochemistry 69:183–215.

Strickland, J.A., Orr, C. and Walsh, T.A., 1995. Inhibition of *Diabrotica* larval growth by patatin, the lipid acyl hydrolase from potato tubers. Plant Physiology 109: 667-674.

Suh, S.G., Peterson, J.E., Stiekema, W.J. and Hannapel, D.J., 1990. Purification and characterization of the 22-kilodalton potato tuber proteins. Plant Physiology 94: 40-45.

Suh, S.G., Stiekema, W.J. and Hannapel, D.J., 1991. Proteinase-inhibitor activity and wound-inducible gene expression of the 22-kDa potato-tuber proteins. Planta 184: 423-430.

Taghizadeh, M. and Ehsanpour, A., 2013. The *in vitro* effects of $CoCl_2$ as ethylene synthesis inhibitor on PI based protein pattern of potato plant (*Solanum tuberosum* L.). Journal of Cell and Molecular Research 5 (1): 42-46.

Takahashi, H., Asanuma, W. and Saito, K., 1999. Cloning of an Arabidopsis cDNA encoding a chloroplast localizing sulphate transporter isoform. Journal of Experimental Botany 50 (340): 1713–1714.

Takahashi, H., Buchner, P., Yoshimoto, N., Hawkesford, M.J. and Shiu, S.H., 2012. Evolutionary relationships and functional diversity of plant sulfate transporters. Frontiers in Plant Science 2 (119): doi: 10.3389/fpls.2011.00119

Takahashi, H., Watanabe-Takahashi, A., Smith, F.W., Blake-Kalff, M., Hawkesford, M.J. and Saito, K., 2000. The roles of three functional sulphate transporters involved in uptake and translocation of sulphate in *Arabidopsis thaliana*. The Plant Journal 23 (2): 171-182.

Takase, M., Mizoguchi, T., Kozuka, T. and Tsukaya, H., 2013. The unique function of the Arabidopsis circadian clock gene PRR5 in the regulation of shade avoidance response. Plant Signaling & Behavior, 8 (4): doi: 10.4161/psb.23534

The Potato Genome Sequencing Consortium, 2011. Genome sequence and analysis of the tuber crop potato. Nature 475: 189-197.

Tieman, D.M. and Klee, H.J., 1999. Differential expression of two novel members of the tomato ethylene-receptor family. Plant Physiology 120: 165–172.

Timmermans, M.M.A., 2016. A novel crosstalk between Flowering Locus T signalling and sugar transport in *Solanum tuberosum*. The interaction between SWEET transporters and the FT homolog StSP6A during tuber initiation. MSc thesis Wageningen UR. 41 pp.

Tonón, C., Daleo, G. and Oliva, C., 2001. An acidic β-1,3 glucanase from potato tubers appears to be patatin. Plant Physiology and Biochemistry 39: 849−854.

Trinidad Ascencio-Ibáñez, J., Sozzani, R., Lee, T.J., Chu, T.M., Wolfinger, R.D., Cella, R. and Hanley-Bowdoin, L., 2008. Global analysis of Arabidopsis gene expression uncovers a complex array of changes impacting pathogen response and cell cycle during geminivirus infection. Plant Physiology 148: 436-454.

Tsai, Y.C., Weir, N.R., Hill, K., Zhang, W., Kim, H.J., Shiu, S.H., Schaller, G.E. and Kieber, J.J., 2012. Characterization of genes involved in cytokinin signaling and metabolism from rice. Plant Physiology 158: 1666–1684.

Twell, D. and Ooms, G., 1988. Structural diversity of the patatin gene family in potato cultivar Desiree. Molecular and General Genetics 212: 325-336.

UniProt, visited may 10, 2017. http://www.uniprot.org/

Valueva, T.A., Revina, T.A., Gvozdeva, E.L., Gerasimova, N.G. and Ozertskovskaya, O.L.,2003. Role of protease inhibitors in potato protection. Russian Journal of Bioorganic Chemistry 29 (5): 454-458.

Valueva, T.A., Speranskaya, A.S., Revina, T.A. and Shevelev, A.B., 2008. Molecular cloning and expression of genes of Kunitz–type C protease inhibitors from potato. Russian Journal of Bioorganic Chemistry 34 (3): 310–317.

Vanholme, B., Grunewald, W., Bateman, A., Kohchi, T. and Gheysen, G., 2007. The tify family previously known as ZIM. Trends in Plant Science 12 (6): 239-244.

Vatansever, R., Koc, I., Ozyigit, I.I., Sen, U., Uras, M.E., Anjum, N.A., Pereira, E. and Filiz, E., 2016. Genome-wide identification and expression analysis of sulfate transporter (SULTR) genes in potato (*Solanum tuberosum* L.). Planta 244: 1167–1183.

Vaughn, J.N., Nelson, R.L., Song, Q., Cregan, P.B. and Li, Z., 2014. The genetic architecture of seed composition in soybean is refined by genome-wide association scans across multiple populations. Genes, Genomes, Genetics 4: 2283-2294.

Vélez-Bermúdez, I.C., Salazar-Henao, J.E., Fornalé, S., López-vedriero, I., Franco-Zorrilla, J.M., Grotewold, E., Gray, J., Solano, R., Schmidt, W., Pagés, M., Riera, M. Caparros-Ruiz, D., 2015. A MYB/ZML Complex Regulates Wound-Induced Lignin Genes in Maize. The Plant Cell 27: 3245–3259.

Von Wirén, N., Gazzarrini, S., Gojon, A., and Frommer, W.B., 2000b. The molecular physiology of ammonium uptake and retrieval. Current Opinion in Plant Biology 3: 254-261.

Von Wirén, N., Lauter, F.R., Ninnemann, O., Gillissen, B., Walch-Liu, P., Engels, C., Jost, W. and Frommer, W.B., 2000a. Differential regulation of three functional ammonium transporter genes by nitrogen in roots hairs and light in leaves of tomato. The Plant Journal 21 (2): 167-175.

Waglay, A., Karboune, S. and Alli, I., 2014. Potato protein isolates: recovery and characterization of their properties. Food Chemistry 142: 373-382.

Waldron, C., Wegrich, L.M., P.A. Owens Merlo and Walsh, T.A., 1993. Characterization of a genomic sequence coding for potato multicystatin, an eight-domain cysteine proteinase inhibitor. Plant Molecular Biology 23: 801-812.

Watanabe, H., Saigusa, M., Hase, S., Hayakawa, T. and Satoh, S., 2004. Cloning of a cDNA encoding an ETR2-like protein (Os-ERL1) from deep water rice (*Oryza sativa* L.) and increase in its mRNA level by submergence, ethylene, and gibberellin treatments. Journal of Experimental Botany 55 (399): 1145-1148.

Weeda, S.M., Kumar, G.N.M. and Knowles, N.R., 2009. Developmentally linked changes in proteases and protease inhibitors suggest a role for potato multicystatin in regulating protein content of potato tubers. Planta 230: 73–84.

Wegener, C.B., Jansen, G. and Jürgens, H.U., 2015. Bioactive compounds in potatoes: Accumulation under drought stress conditions. Functional Foods in Health and Disease 5 (3): 108-116.

Wegener, C.B., Jansen, G. and Jürgens, H.U., 2014. Influence of drought and wounding stress on soluble phenols and proteins in potato tubers. Sustainable Agriculture Research 3 (3).

Wei, W., Zhang, Y., Lü, H., Li, D., Wang, L. and Zhang, X., 2013. Association analysis for quality traits in a diverse panel of Chinese sesame (*Sesamum indicum* L.) germplasm. Journal of Integrative Plant Biology 55 (8): 745–758.

Wenzler, H.C., Mignery, G.A., Fisher, L.M. and Park, W.D., 1989. Analysis of a chimeric class-I patatin-GUS gene in transgenic potato plants: High-level expression in tubers and sucrose-inducible expression in cultured leaf and stem explants. Plant Molecular Biology 12: 41-50.

West, A.H. and Stock, A.M., 2001. Histidine kinases and response regulator proteins in two-component signaling systems. TRENDS in Biochemical Sciences 26 (6): 369-376.

Wilson, R.L., 2014. Unique functions of the ethylene receptors in seed germination. PhD thesis University of Tennessee. 115 pp.

Wu, B., 2004. Oligomerization of ammonium transporter LeAMT1;1 and its interactions with other proteins. PhD Thesis Eberhard-Karls-Universität Tübingen. 74 pp.

Wuriyanghan, H., Zhang, B., Cao, W.H., Ma, B., Lei, G., Liu, Y.F., Wei, W., Wu, H.J., Chen, L.J., Chen, H.W., Cao, Y.R., He, S.J., Zhang, W.K., Wang, X.J., Chen, S.Y. and Zhang, J.S., 2009. The Ethylene Receptor ETR2 Delays Floral Transition and Affects Starch Accumulation in Rice. The Plant Cell 21: 1473-1494.

Xie, C., Zhang, Z.G., Zhang, J.S., He, X.J., Cao, W.H., He, S.J. and Chen, S.Y., 2002. Spatial expression and characterization of a putative ethylene receptor protein NTHK1 in tobacco. Plant and Cell Physiology 43 (7): 810-815.

Xu, X., Lammeren, A.A.M., Vermeer, E. and Vreugdenhil, D., 1998a. The role of gibberellin, abscisic acid, and sucrose in the regulation of potato tuber formation in vitro. Plant Physiology 117: 575–584.

Xu, X., Vreugdenhil, D. and Lammeren, A.A.M. van, 1998b. Cell division and cell enlargement during potato tuber formation. Journal of Experimental Botany 49 (320): 573-582.

Yamaguchi-Shinozaki, K. and Shinozaki, K., 2006. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annual Review of Plant Biology 57: 781–803.

Yau, C.P., Wang, L., Yu, M., Zee, S.Y. and Yip, W.K., 2004. Differential expression of three genes encoding an ethylene receptor in rice during development, and in response to indole-3-acetic acid and silver ions. Journal of Experimental Botany 55 (397) 547-556.

Ye, H., Du, H., Tang, N., Li, X. and Xiong, L., 2009. Identification and expression profiling analysis of TIFY family genes involved in stress and phytohormone responses in rice. Plant Molecular Biology 71: 291-305.

Yokoyama, A., Yamashino, T., Amano, Y.I., Tajima, Y., Imamura, A., Sakakibara, H. and Mizuno, T., 2007. Type-B ARR transcription factors, ARR10 and ARR12, are implicated in cytokinin-mediated regulation of protoxylem differentiation in roots of *Arabidopsis thaliana*. Plant and Cell Physiology 48 (1): 84–96.

Zhang, Y., Gao, M., Singer, S.D., Fei, Z., Wang, H. and Wang, X., 2012. Genome-wide identification and analysis of the TIFY gene family in grape. PLoS ONE 7(9): doi: 10.1371/journal.pone.0044465

Zhang, D., Kan, G., Hu, Z., Cheng, H., Zhang, Y., Wang, Q., Wang, H., Yang, Y., Li, H., Hao, D and Yu, D., 2014. Use of single nucleotide polymorphisms and haplotypes to identify genomic regions associated with protein content and water-soluble protein content in soybean. Theoretical and Applied Genetics 127: 1905-1915.

Zhang, Z., Li, X., Yu, R., Han, M. and Wu, Z., 2015a. Isolation, structural analysis, and expression characteristics of the maize TIFY gene family. Molecular genetics and genomics 290:1849–1858.

Zhang, H. and Wang, S., 2013. Rice versus *Xanthomonas oryzae pv. oryzae*: a unique pathosystem. Current Opinion in Plant Biology 16: 188-195.

Zhang, J.S., Xie, C., Shen, Y.G. and Chen, S.Y., 2001. A two-component gene (NTHK1) encoding a putative ethylene-receptor homolog is both developmentally and stress regulated in tobacco. Theoretical and Applied Genetics 102: 815–824.

Zhang, L., You, J. and Chan, Z., 2015b. Identification and characterization of TIFY family genes in Brachypodium distachyon. Journal of Plant Research 128: 995–1005.

Zhang, Z.G., Zhou, H.L., Chen, T., Gong, Y., Cao, W.H., Wang, Y.J., Zhang, J.S. and Chen, S.Y., 2004. Evidence for serine/threonine and histidine kinase activity in the tobacco ethylene receptor protein NTHK2. Plant Physiology 136: 2971–2981.

Zhao, C., Craig, J.C., Petzold, H.E., Dickerman, A.W. and Beers, E.P., 2005. The xylem and phloem transcriptomes from secondary tissues of the Arabidopsis root-hypocotyl. Plant Physiology 138: 803-818.

Zhu, D., Bai, X., Luo, X., Chen, Q., Cai, H., Ji, W. and Zhu., Y., 2013. Identification of wild soybean (Glycine soja) TIFY family genes and their expression profiling analysis under bicarbonate stress. Plant Cell Reports 32:263–272.

Zuber, H., Davidian, J.C., Aubert, G., Aimé, D., Belghazi, M., Lugan, R., Heintz, D., Wirtz, M., Hell, R., Thompson, R. and Gallardo,K., 2010b. The seed composition of Arabidopsis mutants for the group 3 sulfate transporters indicates a role in sulfate translocation within developing seeds. Plant Physiology 154: 913-926.

Zuber, H., Davidian, J.C., Wirtz, M., Hell, R., Belghazi, M., Thompson, R. and Gallardo, K., 2010a. Sultr4;1 mutant seeds of Arabidopsis have an enhanced sulphate content and modified proteome suggesting metabolic adaptations to altered sulphate compartmentalization. BMC Plant Biology 10 (78): doi: 10.1186/1471-2229-10-78

Appendix I. QTLs soybean

Table 5. The QTLs for soybean based on Bandillo et al. (2015). Interval sizes for chromosome 15 and 20 were already determined by these authors. For chromosome 6 and 13 the QTL interval was determined on two Mbp upstream and downstream the markers.

QTL	Chromosome	Position marker (bp)	Start (bp)	End (bp)	Length
1	6	5.591.484	3.591.484	7.660.542	4.069.058
	6	5.660.542			
2	6	46.040.638	44.040.638	48.040.638	4.000.000
3	13	24.858.209	22.858.209	26.858.209	4.000.000
4	15	3.828.587	3.820.000	3.960.000	140.000
	15	3.833.574			
	15	3.918.803			
	15	3.919.945			
	15	3.967.324			
5	20	29.594.697	29.060.000	30.040.000	980.000
	20	29.983.050			
6	20	30.930.931	30.380.000	30.930.000	550.000
7	20	31.150.279	31.150.000	32.050.000	900.000
	20	31.243.150			
	20	31.436.069			
	20	31.580.769			
	20	31.610.452			
	20	31.640.038			
	20	31.687.470			
	20	31.972.955			

Appendix II. QTLs rice

Table 6. The QTLs for rice based on Huang et al. (2012). The QTL interval was determined on two Mbp upstream and downstream the markers. If the distance between two markers was less than one Mbp, it was considered to be one QTL. All QTLs, except no. 5, were not found in the indica, neither the japonica population, but only in the 'total' population and are therefore written in italic. See paragraph 3.1 for more details.

QTL	Chromosome	Position marker (bp)	Start (bp)	End (bp)
Indica				
1	*6*	*24.746.851*	*22.746.851*	*26.746.851*
2	*7*	*23.557.460*	*21.557.460*	*25.557.460*
3	*11*	*4.343.017*	*2.343.017*	*6.343.017*
Japonica				
4	*6*	*24.746.851*	*22.746.851*	*26.746.851*
5	7	23.614.414	21.614.414	25.614.414
6	*11*	*4.343.017*	*2.343.017*	*6.343.017*

Appendix III. QTLs potato

Table 7. The QTLs for potato based on Peter Vos (unpublished results). The QTL interval was determined on two Mbp upstream and downstream the markers. If the distance between two markers was less than one Mbp, it was considered to be one QTL.

QTL	Chromosome	Marker	Position marker (bp)	Start (bp)	End (bp)
1	1	PotVar0050066	80.162.603	78.162.603	83.638.789
	1	PotVar0050119	80.467.877		
	1	PotVar0050307	80.597.868		
	1	PotVar0050332	80.598.351		
	1	PotVar0050467	80.683.169		
	1	PotVar0050677	80.940.835		
	1	PotVar0061070	81.638.789		
2	1	PotVar0035163	83.422.333	81.422.333	85.422.333
3	3	solcap_snp_c2_5292	8.263.226	6.263.226	10.263.418
	3	solcap_snp_c2_5289	8.263.418		
4	3	solcap_snp_c2_45699	43.326.576	41.326.576	45.929.298
	3	solcap_snp_c2_45702	43.326.959		
	3	solcap_snp_c2_45703	43.326.982		
	3	solcap_snp_c1_13506	43.929.298		
5	3	PotVar0042905	46.459.725	44.459.725	48.459.725
6	5	PotVar0026091	4.249.963	2.249.963	8.354.190
	5	PotVar0026113	4.250.232		
	5	PotVar0078022	4.406.638		
	5	PotVar0078025	4.406.720		
	5	PotVar0078111	4.409.568		
	5	PotVar0078229	4.411.283		
	5	PotVar0078469	4.418.715		
	5	PotVar0078670	4.432.880		
	5	PotVar0078972	4.447.319		
	5	PotVar0079081	4.489.481		
	5	PotVar0079124	4.490.397		
	5	PotVar0079737	4.550.107		
	5	PotVar0080027	4.709.697		
	5	PotVar0080320	4.724.800		
	5	PotVar0080800	4.790.807		
	5	PotVar0129937	4.921.097		
	5	PotVar0116903	5.363.863		
	5	PotVar0117280	5.691.161		
	5	PotVar0117324	5.691.686		
	5	PotVar0117367	5.693.006		
	5	solcap_snp_c2_47284	6.354.190		
7	5	solcap_snp_c1_1126	51.697.315	49.697.315	53.893.583
	5	PotVar0034407	51.893.583		
8	7	PotVar0069893	40.611.241	38.611.241	43.675.865
	7	PotVar0092426	41.675.865		
9	7	PotVar0119736	45.783.394	43.783.394	47.783.394
10	7	PotVar0133614	47.103.671	45.103.671	49.103.671

Appendix IV. QTLs maize

Table 8. The QTLs for maize based on Cook et al. (2012). The QTL interval was determined on two Mbp upstream and downstream the markers.

QTL	Chromosome	Position marker (bp)	Start (bp)	End (bp)
1	1	41.569.344	39.569.344	43.569.344
2	1	214.607.570	212.607.570	216.607.570
3	1	233.597.309	231.597.309	235.597.309
4	1	264.586.209	262.586.209	266.586.209
5	2	6.003.320	4.003.320	8.003.320
6	2	18.286.061	16.286.061	20.286.061
7	2	184.529.536	182.529.536	186.529.536
8	2	234.059.444	232.059.444	236.059.444
9	3	107.736.175	105.736.175	109.736.175
10	3	225.917.080	223.917.080	227.917.080
11	4	152.317.836	150.317.836	154.317.836
12	4	175.740.882	173.740.882	177.740.882
13	5	88.027.712	86.027.712	90.027.712
14	5	197.933.894	195.933.894	199.933.894
15	6	73.924.500	71.924.500	75.924.500
16	6	106.474.383	104.474.383	108.474.383
17	6	137.612.791	135.612.791	139.612.791
18	6	164.950.817	162.950.817	166.950.817
19	7	128.349.130	126.349.130	130.349.130
20	7	149.948.232	147.948.232	151.948.232
21	8	73.822.546	71.822.546	75.822.546
22	8	145.172.472	143.172.472	147.172.472
23	8	163.510.712	161.510.712	165.510.712
24	9	8.323.233	6.323.233	10.323.233
25	9	102.494.203	100.494.203	104.494.203
26	9	135.383.654	133.383.654	137.383.654
27	9	142.950.644	140.950.644	144.950.644
28	10	50.061.312	48.061.312	52.061.312
29	10	131.716.806	129.716.806	133.716.806
30	10	143.096.411	141.096.411	145.096.411
31	10	148.028.746	146.028.746	150.028.746

Appendix V. Candidate genes soybean

Table 9. Candidate genes of soybean, found by BLAST. Descriptive information originates from EnsemblPlants (2017) and UniProt (2017). A stripe means that that information was unknown.

Candidate gene	Chromosome	Start (bp)	End (bp)	Molecular function	Biological process
GLYMA06G06180	6	4.436.872	4.441.595	phosphorelay sensor kinase activity	-
GLYMA06G06240	6	4.469.630	4.474.904	phosphorelay sensor kinase activity	-
GLYMA06G06730	6	4.807.282	4.812.403	DNA binding; transcription factor activity, sequence-specific DNA binding	phosphorelay signal transduction system; transcription, DNA-templated
GLYMA13G19871	13	23.352.627	23.363.983	-	phosphorelay signal transduction system
GLYMA13G22320	13	25.863.141	25.866.963	DNA binding; transcription factor activity, sequence-specific DNA binding	phosphorelay signal transduction system; transcription, DNA-templated
GLYMA15G05470	15	3.856.854	3.858.704	sugar transmembrane transporter activity	carbohydrate transmembrane transport; carbohydrate transport
GLYMA15G05760	15	4.082.251	4.087.146	secondary active sulphate transmembrane transporter activity; sulphate transmembrane transporter activity	sulphate transmembrane transport
GLYMA15G05770	15	4.094.166	4.097.741	lipid binding	lipid transport
GLYMA20G21030	20	29.984.895	29,986,397	ammonium transmembrane transporter activity	ammonium transmembrane transport; cellular response to nitrogen starvation; nitrogen utilization; organic cation transport
GLYMA20G21535	20	30.873.110	30.873.943	-	-
GLYMA20G21780	20	31.385.164	31.389.333	phosphorelay sensor kinase activity	-

Appendix VI. Candidate genes rice

Table 10. Candidate genes of rice (for *O. sativa incica* and *O. sativa japonica* separately), found by BLAST. Descriptive information originates from EnsemblPlants (2017) and UniProt (2017). A stripe means that that information was unknown.

Candidate gene	Chromosome	Start (bp)	End (bp)	Molecular function	Biological process
Indica					
BGIOSGA023251	6	25.335.798	25.338.635	DNA binding	regulation of transcription, DNA-templated; transcription, DNA-templated
BGIOSGA023881	7	23.981.101	23.982.851	lipid binding	lipid transport
BGIOSGA023880	7	23.984.269	23.985.119	lipid binding	lipid transport
BGIOSGA034841	11	2.643.578	2.647.677	-	phosphorelay signal transduction system
Japonica					
P0556B08.32 (OS06G0609500)	6	24.268.909	24.273.342	DNA binding	regulation of transcription, DNA-templated; transcription, DNA-templated
Os06g0647150	6	26.450.752	26.454.632	-	-
Os06g0647200	6	26.450.761	26.455.305	DNA binding; transcription factor activity, sequence-specific DNA binding	phosphorelay signal transduction system; regulation of transcription, DNA-templated; transcription, DNA-templated
Os06g0654300	6	26.809.615	26.811.574	phosphorelay sensor kinase activity	-
OS11G0157600	11	2.789.011	2.793.728	phosphorelay signal transduction system	-

Appendix VII. Candidate genes maize

Table 11. Candidate genes of maize, found by BLAST. Descriptive information originates from EnsemblPlants (2017) and UniProt (2017). A stripe means that that information was unknown.

Candidate gene	Chromosome	Start (bp)	End (bp)	Molecular function	Biological process
GRMZM2G379656	1	234.479.845	234.481.852	DNA binding	regulation of transcription, DNA-templated; transcription, DNA-templated
GRMZM2G073668	1	264.657.422	264.662.580	phosphorelay sensor kinase activity	-
GRMZM2G395114	2	4.895.674	4.900.685	secondary active sulphate transmembrane transporter activity	-
GRMZM2G341405	2	183.158.827	183.173.609	hydrogen-translocating pyrophosphatase activity; inorganic diphosphatase activity; phosphorelay signal transduction system	phosphorelay signal transduction system; proton transport; regulation of transcription, DNA-templated
GRMZM2G176347	2	234.302.197	234.303.206	-	-
GRMZM2G179349	3	108.721.006	108.722.375	-	carbohydrate transport
GRMZM2G157675	3	223.847.899	223.851.013	-	carbohydrate transport
GRMZM2G052544	5	86.297.888	86.301.099	DNA binding	regulation of transcription, DNA-templated; transcription, DNA-templated
GRMZM2G148772	5	89.751.516	89.753.301	zinc ion binding	-
GRMZM2G100318	8	163.129.203	163.133.084	DNA binding; transcription factor activity, sequence-specific DNA binding	phosphorelay signal transduction system; transcription, DNA-templated
GRMZM2G360523	9	101.990.935	101.999.695	DNA binding	phosphorelay signal transduction system; regulation of transcription, DNA-templated; transcription, DNA-templated
GRMZM2G175140	10	130.101.668	130.103.747	ammonium transmembrane transporter activity	-
GRMZM2G392101	10	147.279.947	147.281.251	-	phosphorelay signal transduction system

Appendix VIII. Literature review ZML

Both PGSC0003DMG400033576 and PGSC0003DMG400010684 are GATA transcription factors (EnsemblPlants, 2017) containing a tify, CCT and GATA zinc-finger (ZML) domain (UniProt, 2017). Except the discovery of the potato genome (The Potato Genome Sequencing Consortium, 2011), no information about these specific genes in potato was found in literature. BLAST searches with the total Arabidopsis genome however resulted for both genes in high scores (data not shown) with GATA25 (also named TIFY1 or ZIM), GATA28 (also named TIFY2a or ZML2) and GATA24 (also named TIFY2b or ZML1). These genes contains also a tify, CCT and GATA zinc-finger (ZML) domain (UniProt, 2017). Because of the GATA zinc-finger and the tify domain, these genes belong both to the GATA (Reyes et al., 2004) and tify (Vanholme et al., 2007) gene family. Most research concerning these genes is done as members of the tify gene family.

The Arabidopsis tify family consists out of 29 genes. Vanholme et al. (2007) divided the Arabidopsis tify family in two groups; group I (TIFY1 and 2) contains a CCT, GATA and tify domain and group II (TIFY3 till 11) which lack the GATA domain. Bai et al. (2011) divided the tify family in four groups and gave group I of Vanholme et al. the name ZML, group II was divided in the TIFY (only tify domain), JAZ (tify and Jas domain) and the PPD (PPD, tify and a truncated Jas domain) subfamily. Because PGSC0003DMG400033576 (putative ZIM) and PGSC0003DMG400010684[10] (putative ZML1) also contains a CCT, GATA and tify domain (UniProt, 2017) and showed high BLAST scores (data not shown) with the genes of this group, these genes probably also belongs to the ZML subfamily. Further research however is needed to confirm this. To prevent for confusion and to distinguish from the other tify members, in this report the ZML annotation will be used.

ZIM was firstly discovered in Arabidopsis (Nishii et al., 2000). During the vegetative phase of Arabidopsis ZIM is most strongly expressed in the shoot apices and roots, but during the generative phase most strongly in the inflorescences (Shikata et al., 2004). ZIM, ZML1 and ZML2 show similar expression patterns, implying that these genes function redundant or co-operative (Shikata et al., 2004). In apple however the expression pattern differs between the genes; ZML2 is expressed in roots, stems, leaves, flowers, fruits and seeds, but ZML1 only in roots, stems, leaves and flowers (Li et al., 2015). The ZML genes in rice were expressed in almost all tissues, but most strongly in the flag leaves (Ye et al., 2009). In *Gossypium raimondii* the expression of the eight ZML genes in this species differs between plant organ and developmental stages. ZML1 for example is strongly expressed in mature leaves and ZML2 in fiber at 20 day post anthesis (He et al., 2015).

In Arabidopsis ZIM is localized in the nucleus and functions as a transcriptional activator (Nishii et al., 2000; Shikata et al., 2003). In maize, however ZML2 functions as a transcriptional repressor (Vélez-Bermúdez et al., 2015). It is not clear if this difference is caused by the crops or by the genes. The activation by ZIM in Arabidopsis was not strong, suggesting that this gene functions in a complex (Shikata et al., 2003). Vélez-Bermúdez et al. (2015) confirmed this hypothesis for ZML2 in maize were MYB11 and ZML2 repress the expression of lignin genes. After wounding, MYB11 and ZML2 are degraded and transcription of lignin genes is started. It needs to be confirmed of this is also the case for ZIM in Arabidopsis.

Overexpression of ZIM in Arabidopsis resulted in elongated hypocotyls and petioles, smaller leaves and a more upward position of the leaves (Shikata et al., 2004). The elongation was inhibited by light, gibberellin (GA) and brassinosteroid (BR), suggesting that overexpression of ZIM does not affect the regulation of light response and signalling and is independent of GAs and BRs (Shikata et al., 2004). By overexpression of ZIM, more than 600 genes were upregulated more than twofold (Shikata et al., 2004).

Besides plant development, the ZML subfamily also seems to be involved in resistance against biotic and abiotic stresses. In pigeon pea (*Cajanus cajan*) the transcript levels of two members of the ZML gene family was upregulated under copper stress, while the third was hardly affected (Sirhindi et al.,

[10] PGSC0003DMG400033576 has two transcripts and PGSC0003DMG400010684 six. It should be noted that not all transcripts contain all three domains.

2016). Ethylene treatment in apple resulted in strong upregulation of ZML1 and ZML2 the first 6h, after which expression decreased again. Both ZML1 and 2 in apple showed only weak response to drought stress and to ABA and salicylic acid (SA) treatment. Salt stress hardly affected the expression of ZML1, but strongly increased the expression of ZML2 at 6 hours after treatment. Methyl jasmonate (MeJa) and ethylene treatment resulted in an increased expression of both genes for about six hours (Li et al., 2015). In wild (*Glycine soja*) and cultivated (*G. max*) soybean most members of the ZML gene family were affected by bicarbonate stress (Zhu et al., 2013). The response however was not the same in leaves and roots and differed moreover between the separate genes. The six ZML members in purple false brome (*Brachypodium distachyon*) responded to at least one of the abiotic stress (drought, salt, cold and heat) or hormone (ABA, ethylene, JA and SA) treatments (Zhang et al., 2015b). The response however differed between the genes. Heat stress for example strongly induced expression of ZML6 is, while it had almost no influence on expression of ZML5 (Zhang et al., 2015b). The expression of the four rice ZML genes was only slightly affected by JA, ABA treatments and by wounding or cold stress, but increased by drought and salt stress (Ye et al., 2009). In maize three ZML members were found (Zhang et al., 2015a). Drought stress suppressed the expression of ZML1 in both investigated maize lines, while it increased the expression of ZML2 and ZML3 in only one line and hardly affected them in the other. The effect of *Fusarium moniliforme*, *Sphacelotheca reiliana* and *Colletotrichum graminicola* on the expression of the ZML genes was investigated in one maize line. ZML2 and ZML3 were suppressed after infection of each of these fungi, while ZML1 was repressed by *F. moniliforme*, induced by *S. reiliana* and hardly affected by *C. graminicola* (Zhang et al., 2015a). Zhang et al. (2012) investigated four ZML members in grape. The expression of ZML1, 3 and 4 was increased under PEG (polyethylene glycol), salinity and drought stress, while the expression of ZML2 was decreased. These authors found that the expression of ZML1 was decreased under cold stress. After inoculation with *Plasmopara viticola*, expression of ZML3 was up-regulated, but ZML4 down-regulated. Infection with *Uncinula necator*, Bois Noir (an emerging grapevine yellows disease caused by phytoplasmas) and leaf roll-associated closeterovirus-3 hardly affected the expression of any of the ZML genes. Expression of ZML2 and ZML4 was upregulated after treatments of JA, MeJa and ABA, while ZML3 was downregulated after ABA treatment (Zhang et al., 2012).

Appendix IX. Literature review type B-I response regulators

PGSC0003DMG400023534 showed the highest BLAST score with Arabidopsis RR1, 2, 10, 11 and 12 and with rice RR21, 22, 23, 24, 25 and 26[11] (data not shown). These genes belong to the same subgroup, named subgroup I or B-I (Du et al., 2007; Hwang et al., 2002; Mason et al., 2004; Pareek et al., 2006; Tsai et al., 2012). Therefore it is assumed that PGSC0003DMG400023534 also belongs to this subgroup. Further research however is needed to prove that. Except the discovery of the potato genome (The Potato Genome Sequencing Consortium, 2011), no information of this or any of the other potato RRs was found. Most research about RRs is done in Arabidopsis and to a lesser extend also in rice. This literature review will therefore focus on subgroup I genes of type B RRs of Arabidopsis and rice.

Arabidopsis RR1 and RR2 were discovered by Sakai et al. (1998b). The expression levels of RR1 were slightly higher than that of RR2. In further experiments Sakai et al. (2000) proved that RR1 and RR2 were localized in the nuclei. They moreover found that these genes were able to bind double stranded DNA and functions as transcriptional activators. Under normal conditions the function of Arabidopsis RR1 is suppressed by its receiver domain (Sakai et al., 2001). These authors found that RR1 is directly activated after cytokinin perception, probably by a phosphorelay signal transduction from histidine kinase CRE1. Kim et al. (2006) found that phosphorylation of Arabidopsis RR2 is also induced by cytokinin. Kiba et al. (1999) found that expression of Arabidopsis subgroup I RRs was not upregulated by cytokinin. They measured however expression by northern hybridization analysis, while Sakai et al. (2001) and Kim et al. (2006) measured phosphorylation of the RRs. Together these results suggest that expression of RRs is post translational regulated. Tsai et al. (2012) suggest a similar cytokinin signalling system in monocots and dicots.

Ito and Kurata (2006) and Du et al. (2007) found all rice subgroup I type B RR genes expressed in all tissues, except RR25, which was mainly expressed in callus, stems and the generative parts. Sakai et al. (1998b) found Arabidopsis RR1 and RR2 expressed in all tissues, but the highest levels were observed in the roots. Lohrman et al. (2001) found RR2 predominantly expressed in the flowers and to a lesser extend in the leaves and stems, but not at all in the roots of Arabidopsis. Reason for the different findings of Sakai et al (1998b) and Lohrman et al. (2001) are not known. Imamura et al. (2003) found Arabidopsis RR11 strongest expressed in roots but also at significant levels in the leaves. Mason et al. (2004) found that Arabidopsis subgroup I members were highly expressed in regions in which cytokinins plays an important role. These regions include the apical meristem, young leaves and root tips (especially the parts were cell division or elongation would take place). They suggested that subgroup I members functions (at least partly) redundantly, what was confirmed by next experiments (Mason et al. 2005). Imamura et al. (2003) suggest also redundant functions between Arabidopsis RR1 and RR11. Ishida et al. (2008) however suggest that Arabidopsis RR1, 10 and 12 have a general but essential role in cytokinin signalling, while the other subgroup I RRs play more specific roles. Arabidopsis RR1, 10 and 12 functions redundantly (Ishida et al., 2008). Mason et al. (2005) found that in some cases Arabidopsis subgroup I members could also function antagonistic. Further research is needed to clarify the precise function of the subgroup I members.

Concerning the biological function of subgroup I members, Kim et al. (2006) found Arabidopsis RR2 to be involved in leaf longevity. RR2 overexpressing mutants showed delayed leaf senescence. Knock out mutants however, showed no early leaf senescence suggesting that more RRs or other factors are involved in leaf senescence. Mason et al. (2010) found RR1 and 12 involved in sodium accumulation in the shoots of Arabidopsis, suggesting a role in tolerance against salinity stress. Yokoyama et al. (2007) found that RR1, 10 and 12 play a central role in cytokinin signalling in Arabidopsis roots. They found moreover that RR10 and 12 negatively regulate the development from procambium into protoxylem. RR1 and 12 also play an important role in controlling root meristem growth. Root meristem growth is determined by a balance between cell division and differentiation. RR1 and 12 have an important role in upregulating Arabidopsis root cell differentiation and downregulating the auxin controlled cell division (Dello Ioio et al., 2007 and 2008; Moubayidin et al., 2010).

[11] Especially before 2007, in literature several different names for the rice RRs were used. In this report the 'new' nomenclature according to Schaller et al. (2007) is used. (for synonyms see also that paper).

Appendix X. Literature review pseudo response regulators

PGSC0003DMG400000584 (putative PRR5) showed high BLAST scores with pseudo response regulators (PRR)1, 3, 5, 7 and 9 of Arabidopsis and with PRR1, 37, 59, 73 and 95[12] of rice (data not shown). These genes belong to subgroup 'C' or 'clock' (Hwang et al., 2002; Mason et al., 2004; Tsai et al., 2012). Far the most research to PRRs is done in Arabidopsis. The few papers about rice PRRs indicate that these are reminiscent to that of Arabidopsis (Murakami et al., 2003; Murakami et al., 2007). Therefore in this paragraph we will focus on research performed in Arabidopsis.

For both Arabidopsis and rice, the BLAST score with PRR1 was less strong than with the other four genes (data not shown). The four other genes are phylogenetically also closer related with each other than with PRR1 (Tsai et al., 2012). Pareek et al. (2006) found similar results as the aforementioned authors, but placed Arabidopsis PRR1 even in a distinct subgroup. Because no information was found about putative PRR5 or any of the other potato PRRs, here a brief literature review of the current knowledge of PRR3, 5, 7 and 9 of Arabidopsis will be given.

PRR3, 5 and 7 are all nuclear localized (Fujiwara et al., 2008). According to UniProt (2017) PRR9 was also localized in the nucleus, but no literature was found to support that. PRRs can be distinguished from the other RRs by its receiver like domain and its CCT domain (Makino et al., 2000). Its receiver domain can be discriminated from the classical ones by lacking the phospho-accepting aspartate site, which is replaced by a glutamate site (Makino et al., 2000). Nevertheless PRR3, 5 and 7 are still able to phosphorylate (Fujiwara et al., 2008; Murakami-Kojima et al., 2002). Protein activity however is regulated by both protein abundance and phosphorylation of the proteins (Fujiwara et al., 2008). PRR9 is induced by phytochrome A and B, but it is not known whether this occurs by phosphorylation (Ito et al., 2003).

PRRs are essential factors in the circadian clock (e.g. Matsushika et al., 2000; Makino et al., 2001). The circadian clock is proposed to be the main regulator of plant growth, development and physiology and allows the plant to cope with diurnal and seasonal variations and biotic and abiotic stresses (for review about the circadian clock see Sanchez and Kay, 2016). PRRs are expressed in the order PRR9 →PRR7 → PRR5 → PRR3 → PRR1, with two to three hours in between. PRR9 is strongly induced by light, after which the rhythm start (Ito et al., 2003; Makino et al., 2001). PRR9 however does not directly regulate the rest of the cascade, because in a knock-out mutant of this gene the cascade PRR7 → PRR5 → PRR3 → PRR1 sustained (Ito et al.,2003).

PRR5, 7 and 9 regulate direct and indirect the expression of many genes (Nakamichi et al., 2010; Nakamichi et al., 2012). Genes that have their peak expression between dawn and mid-day are repressed and genes that have their peak expression from dusk to night are activated (Nakamichi et al., 2009). Genes influenced by PRR5, 7 and 9 include among others circadian clock associated genes, genes involved in biotic and abiotic stresses, developmental processes, transport and signal transduction (Nakamichi et al., 2009). Detailed study to PRR5 revealed that this gene directly influenced the expression of 64 genes and indirectly of 1,024 genes (Nakamichi et al., 2012). Direct targets of Arabidopsis PRR5 include genes involved in the regulation of hypocotyl elongation, flowering time and cold stress response (Nakamichi et al., 2012). PRR3, 5, 7 and 9 share some target genes and functions partly redundantly (Farré et al., 2005; Nakamichi et al., 2009; Nakamichi et al., 2010; Nakamichi et al., 2012; Para et al., 2007; Salomé and McClung, 2005).

Nakamichi et al (2005) found flowering time of Arabidopsis delayed in knock out mutants of PRR5, 7 and 9, with the triple mutant showing the most delayed flowering. They also found the sensitivity to red light (and to a lesser extent also to far red light) reduced in some of the mutants, especially in the triple mutant. Arabidopsis PRR5 or 9 overexpressing plants flowered much earlier and showed hypersensitivity to red light (Sato et al., 2002).

PRRs have also a role in responses against abiotic stresses. Reduced leaf expansion is an important parameter for plant shade avoidance response. Arabidopsis PRR5 functions as regulator of shade

[12] Especially before 2007, in literature several different names for the rice RRs were used. In this report we use the 'new' nomenclature according to Schaller et al. (2007). (for synonyms see also that paper).

avoidance response by repressing leaf expansion under shade conditions (Takase et al., 2013). Nakamichi et al. (2009) found PRR5, 7 and 9 triple knock out mutants more tolerant to cold, saline and drought stress. Liu et al. (2013) found that PRR7 directly regulates genes involved in response to cold and drought stress. DREB1 (dehydration-responsive element B1; also called CBF) genes are transcription factors that have a prominent role in the regulation of many genes involved in cold tolerance in Arabidopsis (for review see Yamaguchi-Shinozaki and Shinozaki, 2006). Under normal conditions DREB1 genes are repressed by PRR5, 7 and 9, while under cold stress DREB1 genes are upregulated in Arabidopsis (Nakamichi et al., 2009). Liu et al. (2013) found that overexpression of PRR7 leaded to repression of DREB1 in Arabidopsis supporting a role for PRRs in regulating DREB1 genes. The mechanism how PRR5, 7 and 9 are involved in regulation of tolerance against saline and drought stress needs to be investigated in more detail.

Appendix XI. Literature review ethylene receptors

Except the discovery of the potato genome (The Potato Genome Sequencing Consortium, 2011), there was no literature found about this PGSC0003DMG400023402. BLAST searches resulted in high scores with the ethylene receptor families of among others Arabidopsis, rice, tomato and tobacco (data not shown). Ethylene receptors contains a membrane spanning domain (which contain among others the ethylene binding region), a GAF domain, a kinase domain, and a domain receiver (also called response regulatory domain). Exceptions are ERS1 and 2 who did not contain a receiver domain (Hua et al., 1995 and Hua et al., 1998). The ethylene receptors can be divided in two subfamilies (
Table 12). Subfamily I members contain all five motifs (H, N, G1, F, and G2-box) of the kinase domain, have three hydrophobic stretches near the N terminus and possess histidine kinase activity (exception is ERS1 (ethylene response sensor 1) which possess also serine kinase activity). Subfamily II members lack most motifs of the kinase domain, contain four hydrophobic stretches near the N terminus and possess only serine kinase activity. Moreover the intron distribution differs between the two subfamilies (Chang et al., 1993; Hua et al., 1995; Hua et al., 1998; Hua and Meyerowitz, 1998; Moussatche, 2004; Moussatche and Klee, 2004; Sakai et al., 1998a).
On a few exceptions left, PGSC0003DMG400023402 shows the highest BLAST scores with the subfamily II members of the ethylene receptor of the mentioned crops (data not shown)[13]. According to UniProt (2017), PGSC0003DMG400023402 contains at least a histidine kinase domain and a receiver domain. UniProt (2017) made however not clear whether this gene contains all five motifs of the kinase domain. PGSC0003DMG400023402 is probably no ERS gene because it contains a receiver domain. This is supported by the fact that tomato has no ERS gene at all (Table 12). Therefore it is assumed that this gene belongs to the ETR (ethylene response or ethylene resistant) genes of subfamily II and will therefore in the rest of this report be annotated as putative ETR. Further research however is needed to prove that.

Table 12. The ethylene receptor family of four crops divided in two subfamilies. Synonyms are written in the brackets (Cao et al., 2003; Moussatche, 2004; O'Malley et al., 2005; Wuriyanghan et al., 2009).

Crop	Subfamily I	Subfamily II
Arabidopsis	ETR1	ETR2
	ERS1	ERS2
		EIN4
Rice	ERS1	ETR2 (PK1 or ERL1)
	ERS2	ETR3 (PK2)
		ETR4
Tomato	ETR1	ETR4
	ETR2	ETR5
	ETR3 (NR or never ripe)	ETR6
Tobacco	ERS1	HK1
	ETR1	HK2

There is some discussion whether ethylene receptors belong to a phosphorelay system. Ethylene receptors are related to histidine kinases (Schaller et al., 2011). Moussatche and Klee (2004) however suggest that ethylene signal transduction does not use a phosphorelay system, because histidine kinase activity is not needed for ethylene receptor function. Shi et al. (2012), in opposite, found Arabidopsis type A RR involved in the ethylene signalling pathway. Hass et al. (2004) found RR2

[13] Remarkably the highest BLAST score with tomato was with Solyc05g055070.2, a gene of unknown function with both a histidine kinase and response regulatory domain. This raises the question whether a seventh tomato ethylene receptor exists. Further research is needed to prove that.

(Arabidopsis type B) to be regulated by ETR1. Both suggesting a phosphorelay system. Binder and Schaller (2015) propose two distinct pathways: one phosphorelay pathway and one in which CTR1 (constitutive triple response 1), EIN2 (ethylene insensitive 2) and EIN3 plays a crucial role. However currently still many questions about the ethylene signalling pathway(s) remains to be resolved.

In Arabidopsis, the subfamily II members ETR2, ERS2 and EIN4 were expressed in embryos, etiolated seedlings, leaves, roots (except ETR2), stems (except EIN4) and inflorescences. Expression pattern within the inflorescences differs however between the receptors. ETR2 was strongly expressed in the developing carpels (mainly in the funiculi and ovules), EIN4 in the locules of stamens, including developing pollen and tapetum cells and ERS2 in all mentioned flower parts. Later in flower development ERS2 was the only gene with strong expression in the epidermal layers of the septum (Hua et al., 1998). Sakai et al. (1998a) found that *Arabidopsis* ETR2 is involved in etiolated seedling elongation, leaf expansion and leaf senescence. ERS2 is also involved in *Arabidopsis* leaf expansion (Hua et al., 1998).
Hua and Meyerowitz (1998) screened loss of function mutants of ETR2, EIN4 and ERS2, which also showed reduced size of shoots and roots both. This reduction was because of inhibition of cell elongation. The strongest effect was observed in the quadruple mutant and to a lesser extent also in the triple and double mutant, but almost no effect was observed in the single mutants, suggesting that they at least partly functions redundantly.
Besides functions in developmental processes, ethylene receptors are also involved resistance against biotic and abiotic stresses. O'Donnell et al. (2003) found in Arabidopsis that ETR2 is induced after *Xanthomonas* infection, but that ERS2 and EIN4 were not induced. Wilson (2014) found that ETR2 stimulates Arabidopsis seed germination under salt stress and far red light treatment. Manaa et al. (2014) found that transgenic Arabidopsis lines, antisensed for coffee (*Coffea canephora*) ethylene receptor EIN4, had higher germination and less susceptibility under salt stress compared to wild type.

Yau et al. (2003) found the highest expression of rice ETR2 in anthers, suggesting a role in pollen development. They also found that ETR2 is induced by auxin and ethylene. Expression of ETR3 and ETR4 was too low to be detected by northern blot analysis. RT-PCR analysis showed that the mRNAs of these genes were present in young green seedlings and anthers (Yau et al., 2003). Watanabe et al. (2004) found ETR2[14] of deep water rice to be upregulated by flooding, ethylene and GA treatment. According to these authors ETR2 might also play an important role in internode elongation. Wuriyanghan et al. (2009) found that ETR2 and ETR3 affect flowering time in rice. They found ETR2 moreover to influence flower development, starch accumulation, sugar transport and grain filling. In rice seedlings ETR3 was induced in shoots by wounding and both in shoots and roots under drought stress. ETR3 was not significantly affected by ABA and salt treatment (Cao et al., 2003).

In tomato ETR4 and ETR5 shows high levels of expression in the reproductive tissues, but low levels in the vegetative tissues (Tieman and Klee, 1999). ETR4 and ETR6 are important for fruit ripening. During ripening the expression of this genes in the fruits increases strongly, but the protein level decreases (Tieman and Klee, 1999; Kevany et al., 2007). Ethylene binding results in fast degradation of the ethylene receptor proteins (Kevany et al., 2007). This suggests that the increase in ethylene during ripening is sufficient to compensate for the increase in RNA expression of the ethylene receptors (Kevany et al. 2008). These latter authors also proved for ETR4 that suppression of this receptor is tissue specific and probably act as biological clock for the start of ripening. Besides an important role in fruit ripening, ETR4 is also involved in resistance against *Xanthomonas* (Ciardi et al., 2001). ETR4 expression is strongly increased after infection with *Xanthomonas*. ETR4 antisense lines show reduced ETR4 expression and increased hypersensitive response and resistance after infection with *Xanthomonas* (Ciardi et al., 2001). Induction of ethylene receptors might therefore be a dampening mechanism, slowing down the ethylene response (Klee and Tieman, 2002).

[14] These authors call this gene ERL1 (ETR2 like gene 1) because its similarity with Arabidopsis ETR2 and EIN4, but the gene is more frequently annotated as ETR2. Therefore in this report this gene is annotated as ETR2.

Tobacco HK1 was solely expressed in the generative tissues (Zhang et al., 2001). These authors also found that this gene was induced after wounding, floating of leaf pieces, drought and salinity stress. After wounding HK1 was also expressed in vegetative organs (Zhang et al., 2001; Xie et al., 2002). HK1 overexpressing tobacco seedlings were more sensitive to salt stress and had under NaCl concentrations of 300 and 450 mM (but not under 150 mM) higher Na^+/K^+ ratios than control plants, from which these authors suggest that HK1 responds to salinity stress by affecting ion accumulation (Cao et al., 2006). The HK1 genes of these authors were controlled by a 35S promotor. Nevertheless these genes were induced by salt stress, suggesting that this response was post translational regulated. HK2 was induced by dehydration and CaCl2 treatment, but not significantly by salinity stress and ABA treatment (Zhang et al., 2004).

Appendix XII. Literature review ammonium transporters

Except the discovery of the potato genome (The Potato Genome Sequencing Consortium, 2011), no literature information was found about putative AMT1;3 or any of the potato AMT1 members. High BLAST scores were found with AMT1 members of Arabidopsis, rice and tomato, especially with tomato AMT1;3 (data not shown). Therefore a brief literature review will be given about the AMT1 members of tomato, especially about LeAMT1;3.

In tomato three AMT1s were found, named LeAMT1;1, LeAMT1;2 and LeAMT1;3 (Table 13). LeAMT1;1 is an orthologue of Arabidopsis AtAMT1;1 (Lauter et al., 1996). LeAMT1;1 and LeAMT1;2 show 75.6 % amino acid identity (Von Wirén et al., 2000a). Both are preferentially expressed in the root hairs and to a lesser extent also in the leaves (Lauter et al, 1996; Von Wirén et al., 2000a). LeAMT1;1 and LeAMT1;2 are supposed to be important for ammonium uptake from the soil (Von Wirén et al., 2000a).

Table 13. The location of expression of the three tomato AMT1s, the influence of nitrogen supply on their expression in the roots and the time of expression in the leaves (Lauter et al, 1996; Von Wirén et al., 2000a).

Gene	Expression Location	Nitrogen supply (roots)	Time (leaves)
LeAMT1;1	Root hairs / leaves	Reduction	Constitutive
LeAMT1;2	Root hairs / leaves	Induction	Day
LeAMT1;3	Leaves	-	Night

LeAMT1;3 has only 62.8 % sequence identity with LeAMT1;1 and LeAMT1;2 (Von Wirén et al., 2000a) and is phylogenetically distantly related from all currently known AMT1 members in plants (Loque and Von Wirén, 2004; Ludewig et al., 2007; Von Wirén et al., 2000b; Wu, 2004). The currently found putative potato AMT (PGSC0003DMG400018761) is the only gene which is closely related to LeAMT1;3, with about 97 % sequence identity (data not shown).

LeAMT1;3 has, in addition to the others, two uORFs in front of the main ORF, which are supposed to control the rate of peptide synthesis. Moreover LeAMT1;3 has a short N-terminus of only 14 amino acids, while the other tomato AMT1s has one of 35-54 amino acids (Von Wirén et al., 2000a). As a consequence of the shorter N-terminus LeAMT1;3 is not able to form a trimer like the other AMT1s (Graff et al., 2011). The formation of a trimer is thought to be important for ammonium transport. Since LeAMT1;3 is a functional ammonium transporter (Von Wirén et al., 2000a) this suggests that ammonium transport by LeAMT1;3 occurs independent of trimer formation (Graff et al., 2011). Expression of LeAMT1;3 was higher under a CO_2 concentration of 400 ppm than under 800 ppm (Von Wirén et al., 2000a).

Appendix XIII. Literature review sulphate transporters

The sulphate transporter family in plants is divided in four groups, named SULTR1-SULTR4 (Takahashi et al., 2012). PGSC0003DMG400018422 and PGSC0003DMG400023515 are sulphate transporters belonging to group 3 and 4 respectively (Vatansever et al., 2016). Potato SULTRs are proteins of 70-75 kDa which contain 8-12 transmembrane domains, suggesting transmembrane transport (Vatansever et al., 2016). Besides the publication of Vatansever et al. (2016) only one paper about a potato sulphate transporter was published (Hopkins et al., 2005) but they investigated only a group 1 member. PGSC0003DMG400018422 and PGSC0003DMG400023515 show high similarity with sulphate transporters of both Arabidopsis and rice (BLAST, data not shown; Vatansever et al., 2016). Therefore in a brief literature review of sulphate transporters of group 3 and 4 also information of Arabidopsis and rice will be included.

Group 3 sulphate transporters

Group 3 is the largest group within the sulphate transporters (Takahashi et al., 2012; Vatansever et al., 2016). From all group 3 sulphate transporters of rice and Arabidopsis, PGSC0003DMG400018422 is closest related to SULTR3;3 of both crops (BLAST, data not shown; Vatansever et al., 2016). PGSC0003DMG400018422 will therefore in the rest of this report be annotated as putative SULTR3;3. In Arabidopsis, SULTR3;3 is only expressed in the leaves and seeds (Takahashi et al., 2000; Zuber et al., 2010b). Its expression is low compared to other SULTR3 members (Zuber et al., 2010b). In rice SULTR3;3 is expressed in all tissues, but predominantly in mature leafs and seeds (Kumar et al., 2011). Zuber et al. (2010b) propose that Arabidopsis SULTR3;3 is involved in the transport of sulphur between different seed tissues. In rice SULTR3;3 and potato putative SULTR3;3 are influenced by hormones, biotic- and abiotic stresses and elicitors (Kumar et al., 2011; Vatansever et al., 2016). Putative SULTR3;3 in potato is for example downregulated by BABA (DL-b-amino-n-butyric acid), BAP (6-benzylaminopurine), mannitol (drought) and heat treatment and upregulated by BTH (acibenzolar-S-methyl), *P. infestans* and GA treatment (Vatansever et al., 2016).

Group 4 sulphate transporters

The group 4 of sulphate transporters is a small group with two members in Arabidopsis (Kataoka et al., 2004; Takahashi et al., 1999) and only one in rice (Kumar et al., 2011) and potato (Vatansever et al., 2016). Rice SULTR4 has four transcripts. Remarkably the tissue expression profile and pattern differs between the four transcripts (Kumar et al., 2011).

In Arabidopsis seedlings SULTR4;1 and SULTR4;2 were expressed both in the roots and the shoots, but predominantly in the hypocotyls and in the pericycle and parenchyma cells of the roots (Kataoka et al., 2004). Zuber et al. (2010a) found SULTR4;1, and to a tenfold lower extend also SULTR4;2, expressed in developing seeds of Arabidopsis. In rice SULTR4 is expressed in all tissue, but predominantly in leaves and seeds (Kumar et al., 2011). In Arabidopsis both genes are expressed in the tonoplast of roots and shoots (Kataoka et al., 2004), but for seeds the cellular localization is not determined yet.

In Arabidopsis shoots mRNA levels of both SULTR4;1 and SULTR4;2 accumulated under sulphur limiting conditions, while in roots only SULTR4;2 accumulated and SULTR4;1 was expressed at a constant level (Kataoka et al., 2004; Takahashi et al., 2000). In contrary expression of SULTR4 in rice roots increased with increasing external sulphate concentrations (Kumar et al., 2011).

Kataoka et al. (2004) suggest that SULTR4;1 and SULTR4;2 of Arabidopsis mediate the efflux of sulphate from the vacuole into the cytoplasm of vasculature. Results of single and double knock out mutants suggested that SULTR4;1 plays a major role, while SULTR4;2 plays only a supplementary role (Kataoka et al., 2004).

In rice and potato SULTR4 is influenced by hormones, biotic- and abiotic stresses and elicitors (Kumar et al., 2011; Vatansever et al., 2016). In potato SULTR4 is upregulated by among others *P. infestans* and salt stress and downregulated by BAP and BTH (Vatansever et al., 2016).

Appendix XIV. Literature review carbohydrate transporters

SWEETs are low affinity sugar transporters (Chen et al., 2012). Plant SWEET family is divided in four clades. The currently found carbohydrate transporters, all belong to clade III of the SWEET family (Manck-Götzenberger and Requena, 2016). The corresponding names and tissue expression profiles are shown in Table 14. Remarkably, for none of the SWEETs expression in tubers could be detected. Timmermans (2016) did a more detailed research to the expression of four potato SWEETs, among which SWEET11b and 12d. They measured the expression of these SWEETs in the developing tubers during five stages of tuberization, ranging from just a hooked, not swollen stolon till a small tuber. At the first stage almost no expression of the SWEET genes was detectable. At the second stage both SWEETs were weakly expressed. From stage three on, expression of SWEET11b was not detectable anymore. The expression of SWEET12d was also not detectable at stage three, but showed a strong increase at stage four. At the last stage the expression of SWEET12d was decreased again, but still showed a moderate expression. SWEET10b, another clade III SWEET, showed constitutive low expression at all stages, but slightly higher at the fourth stage. Differences between Manck-Götzenberger and Requena (2016) and Timmermans (2016) might be related to measurement method (fragments per kilobase of transcript per million mapped reads by Manck-Götzenberger and Requena, 2016 and qPCR by Timmermans, 2016). Also stage of plant development might had influenced the results, but this is not sure because it is unknown at which stage Manck-Götzenberger and Requena (2016) measured gene expression.

Table 14. The six currently found carbohydrate transporters and their names and tissue expression profiles according to Manck-Götzenberger and Requena (2016). ND means not detectable.

		Expression			
PGSC code	**Name**	**Tuber**	**Root**	**Stem**	**Leaf**
PGSC0003DMG402031741	SWEET10c	ND	Very strong	Weak	Moderate
PGSC0003DMG400031742	SWEET10d	ND	Moderate	Weak	Moderate
PGSC0003DMG400033693	SWEET11b	ND	Strong	Weak	Strong
PGSC0003DMG400031738	SWEET12a	ND	Weak	ND	ND
PGSC0003DMG400004335	SWEET12c	ND	Strong	Weak	Weak
PGSC0003DMG400004337	SWEET12d	ND	Moderate	ND	Weak

The cellular location of potato SWEETs still needs to be determined, but Arabidopsis SWEET11 and 12 (both clade III) are localized in the phloem parenchyma cells (Chen et al., 2012). They form a pore in the cell membrane by seven transmembrane helices (Chen et al., 2010). Arabidopsis clade III SWEETs preferentially transport sucrose and to a lesser extend also glucose and fructose, but not maltose (Chen et al., 2012; Hir et al., 2015). Arabidopsis clade III transporters perform the first step in phloem loading; they transport sucrose from the phloem parenchyma cells into the apoplast after which sugar transporters of the SUC/SUT family transport it into the sieve elements and companion cells (Chen et al., 2012). Arabidopsis SWEET1 (clade I) however is a bidirectional glucose transporter, which suggests also phloem unloading (Chen et al., 2010). If clade III SWEETs are able to transport sucrose bidirectional needs to be investigated. SWEET11 and 12 were also supposed to supply sugars to developing xylem cells to support the formation of secondary cell walls in Arabidopsis floral stems (Hir et al., 2015). These authors found that single and especially double knock out mutants of SWEET11 and 12 had smaller xylem and phloem pole area, less xylem and phloem cells per pole area and smaller diameter of xylem cells. Timmermans (2016) produced transgenic potato SWEET11b lines. Overexpressing SWEET11b affected plant architecture and resulted in more sympodial units, more basal stems and in some cases loss of apical dominance in cultivar Andigena. In cultivar Desiree however the number of sympodial units was decreased. Moreover in Andigena the plant length was not significantly affected, while it was increased in Desiree. RNAiSWEET11b plants showed reduced

fitness what appeared among others out of a reduced number of sympodial units and stems and a reduced plant length.
In Arabidopsis SWEET15 (also called SAG29) is strongly upregulated in senescing leaves (Seo et al., 2011). Clade III SWEETs are also involved in the reproduction of plants. Lin et al. (2014) found SWEET9 of Arabidopsis, *Brassica rapa* and *Nicotiana attenuate* involved in nectar production. After synthesis in the nectary parenchyma cells, sucrose is transported into the extracellular space where it is further processed. Chen et al. (2015) found SWEET11, 12 and 15 to play a crucial role in seed filling of Arabidopsis. Each of these genes shows a specific expression pattern in the seed, but are also able to compensate for each other because only the triple knock out mutant shows severe seeds defects.

SWEETs are also affected by biotic and abiotic stresses. Arabidopsis SWEET10, 11, 12, 13 and 15 were upregulated after infection with *Pseudomonas syringae pv. tomato* and/or *Golovinomyces cichoracearum* and/or *B. cinerea* (Chen et al., 2010). The expression of SWEET9 and 14 was not affected after inoculation with any of these pathogens. Fabro et al. (2008) however found Arabidopsis SWEET12 is downregulated 18 hour after infection with *G. cichoracearum*. Chen et al. (2010) found the expression of SWEET12 eight hours after infection with *G. cichoracearum* strongly increased but twelve hours after infection it decreased already. A lower expression as before inoculation was even not observed 72 hours after inoculation, but these authors did not measure the expression of SWEET12 at 18 hours after inoculation. The different methods (qPCR by Chen et al., 2010; microarray by Fabro et al., 2008) to measure gene expression might also be a reason for the different results.
Probably one of the most investigated interactions between SWEET clade III genes and pathogens is that of rice and *Xanthomonas oryzae pv. oryzae* (Xoo). In short Xoo activates SWEETs by transcription activator like effectors (TALs) resulting in a carbohydrate supply to the pathogen. Due to the interaction of SWEET11 (also Xa13 or Os8N3) with copper transporters, copper is removed out of the xylem vessels, which is beneficial for Xoo because its growth is inhibited by this metal. Plants carrying recessive SWEET clade III genes show resistance against Xoo (for brief review see Zhang and Wang, 2013). Not only biotrophic, but also symbiotic microorganisms are able to influence the expression of clade III SWEETs. Manck-Götzenberger and Requena (2016) investigated the effect of a symbiotic AM fungi (*R. irregularis*) on the expression of SWEET genes at six and eight weeks post inoculation. SWEET10c, 10d and 12c were repressed, while SWEET12d was increased at both stages. SWEET11b was hardly affected. SWEET12a was decreased at six weeks post inoculation, but increased at eight weeks. The effects however were mostly not significant. In total 22 of the 35 potato SWEETs were affected by AM fungi inoculation, suggesting a complex carbohydrate reorganisation. The precise role of the individual SWEETs needs still to be investigated. It is also not clear yet whether *R. irregularis* influences potato clade III SWEETs in a similar way as Xoo does with rice SWEETs.
SWEET15 is upregulated in Arabidopsis under cold, salt and drought stress in an ABA dependent manner. This SWEET plays a role in tolerance against these stresses by modulating cell membrane integrity, resulting in maintenance of cell viability (Seo et al., 2011). SWEET11 and 12 are also involved in cold resistance of Arabidopsis floral stems (Hir et al., 2015). They found that double knock out mutants of these genes showed increased cold resistance, probably due to smaller xylem (caused by less xylem cells and decreased diameter of xylem vessel). Feng et al. (2015) investigated the effect of fructose, sucrose, glucose, salt, high temperature and low temperature treatments on the expression of tomato SWEETs in different tissues. The expression of all genes was affected by these stresses, but the response was tissue specific. For example SWEET10a, 10b, 10c, 11a, 11b, 11c, 11d, 12a and 12c showed in general high expression under these treatments in leaves, but low expression in roots, while SWEET12b, 12d and 14 showed opposite pattern. Also in the upstream regions of these tomato clade III genes many stress- and hormone-related elements were found, like ABRE, HSE and P-Box, responsive to ABA, heat temperature and GA respectively (Feng et al., 2015).

Appendix XV. Literature review xylogen

Gong et al. (2015) found the expression of putative xylogen downregulated under drought stress in potato stolons. Further no literature information was found about putative xylogen or any of its closely related potato genes (found by BLAST, data not shown). BLAST searches (data not shown) resulted in high scores with genes of the xylogen family of both Arabidopsis and rice. This review will therefore focus on the xylogen family in these crops.

Xylogen type proteins have three motifs: a N-terminal signal peptide, a non-specific lipid transfer protein (nsLTP) domain and a glycosylphosphatidylinositol (GPI) anchor motif. Moreover most of these proteins can be modified with arabinogalactan moieties (Kobayashi et al., 2011). Xylogen type proteins belong therefore to a subfamily of the arabinogalactan proteins (AGP family), the novel chimeric AGPs (Kobayashi et al., 2011), but are also classified as nsLTP-like proteins (Edstam et al., 2011; 2013) and GPI anchored proteins (Borner et al., 2003).

Kobayashi et al. (2011) divided the xylogen family in seven groups, but Ma et al. (2014) only in four. The highest BLAST score (data not shown) was found with Arabidopsis AtXYP1 (also named AtLTPg31) and AtXYP2 (also named AtLTPg11) followed by rice OsXYLP5 (also named OsLTPg3). Kobayashi et al. (2011) divided these genes in two different subgroups, but according to Ma et al. (2014) these genes belong to the same subgroup, clade A.

In Arabidopsis seedlings AtXYP1 is only expressed in the cotyledons while AtXYP2 is expressed in the lateral root primordia and in the vasculature of hypocotyl, root and shoot (Kobayashi et al., 2011). In mature Arabidopsis plants AtXYP1 is preferentially expressed in the flowers (Hu et al., 2003; Kobayashi et al., 2011; Pandiyan, 2010). Kobayashi et al. (2011) found AtXYP2 in mature Arabidopsis plants expressed in the branching point of the stem and in the torus of the flower, but they did not measure its expression in the mature roots. Pandiyan (2010) found AtXYP2 predominantly expressed in the roots and to a lesser extend also in other tissues of mature Arabidopsis plants. OsXYLP5 is expressed in all tissues, but most dominant in the roots, stems and in later stages also in the panicles of rice (Ma et al., 2014). AtXYP1, AtXYP2 and OsXYLP5 plays an important role in the plants vascular development (Kobayashi et al., 2011; Ma et al., 2014; Motose et al., 2004). Single knockout mutants of Arabidopsis AtXYP1 and AtXYP2 showed no morphological changes, suggesting redundant functions of these genes. Double knockout mutants of AtXYP1 and AtXYP2 resulted in defects in the vascular system like thicker veins and improper connection of the tracheary elements (Motose et al., 2004). These authors hypothesize that AtXYP1 and AtXYP2 have a coordinating, rather than a determining function in vascular development, because the effect on the vascular system is limited and is some tissues not significant. In contrast to results of Motose et al. (2004), Zhao et al. (2005) found AtXYP1 expressed in the phloem and non-vascular tissue of the primary root and hypocotyl eight weeks old Arabidopsis seedlings and not in the xylem, suggesting a possible role for AtXYP1 in phloem development.

Auxin and cytokinin are crucial for differentiation of tracheary elements (Motose et al., 2004). These authors found the XYP1 gene of *Zinnia elegans* indeed upregulated by auxin, while for accumulation of the xylogen protein both auxin and cytokinin were needed. OsXYLP5 is upregulated in rice seedling by 1-naphthylacetic acid (NAA), 6-Benzylaminopurine (6-BA) and GA (Ma et al., 2014).

Cold, drought and salt stress had no effect on the level of expression of OsXYLP5 and AtXYP1 in rice and Arabidopsis respectively (Ma et al., 2014). These authors however found the expression of AtXYP2 in roots of cold stressed Arabidopsis plants upregulated and in roots of salt stressed plants downregulated. Provart et al. (2003) found AtXYP2 in Arabidopsis shoots upregulated under cold stress, compared to control plants. These authors suggest a role for AtXYP2 as lipid transfer protein in changing cellular membranes to increase tolerance against low temperatures. Besides abiotic stress, AtXYP1 and AtXYP2 are influenced by biotic stresses. Kidd et al. (2011) found the expression of AtXYP2 in Arabidopsis leaves reduced after inoculation with *Fusarium oxysporum* compared to control plants. Trinidad Ascencio-Ibáñez et al. (2008) found AtXYP1 downregulated and AtXYP2 upregulated in Arabidopsis leaves after inoculation with the cabbage leaf curl virus.

Motose et al. (2004) found that Arabidopsis AtXYP1 and AtXYP2 *in vitro* were able to bind to stigmasterol and also weakly to brassicasterol. The physiological relevance of this ability is not clear.