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(54) **Title:** ARABINO GALACTAN PROTEINS FOR USE AS AN ANTIPARASITIC AGENT

(57) **Abstract:** The present invention relates to the use of at least one arabinogalactan protein (AGP) as a plant antiparasitic agent. AGP selectively induces chemotaxis, zoospore encystment and inhibit cyst germination. More precisely, AGP interferes in the normal process of infection of pea roots by *Aphanomyces euteiches*. It disrupts the natural and early interaction between the host and the parasite, thus preventing the host to be infected.



Arabinogalactan proteins for use as an antiparasitic agent

FIELD OF THE INVENTION

The present invention relates to the use of at least one arabinogalactan protein as
5 a plant antiparasitic agent.

BACKGROUND OF THE INVENTION

Oomycetes contain some of the most destructive pathogens of wild and domesticated plants. Among oomycetes, *Aphanomyces euteiches* is a major parasite of
10 several plants including peas, alfalfa and clover, and is a threat to the production of proteins of plant origin, for which there is an ever-increasing demand.

Aphanomyces euteiches is responsible for common root rot, including root destruction and necrotic streaking of the hypocotyl. It thus represents the most destructive soil borne disease of pea. The symptoms of the plant infection appear as
15 water soaked lesions, which develop into a straw coloured soft-rot of the cortical cells affecting the entire root system if the infection is severe. Symptoms also appear on the epicotyl, giving the lower stem a shrunken appearance. *Aphanomyces euteiches* is capable of causing disease in a range of different legumes (such as Faba bean, etch, lentil, red clover, Phaseolus bean, alfalfa) and can also complete its life cycle in some
20 plant species from other families; but pea is the crop where the pathogen causes the greatest economic damage.

A key feature of oomycetes is the asexual spore, called the zoospore, which consists of a wall-less cell equipped with two flagella, making the spore mobile in free water. Zoospores have been shown to react to a diversity of chemical and even electrical
25 signals in the environment, and in pathogenic species, this is used to locate favourable

infection sites on the plant surface and accurately dock to the host, positioning the ventral surface of the spore against the host surface.

Pea varieties showing partial resistance have been described, but the resistance does not seem to be equally effective against all strains of the pathogen, and no fully
5 resistant variety is available today for use in commercial pea cultures.

Chemical agents directed against oomycetes, such as fungicides, have thus been explored but did not provide acceptable results, especially since they have been shown to be toxic and harmful against the environment. In addition, many authors as well as environment groups are thoughtful with regards of the health consequence of using such
10 chemicals.

Therefore, due to environmental and health issues, the use of such chemicals is not appropriate and acceptable. To date, treatment for controlling oomycetes are thus ineffective and unacceptable. There is thus a long felt need for an effective prevention against infections of plant by parasites, especially pathogens belonging to the genus of
15 *Aphanomyces*.

SUMMARY OF THE INVENTION

The inventors met the burden to analyze the implication of arabinogalactan proteins (AGP) produced from root caps of plants in plant-parasite interaction and on
20 zoospore behaviour. AGP are very specific glycoproteins of the extra cellular matrix.

The cycle of infection of oomycetes includes zoospores attraction, zoospore encystment and subsequent cyst germination to initiate root infection. The inventors have shown that AGP are involved in the control of interaction of roots with parasites such as *Aphanomyces euteiches*. The inventors evidenced that, when put in contact with AGP,

the mobility of zoospores of *Aphanomyces euteiches* is significantly affected. In addition, treatment with AGP from *Pisum sativum* root caps induces a rapid and significant attraction of zoospores. The inventors have further shown that AGP triggers significant encystment of *Aphanomyces euteiches* zoospores, and surprisingly inhibits
5 the cysts to germinate, thereby preventing infection. Therefore, the invention aims to reduce the negative effects of zoospores of a parasite, such as *Aphanomyces euteiches*, in plants, such as *Pisum sativum*, thanks to arabinogalactan proteins.

The invention thus relates to the use of at least one arabinogalactan protein as a plant antiparasitic agent.

10 In one embodiment, said arabinogalactan protein is extracted from the root cap of pea, preferably of *Pisum sativum*.

In another embodiment, the invention relates to the use of at least one arabinogalactan protein as an antiparasitic agent against parasites of *Pisum sativum*.

In still another embodiment, the invention relates to the use of at least one
15 arabinogalactan protein as a plant antiparasitic agent targeting parasites belonging to the genus of *Aphanomyces*, and preferably targeting *Aphanomyces euteiches*.

DETAILED DESCRIPTION OF THE INVENTION

20 Definition

The term "**antiparasitic agent**" or "**plant antiparasitic agent**", as used herein, refers to a class of products indicated in the treatment or the prevention of parasitic infections in plants. Preferably, this term also encompasses a class of products which prevents parasitic infections in plants. Therefore, antiparasitic agents are highly useful

for controlling, alleviating and preventing the negative effects of a parasite, such as oomycetes, among economically important cultures of plants, such as cultures of peas. Preferably, in the context of the invention, an antiparasitic agent prevents infection of a plant by oomycete. Preferably, said oomycetes belong to the genus of *Aphanomyces*,
5 most preferably said parasite is *Aphanomyces euteiches*. Preferably, said plant belongs to the genus of the *Leguminosae* family, most preferably said plant is *Pisum sativum*.

By "**antiparasitic agent against parasites of *Pisum sativum***", it is meant a plant antiparasitic agent which is able to inhibit, reduce or prevent the infection of *Pisum sativum* by any parasite known to infect *Pisum sativum*.

10 The term "***Pisum sativum***", as used herein, refers to the plant producing peas, also named garden peas. *Pisum sativum* belongs to the *Leguminosae* family, also named the *Fabaceae* family.

The term, "**plant root**", as used herein, refers to the organ of a plant that typically lies below the surface of the soil. Plant roots are highly sensitive to
15 environmental factors such as moisture gradients, minerals and soil microorganisms and they are able to adapt their growth in order to maintain whole plant health. The capacity of the root system to explore the soil in response to abiotic and biotic stimuli is due to the very apical region of the root, termed the root cap. This part of the root is essential for the plant survival.

20 The expression "**root cap**", as used herein, refers to a section of tissues at the tip of a plant root. Root cap cells contain statoliths which are involved in gravity perception in plants. The root cap protects the growing tip in plants. It secretes mucilage to ease the movement of the root through soil, and may also be involved in communication with the soil microbiota. The root cap originates from the root cap meristem, which is regulated

independently from the apical root meristem to give rise to root cap cells specifically. As the cells progress and differentiate during cap development, they are specifically involved in many vital functions such as gravity sensing, hydrotropism, and synthesis of exudates. In most plant species, such as pea (*Pisum sativum*) or cotton (*Gossypium*
5 *hirsutum*), cells at the periphery of the root cap become detached as individual living cells called root border cells.

The sloughing of thousands of root cap cells into the rhizosphere is not only important in assisting the growing root to penetrate the soil but also provides a protecting sheath of living cells surrounding the vulnerable root tip against pathogens and abiotic stresses.

10 **"Arabinogalactan proteins"** or **"AGP"** are highly glycosylated proteins found on the plasma membrane, in the cell wall and in root secretions. These extracellular proteoglycans are structurally complex macromolecules due to the large branched-glycan chains containing up to 98% carbohydrate mainly *O*-linked to the Hyp residues of the protein backbone. AGP consist primarily of (1→3)-β-galactan and (1→6)-β-
15 linked galactan chains with (1→3, 1→6)- linked branched points. Other sugars such as D-glucuronic acid, L-rhamnose, L-fucose or D-xylose can also be present in the side chains carried by the (1→3)-β-galactan backbone. AGP are temporally and spatially regulated during plant development and are involved in many processes including cell-cell interaction, pollen tube guidance, cell expansion and root growth.

20 The inventors have met the burden to show that root tip arabinogalactan proteins have distinct compositional features depending on the species in which they are found.

By **"fraction of an AGP"**, it is meant an oligosaccharidic part, or both an oligosaccharidic and a protein parts of said AGP. It may comprise only a part of the oligosaccharides naturally present on said AGP.

By "**AGP extracted from the root cap**" it is meant the AGP obtained by the extraction or the isolation from the root cap and border-like cells of a plant, preferably of *Pisum sativum*. Techniques for implementing said extraction or isolation fall completely within the abilities of the person skilled in the art.

5 The expression "**AGP from the whole plant**" refers to any AGP indifferently of the origin of the AGP. For example, said AGP include AGP extracted from the whole plant, as well as AGP extracted from the root cap or from the seed. The terms "*Aphanomyces euteiches*" and "*A. euteiches*" refer to a plant pathogen or parasite. The genus *Aphanomyces* belongs to an order of oomycetes (*Saprolegniales*) that is
10 phylogenetically separated from other orders such as the *Peronosporales* and *Pythiales* where other important plant pathogens are found. The oomycete *Aphanomyces euteiches*, is often referred to as one of the most important and destructive parasite of peas, notably due to its persistence in soil and ability to rapidly destroy the crop.

The term "**oomycetes**" as used herein refers to a specific group of micro-
15 organism. Oomycetes is a group which superficially resembles fungi, but is more closely related to photosynthetic brown algae. Indeed, oomycetes resemble fungi in morphology (mycelial growth) and in the pathogenic lifestyle of many species. However, it has long been known that they differ from fungi in many physiological traits. For example, their cell wall is composed mainly of cellulose as opposed to chitin
20 in fungi. Modern DNA based phylogenetic research places the oomycetes far from true fungi in the kingdom *Stramenopila*, which also includes brown algae and diatoms. The original ancestor of the *Stramenopila clade* is believed to have been a photosynthetic organism. The photosynthetic ability was later lost by some groups, such as the oomycetes. As a group, oomycetes are mainly associated with aquatic habitats, where

both saprotrophic and parasitic lifestyles exist. The vegetative stages of their lifecycle is diploid, and both heterothallic (outcrossing) and homothallic (self fertilising) modes of reproduction exist within the group. The female sexual organ is called an oogonium, which when fertilised by the male organ (antheridium) produces a unicellular oospore.

5 Oospores are typically desiccation resistant and capable of long-term survival. In the following, oomycetes will be referred to as a "parasite" or "pathogen" even though phylogenetical analysis considered said family to be micro-organism *per se*.

The term "**zoospore**" as used herein refers to the asexual spore of oomycetes. Zoospores consist of wall-less cells equipped with two flagella, making the spore
10 mobile in free water. Zoospores possess an ability to rapidly shed their flagella and encyst, using preformed substances stored in vesicles to form a spherical walled cyst which is more protected than the motile spore. The cyst is then capable of either germinating to form a mycelium, or releasing a new secondary zoospore.

Besides oospores and zoospores, some oomycete groups have developed additional
15 reproductive organs. Sporangia are capable of germinating on their own, as well as forming and releasing zoospores. These sporangia are easily released from the mycelium and in some species function as propagules for spread of the organism by wind. As a further adaptation to life above ground, the sporangia in the genus *Peronospora* (causing downy mildew diseases) have in some species lost the ability to
20 produce and release zoospores. Another structure present in some oomycetes is the chlamydospore, which is an asexual resting spore with a thickened cell wall.

The expression "**zoospore encystement**" refers to the physiological, developmental and morphological changes that occur in the encysted zoospore.

Generally, said expression refers to a loss of motility as the flagella of the zoospore are detached.

The term "**germination**", as used herein, refers to the stage in which a parasite starts to sprout, grow and develop within a host. It represents the final step of a
5 successful infection of a host by a parasite.

The invention relates to the use of at least one arabinogalactan protein as a plant antiparasitic agent.

The inventors have assessed the impact of root cap arabinogalactan proteins on the behaviour of zoospores of *Aphanomyces euteiches*. The inventors have evidenced that
10 arabinogalactan proteins of *Pisum sativum* and *Brassica napus* root cap cells are able to induce a rapid and significant attraction of zoospores.

They have further demonstrated that in addition to chemotaxis, AGP from root caps of both species also induce a rapid and premature encystment of zoospores.

Finally, they have showed that AGP do not only trigger significant encystment of
15 *Aphanomyces euteiches* zoospores, but they do also inhibit the cysts to germinate and, thereby, prevent root infection. This is highly surprising and unanticipated since Donaldson & Deacon showed that encystment of zoospores of *Pythium ssp* can be induced by arabic gum but did not respond to arabinogalactan (Donaldson & Deacon, 1993).

20 Chemotaxis, zoospore encystment and cyst germination are important steps in the disease cycle and key determinant for the success or failure of the parasite to further colonize the plant.

Therefore, AGP are highly adapted for selectively inducing chemotaxis, zoospore encystment and inhibiting cyst germination. More precisely, AGP interfere in

the normal process of infection by *Aphanomyces euteiches*. They disrupt the natural and early interaction between the host and the parasite, thus preventing the host to be infected. Therefore, AGP application represents a highly promising strategy for preventing infection of plant, such as pea, by pathogens such as *Aphanomyces*
5 *euteiches*, especially since it avoids the use of any toxic chemical compound.

In one specific embodiment, the invention relates to the use of at least one arabinogalactan protein as a plant antiparasitic agent, wherein said arabinogalactan protein is extracted from the root cap of pea, preferably *Pisum sativum*.

Indeed, the inventors have characterized cell walls from root caps, border cells and
10 border-like cells of *Pisum sativum* and *Brassica napus*.

The inventors have found out that root caps, border cells and border-like cells were highly enriched in AGP. Significant differences in root AGP compositions between *Brassica napus* and *Pisum sativum* were revealed by cross-electrophoresis separation, western blotting and linkage analyses. After further analysis, the inventors also
15 evidenced that AGP isolated from pea root tip cells were able to selectively attract zoospores at a higher rate than AGP from *Brassica napus*. Indeed, it appears that AGP are likely part of complex molecular interactions between roots of the plant and zoospores of the parasite and as a consequence, the nature of AGP in root caps is likely to impact the relations between roots and parasites.

20 In another embodiment, the invention relates to the use of at least one arabinogalactan protein as a plant antiparasitic agent against parasites of *Pisum sativum*. Indeed, *Pisum sativum* are widely cultured and suffer from massive infections of various parasites, such as the parasites belonging to the genus selected from *Aphanomyces*, *Fusarium*, *Phoma*, *Phytophthora* or *Pythium*. Those infections lead to

extensive economical damages. By providing a new and environmental-friendly antiparasitic agent, the inventors fulfilled a long time felt need.

In a preferred embodiment, the invention relates to the use of at least one arabinogalactan protein as a plant antiparasitic agent targeting parasites belonging to the
5 genus selected from the group consisting of *Aphanomyces*, *Fusarium*, *Phoma*, *Phytophthora* and *Pythium*.

In another embodiment, the invention relates to the use of at least one arabinogalactan protein as a plant antiparasitic agent targeting parasites selected from the group consisting of *Aphanomyces euteiches*, *Fusarium solani* sp *pisi*, *Phoma*
10 *medicagines*, *Phytophthora* (*Phytophthora infestans*, *Phytophthora parasitica*) and *Pythium* spp.

In a still preferred embodiment, the invention relates to the use of at least one arabinogalactan protein as a plant antiparasitic agent targeting parasites belonging to the genus of *Aphanomyces*, preferably targeting *Aphanomyces euteiches*. Therefore, the
15 invention also relates to the use of at least one arabinogalactan protein as a plant antiparasitic agent targeting *Aphanomyces euteiches*.

The invention also relates to the use of at least one arabinogalactan protein for reducing the mobility of the zoospores of a parasite, preferably *Aphanomyces euteiches*, and/or for enhancing encystement, and/or for inhibiting germination of said zoospores.

20 Typically, the use of arabinogalactan proteins of *Pisum sativum* increases zoospore encystement of at least 60%, preferably of at least 70%, more preferably of at least 80%, and most preferably of at least 90% when compared to the zoospore encystement in absence of said arabinogalactan proteins. In a preferred embodiment, said increase occurs in about 15 minutes.

Typically, the use of arabinogalactan proteins of *Pisum sativum* decreases the rate of cyst germination of at least 40%, preferably at least 50%, preferably of at least 60%, more preferably of at least 70%, and most preferably of at least 80%, when compared to the rate of cyst germination in absence of said arabinogalactan proteins. Therefore, the
5 quantity of germinated zoospores put in contact with an AGP according the invention is less than 10%, preferably less than 8%, preferably 7%.

The invention related to the use of arabinogalactan proteins of *Pisum sativum* as a plant antiparasitic agent targeting parasites selected from *Aphanomyces euteiches*, *Phytophthora infestans* and *Phytophthora parasitica*. The invention also related the use
10 of arabinogalactan proteins of *Brassica napus* as a plant antiparasitic agent targeting parasites selected from *Aphanomyces euteiches* and *Phytophthora infestans*.

The invention also relates to arabinogalactan proteins according to the invention, wherein said arabinogalactan proteins comprise arabinose, galactose and galacturonic acid. Indeed, arabinogalactan proteins present differences in structure and composition,
15 for example depending on the species in which they are produced. The inventors have shown that arabinogalactan proteins which comprise arabinose, galactose and galacturonic acid are highly adapted for carrying out the invention. The inventors identified and characterized the carbohydrate composition of the AGP according to the invention. Without being bound to any theory, the inventors assume that the very
20 specific carbohydrate composition of the AGP is responsible for their unexpected properties and their ability to inhibit the infection of a plant by a parasite.

Preferably, said AGP have the following carbohydrate composition, in molar percentages:

- from about 18 to about 24 % of arabinose, preferably from about 19 to about 23%, preferably from about 20 to about 22%, most preferably about 21%;
- from about 6 to about 9 % of rhamnose, preferably from about 7 to about 8%, most preferably about 7.5%;
- 5 ➤ from about 1 to about 3% of fucose, preferably from about 1.5 to about 2.5%, most preferably about 2%;
- from about 12 to about 18% of xylose, preferably from about 13 to about 16%, most preferably about 15%;
- from about 16 to 20% of galacturonic acid, preferably from about 17 to about 19%, most preferably about 18%;
- 10 ➤ from about 3 to about 5% of glucuronic acid, preferably from about 3.5 to about 4.5%, most preferably about 4%;
- from about 1 to about 3% of mannose, preferably from about 1.8 to about 2.2%, most preferably about 2%;
- 15 ➤ from about 24 to about 30% of galactose, preferably from about 25 to about 29%, preferably from about 26 to about 28%, most preferably about 27%; and
- from about 2 to 5% of glucose, preferably from about 3 to 5%, preferably from 2.8 to 4.2%, most preferably about 3.5%.

The invention further relates to a composition of carbohydrates comprising, in
20 molar percentages of the said composition:

- from about 18 to about 24 % of arabinose, preferably from about 19 to about 23%, preferably from about 20 to about 22%, most preferably about 21%;
- from about 6 to about 9 % of rhamnose, preferably from about 7 to about 8%, most preferably about 7.5%;

- from about 1 to about 3% of fucose, preferably from about 1.5 to about 2.5%, most preferably about 2%;
- from about 12 to about 18% of xylose, preferably from about 13 to about 16%, most preferably about 15%;
- 5 ➤ from about 16 to 20% of galacturonic acid, preferably from about 17 to about 19%, most preferably about 18%;
- from about 3 to about 5% of glucuronic acid, preferably from about 3.5 to about 4.5%, most preferably about 4%;
- from about 1 to about 3% of galactose, preferably from about 1.8 to about 2.2%,
10 most preferably about 2%;
- from about 24 to about 30% of mannose, preferably from about 25 to about 29%, preferably from about 26 to about 28%, most preferably about 27%; and
- from about 2 to 5% of glucose, preferably from about 3 to 5%, preferably from 2.8 to 4.2%, most preferably about 3.5%.

15 In one embodiment, the invention relates to the use of said composition as a plant antiparasitic agent.

In still another embodiment, the invention relates to the use of said composition as an antiparasitic agent against parasites of *Pisum sativum*.

The invention further relates to a method for inhibiting the infection of a plant
20 comprising the step of putting in contact the root cap of said plant with at least one arabinogalactan protein.

By "**putting in contact**", it is meant a physical contact between the AGP and the plant. Typically, said physical contact may be performed by watering or spraying the AGP on culture of plants, preferably on early culture of plant highly sensitive to pathogens. Said

physical contact may also be performed by coating seeds of peas before culture of said peas. Said expression also encompasses the insertion of AGP within a plant, for example through genetic manipulation so that AGP is expressed. Said manipulations therefore lead to the expression of a higher amount of AGP by the plant. Techniques for transforming plants so as to produce AGP fall within the ability of the person skilled in the art.

In one embodiment, said arabinogalactan protein is extracted from the root cap of pea, preferably *Pisum sativum*.

Said method is challenging since it allows the acceleration of encystment of the zoospores of the parasite, as well as the inhibition of cyst germination, still being free of the use of any harmful chemical compound.

FIGURES LEGENDS

Figure 1: Western blot analysis with mAbs specific for AGP (JIM8, JIM13) and pectins (JIM5) epitopes on isolated AGP fractions and the pectin-containing extracts from *B. napus* and *P. sativum* root caps (including BLC or border cells). Molecular mass is indicated in kilodaltons (kDa) on the left.

Lanes 1 and 4 correspond to gum arabic (used as a positive control), lanes 2-5-7 and lanes 3-6-8 to isolated AGP from respectively *B. napus* and *P. sativum* root caps (including BLC or border cells), lane 9 to commercial citrus pectin (Sigma), lane 10-11 to ammonium oxalate-enriched pectin extract from *B. napus* and *P. sativum* root cap cell walls (including BLC or border cells) respectively.

Figure 2: Chemotactic response of *A. euteiches* zoospores to various substances over a period of 4h. Effects of AGP, exudates and root extracts from *B. napus* and *P. sativum* on zoospores attraction (A). Control experiments on chemotaxis using water, pectin, gum arabic and β -GlcY reagent (B).

5 *Data are means of three replicates based on 200 zoospores per replicate.*

Figure 3: Percentage of zoospores encysted at 5 and 15 minutes in the presence of AGP extracted from root caps, AGP + β -GlcY reagent, gum arabic (0.1%) or pectin. *Data are means of three replicates based on 200 zoospores per replicate.*

10

Figure 4: Germination of zoospores from cysts of *A. euteiches*. Micrographs showing the germination of zoospore cysts into *P. sativum* root exudates. bar = 10 μ m. (A). Insert showing a germinating zoospore stained with wheat-germ agglutinin (WGA) - FITC probe, bar = 5 μ m. Percentage of germinated zoospores at 1h, 2h and 4h after
15 treatment with AGP from *B. napus*, AGP from *P. sativum* or pea root exudates (B). Zoospores were first AGP-encysted for 5 min before being treated with *P. sativum* root exudates. Note that the presence of exudates partially reverted the inhibitory effect of AGP on zoospores germination (C). Data are means of three replicates based on at least 200 zoospores per replicate.

20

Figure 5: Effects on encystment and germination of *P. infestans* zoospore, in contact with:

- water;
- β -GlcY (5 mg/mL);

- arabic gum (1 mg/mL);
- AGP from the apex of peas, i.e from the root cap of peas (0,2 mg/mL);
- AGP from the apex of peas + β -GlcY;
- AGP from the whole plant of pea (0,5 mg/mL);
- 5 - AGP from the whole plant of pea (0,25 mg/mL);
- AGP from the whole plant of pea (0,125mg/mL);
- AGP from the whole plant of pea + β -GlcY;
- AGP from the seeds of pea (0,75 mg/mL);
- AGP from the seeds of peas (0,375 mg/mL);
- 10 - AGP from the seeds of peas (0,19 mg/mL); and
- AGP from the seeds of peas + β -GlcY.

Figure 6: Effects on encystment and germination of *P. parasitica* zoospores, in contact with:

- 15 - water;
- β -GlcY (5 mg/mL);
- arabic gum (1 mg/mL);
- AGP from the apex of peas, i.e from the root cap of peas (0,2 mg/mL);
- AGP from the apex of peas + β -GlcY;
- 20 - AGP from the apex of peas (0,5 mg/mL);
- AGP from the whole plant of pea (0,25 mg/mL);
- AGP from the whole plant of pea (0,125mg/mL);
- AGP from the whole plant of pea + β -GlcY;
- AGP from seeds of pea (0,75 mg/mL);

- AGP from seeds of pea (0,375 mg/mL);
- AGP from seeds of pea (0,19 mg/mL); and
- AGP from seeds of pea + β -GlcY.

EXAMPLE

Example 1 : Effect of AGP on *A. euteiches* zoospores

Materials and Methods

Plant material

5 Brassica napus (Expert variety) and Pisum sativum (Normand variety) seeds were surface sterilized and sown onto Murashige and Skoog medium containing 1.2% Bacto Agar supplemented with 3% sucrose (Durand et al., 2009). Growth conditions were identical to those described in Vicré et al. (2005). In order to avoid the roots penetrating the agar and the subsequent loss of border cells and BLC, plants were grown in
10 vertically orientated Petri dishes. Root caps, border cells and BLC were harvested from 5-day-old seedlings.

Histochemical staining on root cap cells and light microscopy

Roots were mounted on glass microscope slides in a drop of water for border cells and
15 BLC examination using bright field microscopy. Ruthenium red dye (Sigma) was used at 0.05% (w/v) in deionized water for 15 min to detect the presence of pectins. Roots were carefully washed in deionized water and observed using a bright-field microscope (Durand et al., 2009). Staining of β -glucans including cellulose was performed using calcofluor white M2R (Sigma) fluorescent probe (1mg L⁻¹) for 30 min in the dark
20 (Andème-Onzighi et al., 2002). After being carefully washed in deionized water, roots were observed using a microscope equipped with UV fluorescence (excitation filter, 359 nm; barrier filter, 461 nm). Vital staining with 5 μ M calcein-AM (Sigma) was performed as described in Vicré et al. (2005). Roots were stained for 3 hours, carefully washed in deionized water and observed using a microscope equipped with UV

fluorescence (excitation filter, 490 nm; barrier filter, 520 nm). Images were acquired with a Leica DFC 300 FX camera.

Immunofluorescence localization of AGP epitope

5 The mAbs specific for AGP used in this study are JIM13 (Yates et al., 1996), JIM8 (Knox et al., 1991) and JIM14 (Knox et al., 1991; see Table 1 below). Roots from 5-day-old seedlings were fixed for 40 min in 1% glutaraldehyde, 4 % paraformaldehyde in 50 mM Pipes, 1 mM CaCl₂, pH 7 and immunolabeled according to Willats et al. (2001). Roots were washed in phosphate-buffered-saline (PBS) containing 1% bovine
10 serum albumin (BSA) and then incubated overnight in JIM13 (1:5), JIM8 (1:5) or JIM14 (1:5) diluted in PBS-1% BSA containing 1:30 normal goat serum (NGS) as previously described in Vicré et al. (2005). Roots were carefully washed and incubated with anti-rat-IgG (dilution 1:50) coupled to fluorescein isothiocyanate (FITC; Sigma). After washing in PBS-1% BSA, roots were mounted in anti-fade agent (Citifluor; Agar
15 Scientific, Dover, UK) and examined using epifluorescence with a Zeiss Axioscope microscope.

*Table 1 Summary of the immunodot binding assays on the AGP fraction isolated from root caps of B. napus and P. sativum. Minus (-), (+/-), and (+) refer to the negative, moderate, or highly positive
20 labeling. Control samples were lime pectin (LM) and gum arabic AGP (GA).*

Antibody	Epitopes	References	<i>B. napus</i>	<i>P. sativum</i>	Controls	
					LM	GA
JIM5	Partially Me-HG/de-esterified HG	Willats et al. (2000)/ Clausen et al. (2003)	+	+	+	-
JIM7	Partially Me-HG	Willats et al. (2000)/ Clausen et al. (2003)	+	+	+	-
LM6	(1→5)- α -L-arabinan (may also bind to AGPs)	Willats et al. (1998)	+	+	+	+/-
LM8	Xylogalacturonan associated epitope	Willats et al. (2004)	+	+	+/-	-
JIM13	β -D-Glc p A-(1→3)- α -D-Gal p A-(1→2)-L-Rha	Yates et al. (1996)	+	+	-	+
JIM8	Carbohydrate portion of AGPs	Knox et al. (1991)	+	+	-	+
JIM14	Carbohydrate portion of AGPs	Knox et al. (1991)	+	+	-	+

Isolation of cell wall material

Root caps (100 mg) were frozen with liquid nitrogen and ground into a fine powder.

- 5 The powder was treated sequentially with boiling EtOH 90% (2 X 30 min), chloroform and MeOH 95% (overnight), MeOH 100% (4 h) and acetone (overnight) according to Aboughe-Angone et al. (2008). The cell wall residue was freeze-dried and kept until use.

10 *Isolation of AGP from root caps and border cells/BLC*

- AGP were isolated by precipitation with the β -GlcY reagent (Biosupplies Australia Pty, Melbourne, Australia) as previously described in Ding & Zhu, (1997). Root caps (including BLC or border cells) from *B. napus* and *P. sativum* were ground to a fine powder in liquid nitrogen and incubated in extraction buffer (50 mM Tris-HCl (pH 8.0), 15 10 mM EDTA, 0.1% β -mercaptoethanol and Triton X-100 1% (w/v) for 16 h at 4°C. The supernatant was mixed by addition of five volumes of ethanol 95 % (v/v) overnight

at 4°C and the precipitate was suspended in 10 mL of 50 mM Tris-HCl pH 8.0. After being dialysed and freeze dried, the AGP-containing material was dissolved in 1% (w/v) NaCl and AGP were precipitated by addition of an equal volume of a solution of 2 mg.mL⁻¹ β -GlcY reagent in 1% (w/v) NaCl (adapted from Gane et al., 1995). The mixture was left to precipitate overnight at 4°C and the AGP- β -glucosyl Yariv complex was collected by centrifugation at 9000 rpm for 1 h. The pellet was washed three times with 1% (w/v) NaCl to remove excess β -GlcY reagent and three times with MeOH. The pellet was dried and dissolved in 500 μ L DMSO and sodium dithionite was added to 10% (w/v). Fractions containing AGP were pooled and dialyzed overnight against deionized water at 4°C and lyophilized.

Monosaccharide analysis

The sugar composition of cell wall material and AGP fractions were determined by gas chromatography (GC) analysis of their trimethyl-silyl methylglycosides according to York *et al.* (1985). Samples were hydrolyzed in 2 M trifluoroacetic acid for 2 h at 110°C and mixed with methanol/ HCl 1M for methanolysis at 80°C for 24 h. Inositol was used as internal standard. Samples were trimethylsilylated with Tri-Sil for 30 min at 80°C and suspended in cyclohexane before being injected on a DB-1 column (DB-1 Supelco). Chromatographic data were integrated with GC Star Workstation software (Varian), each surface being corrected according to its response factor. Sugars detected by GC were arabinose (Ara), fucose (Fuc), galactose (Gal), galacturonic acid (GalA), glucose (Glc), glucuronic acid (GlcA), mannose (Man), rhamnose (Rha) and xylose (Xyl).

Immunoblots

The mAbs used in this study are presented in Table 1. Samples (25 µg) of AGP were blotted on nitrocellulose membranes (Whatman, protan BA 83 nitrocellulose 0.2 µm) and were blocked with 5% (w/v) low fat-dried milk in tris-buffered saline (TBS) (Tris 20 mM, NaCl 500mM pH 7.5). Samples were incubated with primary antibody (JIM5, JIM7, LM6, LM8, JIM13, JIM8 and JIM14) diluted 1:10 in TBS for 2 h. After four washes with TBS containing 1% Tween 20 (TTBS), blots were incubated for 2 h with anti-rat IgG peroxylase conjugate (Sigma) diluted 1:50 in TBS containing 5% low fat milk. After four washes with TTBS followed by one wash with TBS, enzyme activity was visualized with H₂O₂ (30% (v/v) diluted in TBS) and methanol 4-chloronaphtol HRP Color Reagent.

Extraction of pectins from root caps and border cells/BLC

Root caps from *B. napus* and *P. sativum* were ground to a fine powder in 70% ethanol using a pestle and mortar and suspended in boiling ethanol for 1h. The powder was subjected to 0.8% boiling ammonium oxalate for 1h and the oxalate extracts were lyophilized.

SDS-PAGE analysis and electroblotting

AGP were separated on 8% polyacrylamide minigels (Bio-Rad). Electroblotting were performed as described previously (Willats & Knox, 1996). Blots were probed with the anti-AGP mAbs JIM13 and JIM8 and the anti- homogalacturonan JIM5.

Radial β-GlcY reagent gel diffusion

A 1% agarose gel containing NaCl 0.15 M, 0.02% (w/v) NaNO₃ and β -GlcY reagent (10 μ g/mL) was prepared according to Van Holst & Clarke (1986). The wells were filled with AGP (1 μ g) and incubated overnight at room temperature. Gum arabic from acacia (Fischer Scientific) was used as a standard.

5

Rocket and cross electrophoresis analysis of AGP

Rocket gel electrophoresis was run with 1% agarose containing 90 mM Tris (pH 8.3), 90 mM boric acid, 2 mM EDTA and β -GlcY reagent (20 μ g.mL⁻¹). Aliquots of 0.5 μ g of AGP were loaded and run for 16 h at 10 mA and the gels were rinsed with 2% (w/v) NaCl. Crossed electrophoresis was performed according to Van Holst & Clarke (1986). Samples were run in the first dimension as described above, then the lanes were cut out and run in the second dimension in a gel with 30 μ g.mL⁻¹ β -GlcY reagent for 16 h at 5 mA (Ding & Zhu, 1997; Girault *et al.*, 2000).

15 *Sugar linkage analysis*

AGP were methylated using potassium methylsulfinylmethanide (200 μ l, 2.5 M in DMSO as described in Ishii *et al.* (1999). The permethylated polysaccharides were hydrolysed with 2.0 M TFA at 121 °C for 1 h, reduced with 1 M NaBD₄ in 1 M NH₄OH for 1 h at room temperature and acetylated using acetic anhydride at 121°C for 3 h. The partially methylated alditol acetates were analysed by gas chromatography coupled to an electron impact mass spectrometry (GC–MS) using SP-2330 columns.

20

Zoospore production and experiments on zoospore chemotaxis.

Experiments were performed using the French reference pea isolate *A. euteiches* (RB84) kindly provided by B. Tivoli (INRA Rennes, France) previously described as being highly virulent. Zoospores were produced according to Moussart *et al.* (2001). The zoospores were used for the experiments at a concentration of about 1×10^5 zoospores ml^{-1} water. Zoospores could be observed microscopically moving freely on the surface. Effects of root cap AGP and exudates on chemotaxis of *A. euteiches* were performed by spreading zoospores suspension (20 μl containing 500 zoospores) onto the surface of glass slides (adapted from Zhao *et al.* 2000). AGP or root exudates was added to the opposite side of the slide. The treatments included water, pectin, AGP from *B. napus*, AGP from *P. sativum*, root exudates from *B. napus* or *P. sativum*, *B. napus* root and *P. sativum* roots. To test the specificity of AGP, 5 μM β -GlcY reagent was used as control. Three replicare slides were icluded for each treatment and the whole test was repeated three times.

Zoospore production and experiments on zoospore chemotaxis and encystment and cyst germination.

Zoospores suspension (20 μl) was added to 20 μl of a test substance on a slide as previously described (Deacon & Saxena, 1998). The following substances were tested for stimulation of zoospore encystment and cyst germination: citrus pectin (1mg/mL, Sigma), 0.1% gum Arabic, AGP from *P. sativum* root caps (200 $\mu\text{g.mL}^{-1}$), AGP from *B. napus* root caps (200 $\mu\text{g.mL}^{-1}$), 5 μM β -GlcY reagent, *P. sativum* and *B. napus* root seedlings. Observations were made with a 40x objective and images were acquired with a Leica DFC 300 FX camera. Data are means of three replicates based on at least 200 zoospores per replicate.

Statistical analysis

Significant differences ($p < 0.05$) were calculated by using one-way analysis of variance (ANOVA) and the Newman-Keuls test.

5

Results

*Formation of border-like cells in *B. napus* and border cells in *P. sativum*.*

Microscopical examination of *B. napus* root tips revealed the presence of layers of cells surrounding the root cap. While *P. sativum* is known to release large numbers of individual root border cells (Hawes *et al.*, 2000), *B. napus* produced BLC that do not disperse into solution and remained attached to each other as previously described for *A. thaliana*. A few BLC appeared first at the root apex in 2 day-old seedlings and their number increased to reach 370 ± 115 cells per root tip in 6 day-old plants corresponding to a root length of 89 ± 10 mm. As the presence of mucilage at the root tip could not be detected by direct observation using bright field microscopy, the inventors used ruthenium red, a dye that stains acidic polymers (*e.g.* pectins) known to be generally secreted among root exudates. The dye revealed a quite abundant mucilage associated with border cells of *P. sativum*, but not with BLC produced by *B. napus* root caps. This result is consistent with the previous observation made on BLC of *A. thaliana* that have been shown to be covered only by a thin layer of mucilage (Durand *et al.*, 2009). In order to check for BLC viability in *B. napus*, the inventors used Calcein-AM as previously described in Vicié *et al.* (2005). BLC were strongly stained indicating that these cells were still viable at the time of their detachment from the root cap.

20

Monosaccharide composition of B. napus and P. sativum root cap cell walls.

Monosaccharide composition of the cell wall isolated from *B. napus* and *P. sativum* root caps showed that arabinose, (respectively 28 ± 3.2 % and 29.5 ± 3.5 %) was the most abundant monosaccharide (Table 2 below). Galactose (respectively 21 ± 2.8 % and 12 ± 1.8 %) and galacturonic acid (respectively 15 ± 2.3 % and 14 ± 1.9 %) were also present in significant amounts from both species. Together these data indicate the presence of homogalacturonan, rhamnogalacturonan I and possibly AGP. Although sugar composition of cell wall material is indicative of the presence of pectin, AGP are usually present in such cell wall fractions (Vicré *et al.*, 2004).

Monosaccharide composition analyses revealed that the level of galactose was specifically lower in *P. sativum* (12 ± 1.8 %) when compared to *B. napus* (21 ± 2.8 %) root caps. Rhamnose was present at similar levels in *P. sativum* and *B. napus* apex and at relatively low level (respectively 8 ± 1.1 % and 7 ± 1.4 %) compared to galactose. These data indicate that the higher galactose content in *B. napus* apex could be related to AGP rather than pectins. Indeed, the presence of AGP in the cell wall material was confirmed using β -GlcY reagent staining.

Table 2 Monosaccharide composition of cell wall extracts from *B. napus* and *P. sativum* root caps. The values are expressed as the average of the mol percentage of three independent experiments. The mol percentage is based on the value for the individual sugar divided by the total of the seven neutral sugars and the uronic acids. The standard errors are indicated for each value.

	<i>B. NAPUS</i>	<i>P. SATIVUM</i>
Sugar	mol %	mol %
Arabinose	28 ± 3.2	29.5 ± 3.5
Rhamnose	7 ± 1.4	8 ± 1.1
Fucose	1 ± 0.2	1 ± 0.3

Xylose	14 ± 2	13 ± 1.8
Galacturonic acid	15 ± 2.3	14 ± 1.9
Glucuronic acid	1 ± 0.4	1 ± 0.2
Mannose	6 ± 1	5 ± 0.6
Galactose	21 ± 2.8	12 ± 1.8
Glucose	7 ± 3.2	16 ± 2.4

Distribution of AGP epitopes at the cell surface of BLC and root border cells.

Developmentally regulated expression of AGP epitopes in roots has been reported in a number of studies using immunocytochemistry (Pennel *et al.*, 1989; Knox *et al.*, 1990; Dolan *et al.*, 1995; Andème-Onzighi *et al.*, 2003). An immunocytochemical analysis using a set of mAbs directed against specific AGP epitopes was carried out to investigate their occurrence at the cell surface of root caps and BLC from *B. napus* and isolated border cells from *P. sativum*. Epitopes recognized by mAbs JIM13, JIM8 and JIM14 were detected at the cell surface of BLC from *B. napus*. A similar pattern of labeling was observed over the cell wall of border cells in *P. sativum*.

Carbohydrate composition of the β -GlcY reagent-precipitable AGP.

Most AGP can be specifically precipitated using β -GlcY reagent (Popper 2011). In order to characterize the carbohydrate composition of AGP in root cap cells, total AGP were extracted using β -glucosyl Yariv (β -GlcY) reagent as described in Ding and Zhu (1997). As shown in Table 3 below, the overall monosaccharide content was similar in *B. napus* and *P. sativum* root caps. The isolated AGP from both species contained a high proportion of Gal (respectively $37 \pm 3.9\%$ and $27 \pm 3\%$) and Ara (respectively $23 \pm 2.6\%$ and $21 \pm 2.3\%$) comprising 60% and 48% of the monosaccharide constituents,

respectively. The Ara : Gal ratio was found to be 0.62 and 0.78 for *B. napus* and *P. sativum*, respectively. Interestingly, Gal content was significantly higher in *B. napus* than in *P. sativum* root cap with minor amounts of Man (respectively $4 \pm 0.5\%$ and $2 \pm 0.2\%$) and Rha (respectively $2 \pm 0.4\%$ and $7.5 \pm 1.2\%$). Surprisingly, monosaccharides
 5 analysis also revealed significant amounts of GalA (respectively $16 \pm 1.9\%$ and $18 \pm 1.9\%$) arguing for the presence of pectins in the AGP extracts.

Table 3 Monosaccharide composition of AGP extracts from *B. napus* and *P. sativum* root caps. The values are expressed as the average of the mol percentage of three independent experiments. The mol
 10 percentage is based on the value for the individual sugar divided by the total of the seven neutral sugars and the uronic acids. The standard errors are indicated for each value.

	<i>B. napus</i>	<i>P. sativum</i>
Sugar	mol %	mol %
Arabinose	23 ± 2.6	21 ± 2.3
Rhamnose	2 ± 0.4	7.5 ± 1.2
Fucose	1 ± 0.1	2 ± 0.3
Xylose	15 ± 2	15 ± 2
Galacturonic acid	16 ± 1.9	18 ± 1.9
Glucuronic acid	1 ± 0.3	4 ± 0.5
Mannose	4 ± 0.5	2 ± 0.2
Galactose	37 ± 3.9	27 ± 3
Glucose	1 ± 0.2	3.5 ± 0.7

Presence of pectins in the β -GlcY reagent-precipitable AGP.

To check for the occurrence of pectin in AGP extracts, the inventors performed
 15 immunodot blot assays using mAbs specific for pectic epitopes. The results are schematically represented in Table 1. The presence of homogalacturonan was confirmed by immunoblot binding assays using the mAbs JIM5 and JIM7 specific for

homogalacturonan epitopes. The presence of pectins in AGP extracts indicates interactions between AGP and pectins. Although β -GlcY reagent has been widely used to precipitate AGP, the inventors can not rule out the possibility of extracting pectins. In order to provide additional information on the presence of pectins, AGP fractions were
5 separated by SDS-PAGE and probed with anti-AGP and anti-pectin mAbs on Western blots. Figure 1 shows that JIM8 reacted with a smear of material ranging from 154 to 169 kDa in AGP fractions isolated from both *B. napus* and *P. sativum*. JIM 13 detected various bands with sizes ranging from 70 kDa to more than 170 kDa with different patterns between *B. napus* and *P. sativum*. Interestingly, both JIM13 and JIM5
10 antibodies recognized the same 85 kDa band on Western blots of AGP extracts. Furthermore, pectin fractions were extracted with ammonium oxalate from root caps and border cells/ BLC, separated by SDS-PAGE, subjected to Western blotting and probed with the mAb JIM5. The data clearly demonstrated the presence of a JIM5-reactive smear of 114-170 kDa in size. It is interesting to note that the single JIM5-
15 detected band observed in the AGP extracts does not coincide with the smear observed in the pectin-enriched fraction. These data strongly indicate that homogalacturonan found in the AGP fractions are likely to be crosslinked to AGP rather than being precipitated by β -GlcY reagent.

20 *Characterization of AGP from root caps using electrophoretic methods.*

Total AGP extracts from *B. napus* or *P. sativum* root caps and border cells/ BLC were quantified by rocket gel electrophoresis. The amounts of AGP were estimated to be $0.20 \pm 0.03 \text{ mg.g}^{-1}$ dry weight of root caps. Interestingly, electrophoresis performed on agarose gel revealed different populations of AGP from root cap samples of both plant

species. The inventors have identified two AGP populations of high and low molecular weights in *P. sativum* tips whereas only a low molecular weight fraction was present in *B. napus* samples. Differences between AGP isolated from *P. sativum* and *B. napus* were confirmed by β -GlcY reagent crossed electrophoresis. There further evidenced differences in the native electrophoresis mobility of AGP. Cross electrophoresis profiles indicated distinct AGP patterns with two peaks clearly discerned in AGP extracts from *P. sativum* root caps. The first peak corresponds to AGP having the highest electrophoretic mobility (Rf 0.92) as compared to the second peak (Rf 0.5). AGP from *B. napus* were characterized by having a Rf value of 0.8-0.93. Thus AGP from *B. napus* root caps were qualitatively different from *P. sativum* upon β -GlcY reagent crossed electrophoresis.

Linkage analysis of AGP by methylation.

Sugar linkage analysis revealed that the AGP contained a branched backbone of 3-, 6-, and 3,6-linked Gal residues and the presence of terminal Gal (Table 4 below). Ara was mostly present in the furanose form in both species as terminally-linked Araf and 5-linked Araf residues. In addition appreciable amounts of 2,4 and 2-linked Rha and smaller amounts of terminal Fuc, terminal Xyl, 4-Xyl and 4-Man were also detected, indicating a possible occurrence of RG-I, xylan, and mannan in AGP fractions. Noticeable differences were observed in the linkage patterns of Ara and Gal between root caps (including BLC and border cells) from respectively *B. napus* and *P. sativum* (Table 4). Whereas root cap AGP from both species have similar monosaccharide composition, the nature of linkage is significantly distinct reflecting differences in their structure.

Table 4 Linkage analysis of AGP isolated from root caps (including BLC and border cells) of *B. napus* and *P. sativum*.

	B. napus	P. sativum
Linkage	mol %	mol %
T-Araf	9	3
T-Arap	1	3
5-Araf	15	7
2-Rha	1	6
2,4-Rha	9	3
T-Fuc	4	3
T-Xyl	3	3
4-Xyl	8	10
T-Gal	8	9
3-Gal	12	33
6-Gal	11	7
3,6-Gal	14	3
4-Man	4	9

5 *Chemotactic responses of Aphanomyces euteiches zoospores to root cap AGP.*

A. euteiches is responsible for common root rot, the most destructive soil borne disease of pea. There is no prevalent pathogenic oomycete inducing such root diseases in *B. napus* crops. Therefore, the inventors took advantage of the *P. sativum* - *A. euteiches* pathosystem to investigate the impact of isolated AGP from root caps (including BLC
10 and border cells from respectively *B. napus* and *P. sativum*) on zoospore behaviour. The cycle of infection of oomycetes includes zoospores attraction, zoospore encystment (which corresponds to a loss of motility as the flagella are detached) and subsequent cyst germination to initiate root infection (Hardham and Suzaku, 1986; Tyler 2002). The inventors performed a quantitative chemotaxis test to assess the mobility of
15 zoospores in the presence of isolated AGP, whole root exudates or whole seedlings of

both *B. napus* and *P. sativum* species (Fig. 2). When placed onto slides in a drop of water only and observed under the microscope, zoospores of *A. euteiches* moved randomly without any preferential direction. In contrast when AGP fractions were used, the random mobility of zoospores was significantly affected. Whereas treatment with *B.*
5 *napus* AGP had little effect on zoospore mobility ($28 \pm 3\%$), treatment with AGP from *P. sativum* root caps induced a rapid and significant attraction of zoospores ($58 \pm 3\%$). When AGP fractions were replaced by whole seedlings, the majority of zoospores ($84 \pm 4\%$) were attracted to pea root tips, whereas a relatively small proportion ($44 \pm 6\%$) of the zoospores moved toward *B. napus* roots. Treatment with the β -GlcY reagent, that
10 binds and precipitates AGP, reduced the attractiveness of both root exudates and pea AGP to zoospores by 11% and 26% respectively. Control experiments using pectin and gum arabic as stimuli did not show any attraction of zoospores by both polysaccharides (Fig. 2b).

15 *Effects of AGP from root on A. euteiches zoospore encystment and cyst germination.*

When suspended in water, *A. euteiches* zoospores remained motile and did not encyst immediately. The time course of encystment and germination of zoospores in water revealed that 80 % of zoospores encysted within 4h but the cysts did not germinate autonomously. The inventors compared the *in vitro* encystment response of *A. euteiches*
20 zoospores to exogenous application of AGP isolated from root caps of *B. napus* or *P. sativum*, root exudates, gum arabic and pectins. When placed in the presence of AGP fractions, about 50% of motile zoospores became immobilized while losing their flagella within only 5 minutes (Fig. 3). After 15 min of treatment with AGP from *B. napus* or *P. sativum*, the observed encystment increased by 16% and 25%, respectively

(Fig. 3). *P. sativum* root exudates significantly increased zoospore encystment (79 ± 7 % after 15 minutes). Treatment with the β -GlcY reagent caused a significant reduction of zoospore encystment confirming the effect of AGP. Gum arabic has been previously reported to induce encystment of *A. euteiches* (Deacon & Saxena, 1998) and was also effective under our conditions (Fig. 3). In contrast, treatment with citrus pectin did not induce any significant effect on zoospores encystment.

Although AGP from root caps of both species accelerate zoospores encystment, they did not trigger the subsequent germination of the cysts. As shown in figure 4, zoospores remained quiescent after 4h incubation with AGP from *P. sativum* or *B. napus* root caps. The inventors observed that cyst germination could not occur as long as AGP were present (observation made during 24h). In contrast to the effect of isolated AGP, cyst germination could be induced in the presence of root exudates of both species although at different rates. The rate of cyst germination increased over time (Fig. 4) and was much higher in the presence of pea root exudates (70 ± 3 % at 4 hours) than in the presence of those of *B. napus* (only 22 ± 4 % at 4 hours). Interestingly, the inhibition of cyst germination by AGP could be partially reverted upon treatment with root exudates of both species (Fig. 4).

Exemple 2: Effects of AGP from root cap, from seeds and from the whole plant on *Phytophthora spp* zoospore encystment and cyst germination.

The inventors further investigated the effect of AGP from the apex of *P. sativum* on *Phytophthora spp*, more precisely, on:

-*Phytophthora infestans*, and

-*Phytophthora parasitica*.

Oomycetes of *Phytophthora infestans* and *Phytophthora parasitica* are among the most devastating pathogens in plants. More specifically, *Phytophthora spp.* is responsible for considerable economic damages to important crop species such as potato, tomato, pepper, soybeans, and alfalfa as well as environmental damages in natural ecosystems.

5 Applying the same protocols than those described in example I, the inventors evidenced that :

- i) AGP from root cap of *P. sativum* interferes with the development of zoospores of *Phytophthora spp.*,
 - ii) the AGP from seed and AGP from the whole plant also show a biological effect of
- 10 inhibiting cyst germination of *P. infestans*.

Example 3: Effect of AGP from *P. sativum* (pea) and *B. napus* (canola) on *Phytophthora infestans*

Zoospores of *P. infestans* were encysted to 17% after 15 min in the presence of water

15 (negative control), and nearly 40% germinated after 4 h.

The inventors tested:

- AGP extracted from the apex of pea, i.e from the root cap of pea (0.2 mg/L),
- AGP extracted from the apex canola, i.e from the root cap of canola (0.2 mg/mL),
- 20 - AGP from the whole plant of pea, and
- AGP from the seeds of peas.

As shown in Figure 5, they evidenced that all of these AGP accelerate encystment of zoospores.

In addition, they showed that at an equivalent concentration (0.2 mg / mL), the AGP from the whole plant induce the higher rate of zoospore encystment. Indeed, 90% of the zoospores were found to be encysted after 15 min.

As for the germination rate, the inventors showed that all the tested AGP have an inhibitory effect after 4h, with a more pronounced effect (6.63% of germination) for the AGP from the whole plant.

They further observed a dose-dependent effect. Indeed, if one uses AGP from the whole plant of pea at a concentration of 0.125 mg / mL, the germination rate is about 13.33% , which is, which is still less than the control.

Therefore, the inventors have shown that AGP are highly suitable for use as antiparasitic agent against parasites of *Phytophthora infestans*.

Example 4: Effect of AGP from *P. sativum* and *B. napus* on *Phytophthora parasitica*

Zoospores of *P. parasitica* behave in the presence of water (negative control) similarly to *P. infestans*.

However, as shown in Figure 6, they are encysted more slowly, with 6% of encystment after 15 min and 43% germination after 4 h.

The inventors tested:

- AGP extracted from the apex of pea, i.e. from the root cap of peas (0.2 mg/mL);
- AGP extracted from the apex of canola, from the root cap of canola (0.2 mg/mL);
- AGP from the whole plant of pea; and
- AGP from the seeds of peas.

The inventors have shown that AGP extracted from the apex of peas, AGP from the whole plant, and AGP from the seeds of peas accelerate encystment of zoospores of *P. parasitica*. They observed that AGP extracted from the apex of pea provide the highest encystment rate (91,8%).

- 5 However, it appears that AGP extracted from the apex of canola showed no effect on the encystment of *P. parasitica*.

Therefore, the inventors have shown that AGP extracted from the root cap of pea, from the whole plant of pea and from the seeds of peas are highly suitable for use as antiparasitic agent against parasites of *Phytophthora infestans*.

CLAIMS

1. Use of at least one arabinogalactan protein or one of its fractions as a plant antiparasitic agent.
- 5 2. Use according to claim 1, wherein said arabinogalactan protein is extracted from the root cap of pea, preferably *Pisum sativum*.
3. Use according to claim 1 or 2, as an antiparasitic agent against parasites of *Pisum sativum*.
- 10 4. Use according to any one of claims 1 to 3, as a plant antiparasitic agent targeting parasites belonging to the genus selected from the group consisting of *Aphanomyces*, *Fusarium*, *Phoma*, *Phytophthora* and *Pythium*.
- 15 5. Use according to any one of claims 1 to 4, as a plant antiparasitic agent targeting parasites selected from the group consisting of *Aphanomyces euteiches*, *Fusarium solani* sp *pisi*, *Phoma medicagines*, *Phytophthora*, *Phytophthora infestans*, *Phytophthora parasitica* and *Pythium* spp.
- 20 6. Use according to any one of claims 1 to 4, as a plant antiparasitic agent targeting *Aphanomyces euteiches*.

7. Use according to any one of claims 1 to 6, wherein said at least one arabinogalactan protein comprises arabinose, galactose and galacturonic acid.

8. A composition of carbohydrates comprising, in molar percentages of said
5 composition:

- from about 18 to about 24 % of arabinose, preferably from about 19 to about 23%, preferably from about 20 to about 22%, most preferably about 21%;
- from about 6 to about 9 % of rhamnose, preferably from about 7 to about 8%, most preferably about 7.5%;
- 10 ➤ from about 1 to about 3% of fucose, preferably from about 1.5 to about 2.5%, most preferably about 2%;
- from about 12 to about 18% of xylose, preferably from about 13 to about 16%, most preferably about 15%;
- from about 16 to 20% of galacturonic acid, preferably from about 17 to about 19%, most preferably about 18%;
- 15 ➤ from about 3 to about 5% of glucuronic acid, preferably from about 3.5 to about 4.5%, most preferably about 4%;
- from about 1 to about 3% of mannose, preferably from about 1.8 to about 2.2%, most preferably about 2%;
- 20 ➤ from about 24 to about 30% of galactose, preferably from about 25 to about 29%, preferably from about 26 to 28%, most preferably about 27%; and
- from about 2 to 5% of glucose, preferably from about 3 to 5%, preferably from 2.8 to 4.2%, most preferably about 3.5%.

9. Use of the composition according to claim 8 as a plant antiparasitic agent.

10. Use of the composition according to claim 8 as an antipathogenic agent against parasites of *Pisum sativum*.

5

11. Method for inhibiting the infection of a plant comprising the step of putting in contact the root cap of said plant with at least one arabinogalactan protein.

12. Method according to claim 11, wherein said arabinogalactan protein is extracted
10 from the root cap of pea, preferably *Pisum sativum*.

FIGURES

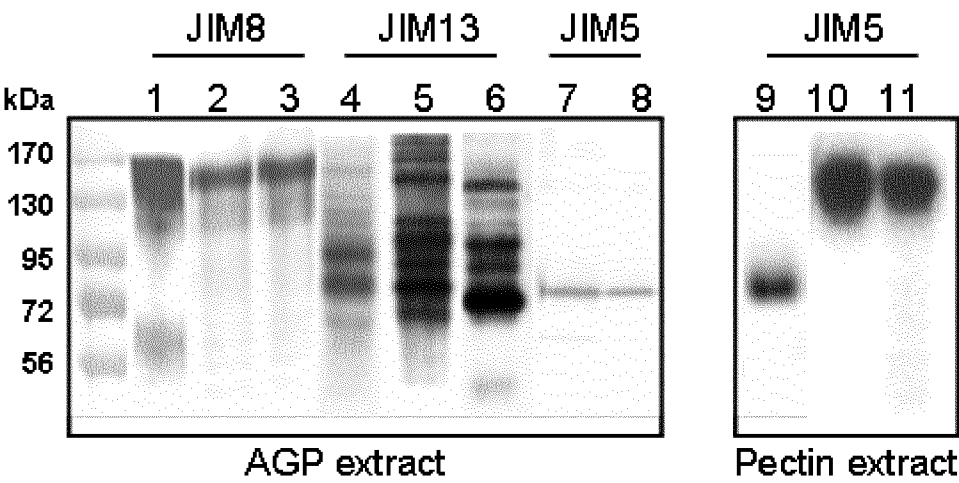
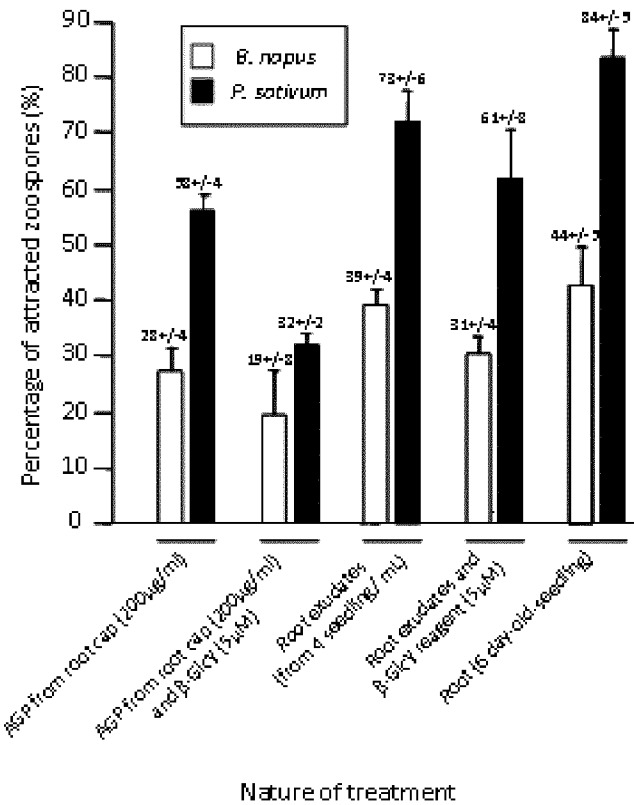


Fig. 1

A



B

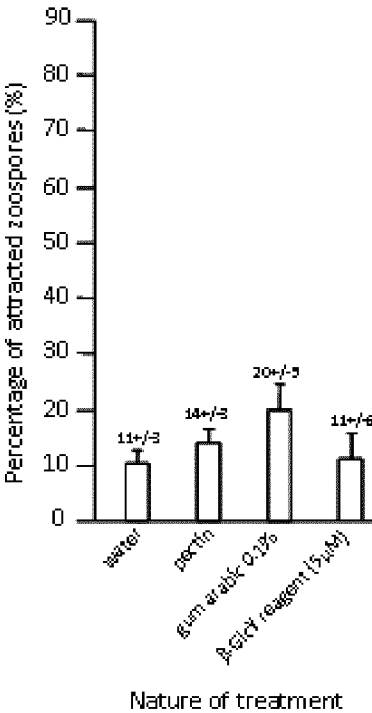


Fig. 2

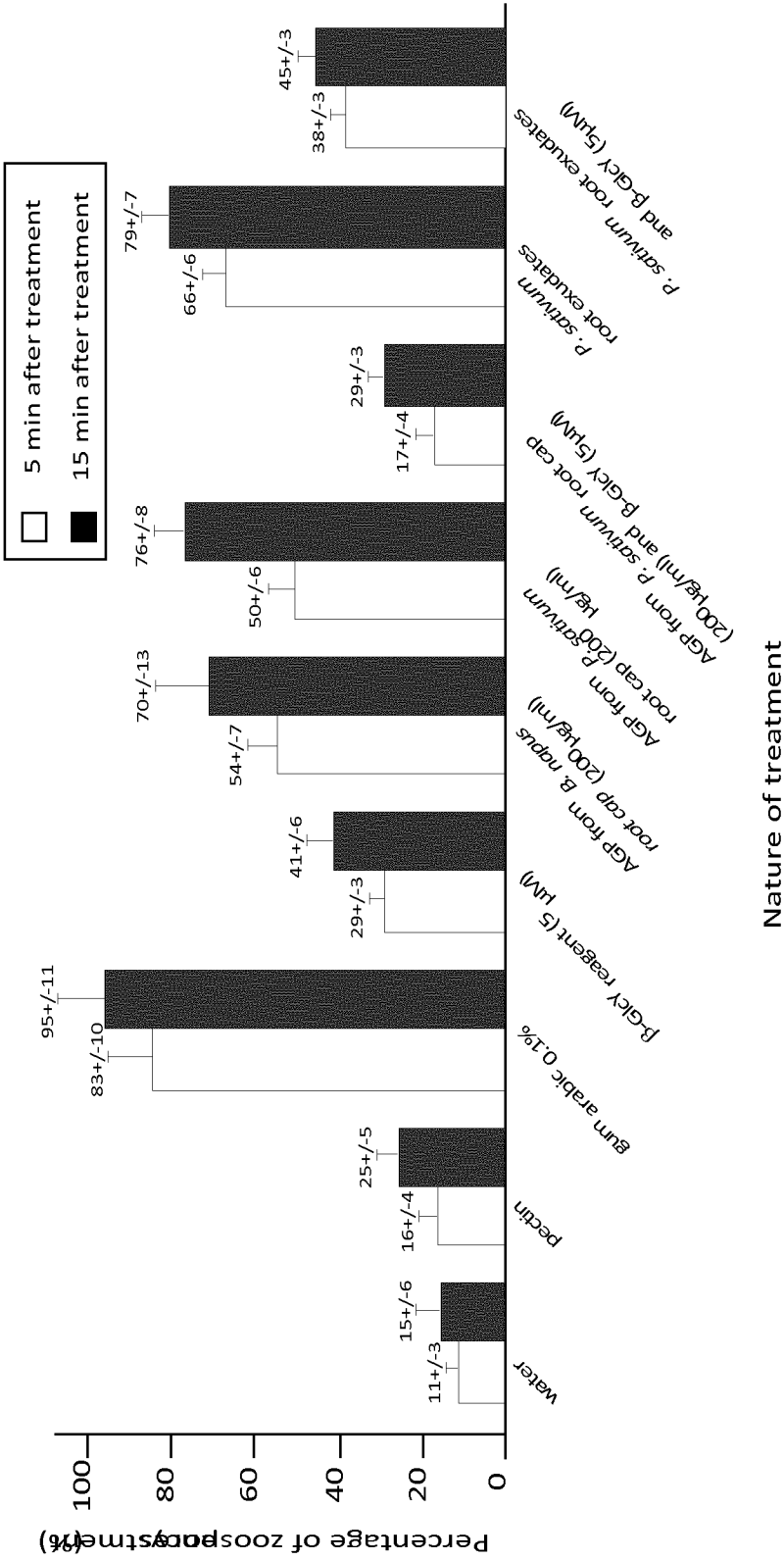


Fig. 3

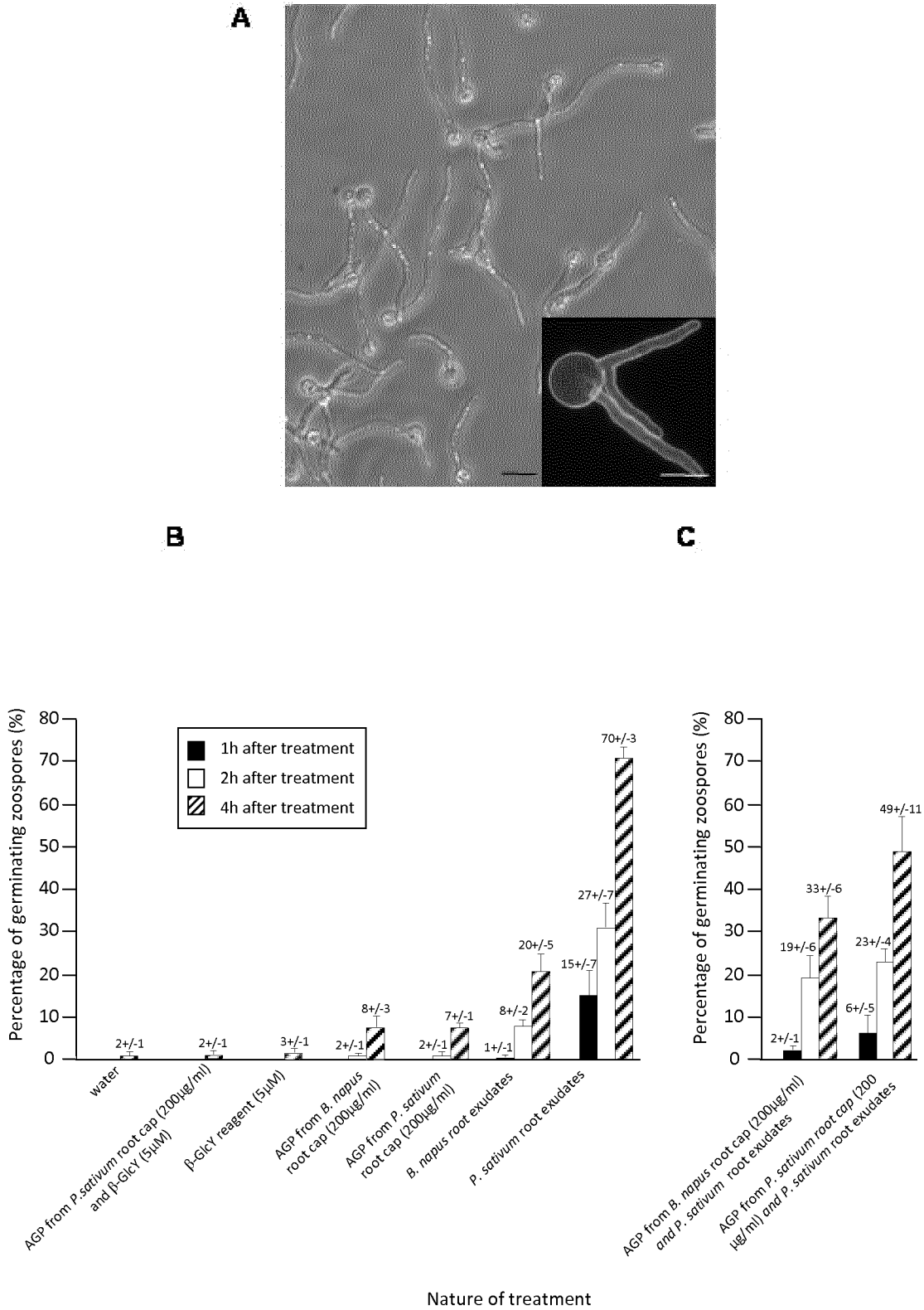


Fig. 4

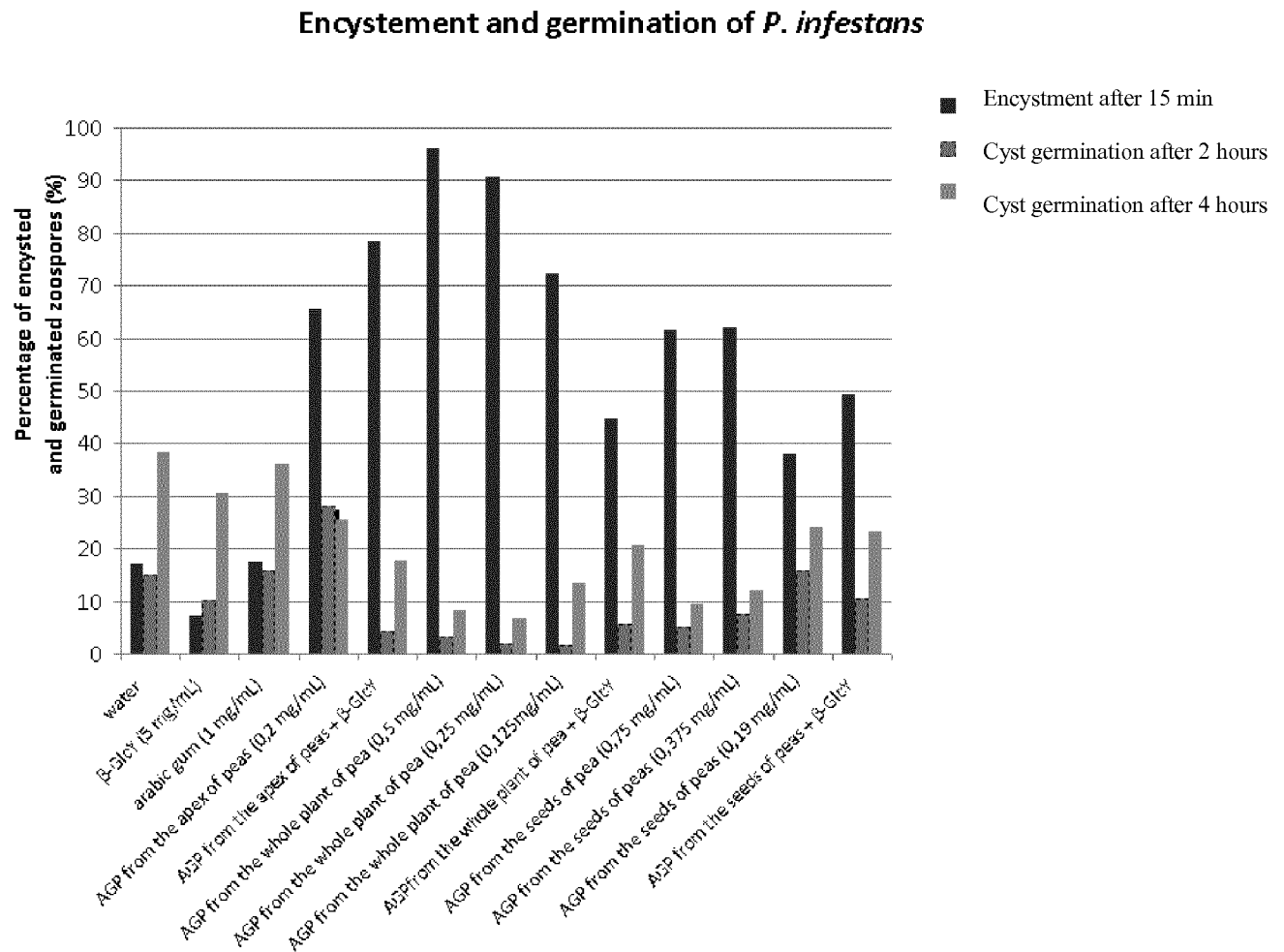


Figure 5

Encystment and germination of *P.parasitica*

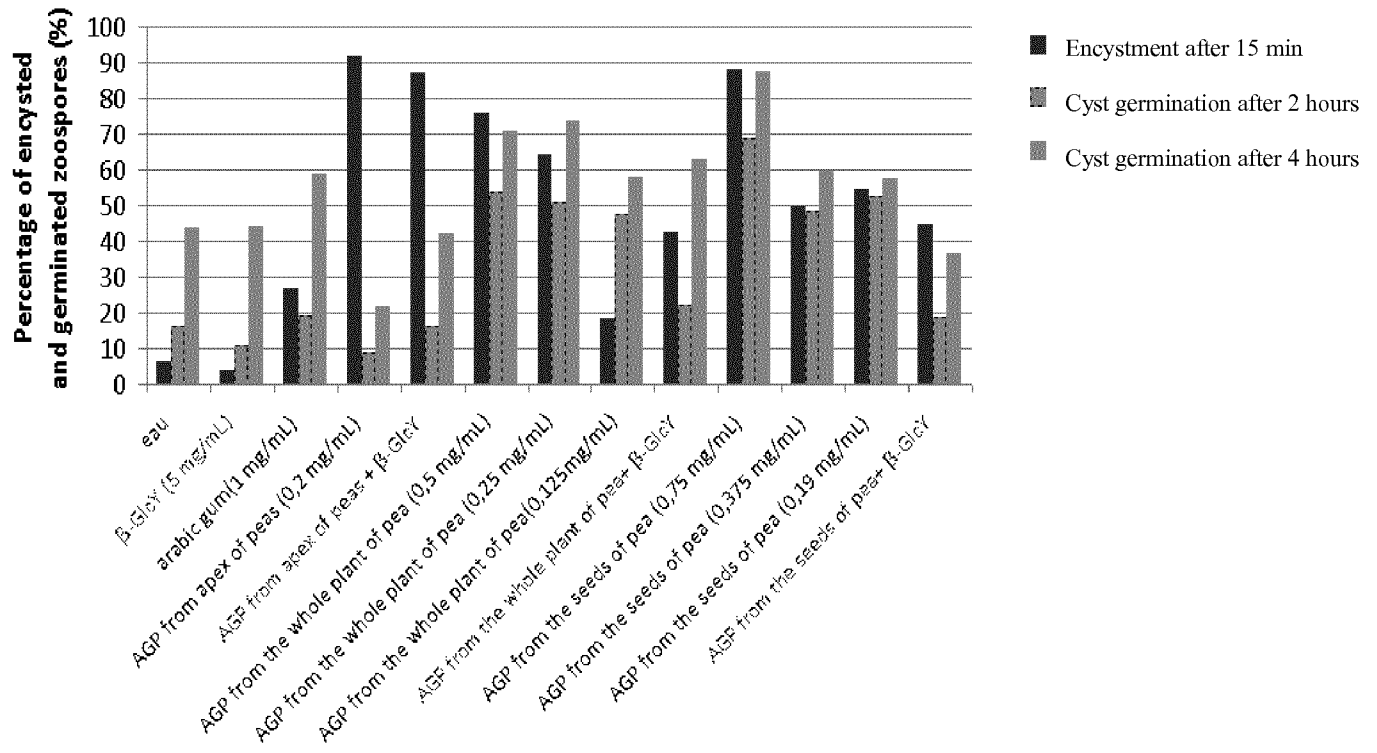


Figure 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/062032

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01N31/06
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WYDRA ET AL: "Immunohistochemical changes in methyl-ester distribution of homogalacturonan and side chain composition of rhamnogalacturonan I as possible components of basal resistance in tomato inoculated with <i>Ralstonia solanacearum</i>", 18 September 2007 (2007-09-18), PHYSIOLOGICAL AND MOLECULAR PLANT PATHOLOGY, ACADEMIC PRESS LTD, GB, PAGE(S) 13 - 24, XP022258361, ISSN: 0885-5765 page 14 page 21, left-hand column ----- -/--	1-12



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search

10 July 2012

Date of mailing of the international search report

19/07/2012

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/062032

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WYDRA K ET AL: "Structural changes of homogalacturonan, rhamnogalacturonan I and arabinogalactan protein in xylem cell walls of tomato genotypes in reaction to <i>Ralstonia solanacearum</i>", PHYSIOLOGICAL AND MOLECULAR PLANT PATHOLOGY, ACADEMIC PRESS LTD, GB, vol. 68, no. 1-3, 1 January 2006 (2006-01-01), pages 41-50, XP024930258, ISSN: 0885-5765, DOI: 10.1016/J.PMPP.2006.06.001 [retrieved on 2006-01-01] Discussion; abstract	1-12
X	K.ENKERLI ET AL: "Immunogold localization of callose and other plant cell wall components in soybean roots infected with the oomycete <i>Phytophthora sojae</i>", CAN.J.BOT., vol. 75, 1997, pages 1509-1517, XP009153930, discussion; page 1513, left-hand column	1-12
X	WO 2011/060123 A1 (NESTEC SA [CH]; ROUGHHEAD ZAMZAM FARIBA [US]; BENYACOUB JALIL [CH]; ROE) 19 May 2011 (2011-05-19) paragraphs [0024] - [0029], [0085], [0144]; claims	1-12
X	CELIO G J ET AL: "Ultrastructure and immunocytochemistry of the host-pathogen interface in poinsettia leaves infected with powdery mildew", 1 January 2004 (2004-01-01), CANADIAN JOURNAL OF BOTANY / JOURNAL CANADIEN DE BOTANIQUE, NATIONAL RESEARCH COUNCIL, OTTAWA, CA, PAGE(S) 421 - 429, XP009153929, ISSN: 0008-4026 Discussion	1-12
X	CLARKE ADRIENNE ET AL: "Tissue-specific responses to infection", UCLA SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, NEW YORK, NY, US, vol. 48, 1 January 1987 (1987-01-01), pages 35-48, XP009153918, ISSN: 0735-9543 abstract Results and Discussion	1-12
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/062032

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 241 983 B1 (PAUL STEPHEN M [US] ET AL) 5 June 2001 (2001-06-05) column 9, line 34 - line 50; claims; examples column 4, line 1 - line 15 -----	1-12
X	WO 2007/034495 A2 (HADASIT MED RES SERVICE [IL]; YISSUM RES DEV CO [IL]; DOMB ABRAHAM J []) 29 March 2007 (2007-03-29) claims; examples -----	1-12

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International application No

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