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Screening for Fusarium oxysporum *resistance in pea*

A detailed evaluation method to identify sources of quantitative resistance to *Fusarium oxysporum* f. sp. *pisi* race 2 within a *Pisum* spp. germplasm collection.

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Abstract (250 words)

F. oxysporum Schl. f. sp. *pisi* Snyd. & Hans. (*Fop*) is an important pathogen of field pea (*Pisum sativum* L.) worldwide. The constant evolution of the pathogen drives the necessity to broaden the genetic basis of the resistance to *Fop.* To this aim, it is important to have an efficient screening method that requires the availability of large germplasm collections and an accurate method for disease assessment. Here, a detailed evaluation method coupling disease incidence, disease rating over time and its related AUDPC was established and used to screen a *Pisum* spp. germplasm collection against one isolate of *Fop* race 2. Large variation in the disease response of specific pea accessions ranging from highly resistant to susceptible were observed within the collection indicating the quantitative expression of the resistance. The repetition of the inoculation experiments on a subset of 19 accessions, including two susceptible accessions, indicated that the scoring method was robust and reproducible to accurately assess the disease response of these pea accessions and confirmed the highly resistant phenotypes of 11 accessions. To initiate the characterisation of resistance mechanisms acting within these accessions, the external and internal stem symptoms were compared between these selected pea accessions together with the extension of fungal colonisation within plants. All these tests indicated that, in all resistant accessions, the resistance mechanisms efficiently stopped the pathogen progression at the crown level. Incorporation of these sources of resistance to breeding program will contribute to improved *Fop* resistance in pea cultivars.

Keywords

Pisum sativum; Fusarium oxysporum f. sp. *pisi;* Quantitative resistance; Resistance mechanisms; Screening method

Introduction

Fusarium wilts are among the most important diseases affecting grain legumes throughout the world (Kraft *et al.*, 1998). *Fusarium oxysporum* Schl. f. sp. *pisi* Snyd. & Hans. (*Fop*) is an important and destructive pathogen of field pea (*Pisum sativum* L.). It has been reported in every country where pea is grown (Kraft & Pfleger, 2001). This soil-borne pathogen can survive as thick-walled chlamydospores, which remain viable in the soil for more than 10 years (Kraft, 1994). The infection cycle of *F. oxysporum* is initiated by the germination of spores in the soil in response to an undetermined signal within the host root exudates (Di Pietro *et al.*, 2003). Upon germination infective hyphae adhere to the root surface and penetrate root epidermis directly without the formation of any distinctive structure (Bishop & Cooper, 1983a; Rodriguez Galvez & Mendgen, 1995). The mycelium then advances inter- or intracellularly through the root cortex, until it reaches the xylem vessels and enters them through the pits (Bishop & Cooper, 1983b; Beckman, 1987). At this point, the fungus switches to an endophytic mode of host colonisation, during which it remains exclusively within the xylem vessels, using them as avenues to rapidly colonise the host (Bishop & Cooper, 1983b). At this stage, the characteristic wilt symptoms appear as a result of severe water stress, which ultimately lead to complete plant death. Upon plant death, pathogenic hyphae grow outward from the vascular tissue and begin to intensely sporulate on the plant surface (Di Pietro *et al.*, 2003).

Characterisation of *Fop* isolates according to their capacity to induce disease in a set of differential lines, their assignment to specific vegetative compatibility groups and the establishment of their molecular fingerprint profiles allowed the identification of four

different races of *Fop* (races 1, 2, 5 and 6) (Haglund & Kraft, 1979; Correll *et al.*, 1987; Grajal-Martin *et al.*, 1993). Races 1 and 2 occur worldwide, while races 5 and 6 are, to date, only important in western Washington State (Infantino *et al.*, 2006). In addition, *Fop* is continually evolving with new variants of the pathogen that continue to emerge (Bodker *et al.*, 1993; Kraft & Pfleger, 2001). As for many soil-borne pathogenic fungi, the use of fungicides is not necessarily effective in controlling Fusarium wilt (Sharma *et al.*, 2010). As a consequence, control of this disease is achieved mainly by integration of different disease management procedures including agronomic and farming practices (Navas-Cortes *et al.*, 1998), soil disinfestation (Momma *et al.*, 2010), biocontrol (Alabouvette *et al.*, 2009) and breeding for resistance (Sharma *et al.*, 2010). Among these methods, the use of resistant cultivars is widely recognized as the safest, most economical and effective method for protecting crops from this disease. Fortunately, resistance to *Fop* in pea is conferred by single race-specific genes that have been successfully transferred to pea cultivars (Infantino *et al.*, 2006). Although the use of these resistant pea cultivars has proven effective in controlling this disease, there is a constant risk of resistance breakdown, since monogenic resistance can be easily overcome by the emergence of new pathogen variants. A continuous search for novel resistance sources to complement and strengthen the resistance of elite cultivars is thus essential with an emphasis on resistance sources based on quantitative and polygenic mechanisms. However, sources of Fusarium wilt resistance in pea are limited (Ali *et al.*, 1994). To broaden the genetic basis of resistance it is important to evaluate large and diverse germplasm collections, including wild species and to use precise and accurate screening techniques (Infantino *et al.*, 2006).

Different screening methods for *Fop* resistance have been described although most of them only consider the disease incidence (DI) or the proportion of asymptomatic plants to classify accessions as resistant or susceptible (Haglund, 1989; McPhee *et al.*, 1999; Sharma *et al.*, 2010). This disease scoring method may not be adequate for quantitative resistance giving the continuous gradient of symptom severity (Russell, 1978). Here, a more detailed disease scoring method that considered not only DI, but also the progression of disease symptoms was established and tested on a set of differential lines and a *Pisum* spp. germplasm collection for resistance to *Fop* race 2. This screening method detected the existence of quantitative resistance to the pathogen within this collection and identified several *Pisum* accessions with high resistance that have a good potential for improving pea resistance to race 2 of *Fop*.

Material and Methods

Fungal isolates and cultural conditions

Fusarium oxysporum f.sp. *pisi* (*Fop*) race 2 strain R2F42 was kindly provided by Dr. W. Chen (USDA-ARS, Pullman, USA) for use in all the experiments. The fungal strain was stored as microconidial suspensions at –80ºC in 30% glycerol. For microconidia production, cultures were grown in potato dextrose broth (PDB; Difco, Detroit, MI) at 28ºC in a shake culture set at 170 rpm (Di Pietro & Roncero, 1998). To determine the extend of *Fop* colonization, the fungus was re-isolated from the root and the basal, middle and apical stem regions of three inoculated plants of four susceptible and 13 resistant accessions after 7 dpi. pea tissue following the method described by Lichtenzveig *et al.* (2006) except that the 1 cm long fragment were plated on PDA containing 0.1 mg/ml kanamycin and incubated at 28ºC for 3 days. With this method,

typical colonies of *Fop* can be easily detected as white filaments emerging from the plant tissue and colonizing the PDA medium.

Plant material and growing conditions

A collection of eighty accessions of *Pisum* spp. of diverse origins was used in this study. The collection was composed of 7 *P. sativum* cultivars from the USDA core collection of the differential set for the four races of *Fop* (Table 1) and 73 accessions from the John Innes pea collection that had been obtained from different countries.

Pea seeds were surface-sterilized for 20 min in a 20% solution of sodium hypochlorite and then rinsed three times with sterile water. Then the seeds were wrapped in wet filter paper in a petri dish, stratified for two days at 4°C in the dark and incubated at 26 ± 2°C until germination. Once germinated, the seedlings were transferred to pots (36 cm^2 x 8 cm) containing sterile vermiculite (1-3 mm diameter) and grown in a controlled environmental chamber under a 16/8 h light-dark photoperiod at 26 ± 2 °C temperature regime with 200 µmol m^{-2} s^{-1} of illumination. Plants were watered every three days with tap water.

Inoculation and disease assessment

Seven-day-old *Pisum* spp. seedlings (2-3 node stage) were inoculated following a modified version of the dip technique described by Haglund (1989). For this procedure, vermiculite was removed from the roots which were trimmed by a third and immersed for 5 min in a suspension containing 5 x 10^6 microconidia per ml of water. Control plants were treated in the same way and were immersed in sterile water. Seedlings were

planted in individual pots containing sterile vermiculite and maintained in the same growth chamber.

Screening of the whole collection including the differential lines was first performed on five seedlings per *Pisum* accessions. Then the 15 most resistant accessions were tested twice in independent experiments along with two partially resistant and two susceptible accessions with five seedlings per accession and per experiment.

Disease symptoms were assessed every three days from 10th to 30th days post-inoculation (dpi). Two different leaf symptom-based approaches were used to estimate the disease symptom rate (DR) at the leaf and the whole plant level. At the whole plant level DR was evaluated as the percentage of symptomatic leaves for each individual plant (PSL) (Fig. 1a). At the leaf level, evaluation of DR was established by assigning a visual index ranging from 1 (healthy leaf) to 5 (dead leaf) to each leaf within a plant and reporting these values for each individual plant by calculating the mean value of the visual index (MVI) of all its leaves (Fig. 1b). These data were used to calculate the area under the disease progression curve (AUDPC) using the formula

$$AUDPC = \Sigma[(x_i + x_{i+1}) / 2] * (t_{i+1} - t)$$

where x_i = estimated proportion of disease severity at date i, x_{i+1} = estimated proportion of disease severity at date i+1, and $t_{i+1} - t_i$ = number of days between scoring dates i and i + 1.

Disease incidence (DI) determined as the proportion of dead plants was also scored at 30 dpi.

To classify accessions as resistant or susceptible, their disease symptoms were compared to those of the accessions P627 and P21 used as resistant and susceptible controls respectively. In addition, the differential lines New Season (resistant) and Little Marvel (susceptible) were also included in order to confirm their response to the infection.

Whole plant staining

To detect the extent of fungal colonization, three plants of the 12 most resistant accessions and the susceptible accession P21 were harvested 30 dpi, washed with sterile water to remove any un-adhered *Fop* microconidia, cleared with 2.5% KOH at 90ºC for 1 h, rinsed twice with deionized water and incubated overnight at room temperature in a solution of 1% HCl. The root and stem regions were then stained in a 1% Parker blue Quink ink aqueous solution for 30 min at 60ºC and destained 16 h at room temperature in lactoglycerol. The resulting stained tissues were then stored at room temperature in 100% glycerol until observation under a binocular microscope. Following this treatment stained fungal structures were clearly visible.

Detection of internal symptoms

A red-brown discoloration within plant tissue has been previously shown to be associated with *F. oxysporum* infection in field peas (Kraft & Pfleger, 2001). In order to observe this red-brown discoloration within pea plant tissue, the basal and middle part of the stem and the upper part of the root system of three plants from the 12 most resistant accessions were harvested at 30 dpi, longitudinally cut with a razor blade and

observed under a Nikon SMZ1000 binocular microscope (Nikon Europe B.V., Badhoevedorp, The Netherlands). For comparison, three plants of the susceptible accession P21 were harvested at 10 dpi and treated in the same way.

Statistical analysis

To analyse the significance of the differences in DR and DI between the different pea accessions to *Fop*, all data obtained from the DI, MVI, PSL and AUDPC values were subjected to an analysis of variance (One-way ANOVA). Percentage PSL and DI data were subjected to an angular transformation to normalise the data and stabilize the variances before being subjected to the analysis of variance (Baird *et al.*, 2002). Whenever the ANOVA was statistically significant ($p \leq 0.05$) for a specific variable, a Duncan's multiple range test was conducted to assess the differences of the means between each accession. The coefficient of correlation existing between the different disease parameters were calculated using the non parametric Spearman's rank correlation coefficient analysis. All statistical analyses were performed with the Genstat release 11.1 software (VSN International Ltd., Hemel Hempstead, UK).

Results

Disease development

The assessment of susceptible cultivars including Little Marvel and Dark Skin Perfection differential lines showed that the initial symptoms appeared on the primary leaves around 10-15 dpi and sequentially reached the later-formed leaves until the whole plant withers and dies (Fig. 1a). At the leaf level, the disease symptoms initiated at the leaf margins, which yellowed and/or curled downward. Leaf yellowing was associated with necrosis until the whole leaf wilted and became dry and brittle (Fig. 1b).

These observations allowed the development of the two scales to estimate Disease Rate (DR).

Validation of the scoring method on differential lines of *P. sativum*

Before applying these screening methods to the *Pisum* spp. germplasm collection they were tested on seven well-described differential lines. As expected, Little Marvel and Dark Skin Perfection lines were highly susceptible to our isolate of *Fop* race 2 showing a DI of 100% and DR values at 30 dpi of 5 and 100% according to the MVI and PSL evaluation method respectively (Table1). Similarly, the resistant differential cultivars (Haglund and Krafts, 1979) were all resistant with DI value ranging from 0 for New Season to 40% for Mini93. DR scores at 30 dpi ranged from 2.3 for New Season to 3.4 for Mini93 for the MVI scale and from 43.4% for New Season to 66.9% for 74SN5 according to the PSL scale. The AUDPC values calculated from the DR datasets ranged from 12.1/438 (AUDPC MVI/AUDPC PSL) for Mini to 24.7/846.4 for Wsu31 over the whole experiments according to the MVI/PSL scales respectively (Table 1). The statistical analysis performed on both DR scales and their associated AUDPC values indicated significant differences between race differential cultivars ($p < 0.001$) and a clear discrimination between the susceptible and resistant genotypes (Table 1).

Screening of wild *Pisum* spp. collection against race 2 of *F. oxysporum* f. sp. *pisi*

The same scoring parameters were used to screen a collection of 73 *Pisum* spp. accessions to identify new sources of resistance to *Fop* race 2 (Tables 2 and 3). Large variation in the disease response was detected among the *Pisum* spp. accessions for all the parameters monitored (Fig. 2 and Table 2). Thus, DI ranged from 0 to 100%, DR

ranged from 1.3/20% to 5/100% according to MVI / PSL parameter and from 4.0/125 to 90.0/2220 for their respective AUDPC values (Table 2). The continuous distribution of the pea accessions for the different parameters monitored, as shown for their AUDPC values, indicated that resistance in this germplam collection is quantitative (Fig. 2). The one-way ANOVA performed for all parameters detected statistically significant differences between accessions. Mean comparison analysis performed for the DR and AUPDC parameters failed to separate them in discrete groups except for the most resistant and susceptible accessions. To simplify classification, we separated the accessions in three main groups according to the result of the mean comparison test of their AUDPC values. All accessions not significantly different according to the Duncan's multiple range test for both AUDPC parameters, to the resistant check P627 were considered resistant. Accessions not significantly different from the susceptible check P21 were considered susceptible and the rest of the accessions were considered partially resistant. As expected the differential line New season and Little Marvel were classified within the resistant and susceptible group respectively (Table 2). According to this classification, 18 accessions were categorized as resistant (24.7% of the collection), 25 accessions as partially resistant (34.3% of the collection) and 30 as susceptible (41% of the collection)(Fig. 2).

The independent repetitions of the assessment of 17 resistant and partially resistant accessions confirmed their low incidence and severity compared to the highly susceptible accessions P21 and P662 (Table 3). As expected, P21 and P662, the susceptible accessions showed a DI value of 100% and DR values of 5 and 100% for MVI and PSL measurements, respectively. Conversely, the DI of the resistant accessions ranged from 0 to 53% while MVI and PSL values varied from 1.8 and 30.0%

for P633 to 3.5 and 68.8% for P18 (Table 3). Similarly, the AUDPC values of resistant accessions remained relatively low ranging from 9.7/343.2 for the highly resistant P23 to 37.1/1264.6 for the partially resistant P316 according on the MVI- and PSL scales while these values ranged from 73.9/2028 and 83.5/2274 for the susceptible accessions P662 and P21 respectively(Table 3). Statistical analysis confirmed the differences between genotypes at $p < 0.001$ for all parameters evaluated. In addition, the mean comparison tests allowed separation of the different accessions in three groups for all parameters confirming our classification. The results obtained largely supported the data of the initial screening with very few exceptions. For instance, 11out of the originally 15 resistant accessions were confirmed as resistant while the remaining four were classified as partially resistant along with P18 and P316 (Table 3)

To determine the most adapted and easiest method of disease scoring for future screening of pea resistance to *Fop*, we examined the correlation existing between the different parameters evaluated using a non-parametric Spearman's correlation rank analysis. The highest and significant correlations were obtained between both AUDPC values ($r = 0.942$; $p < 0.001$) and both DR measurements ($r = 0.925$; $p < 0.001$). Similarly, a good positive correlation were observed between the AUDPC values and their respective DR evaluations at 30 dpi with Spearman's rank correlation of $r = 0.803$ and 0.716 ($p < 0.001$) for MVI and PSL, respectively (Table 4). By contrast, only a low correlation with $r \leq 0.524$ was observed between DI and any of the other parameters (Table 3).

Detection of *F.* oxysporum f.sp. *pisi* within plant tissue

To detect the extension of *Fop* colonisation within plants, we re-isolated the fungi from the different parts (root and basal, middle and apical stem regions) of inoculated plants of 15 of the selected accessions. In the susceptible genotype P21 and the partially resistant accession P316, *Fop* colonies were detected at both extremities of all plated plant segments indicating that as early as 7 dpi, *Fop* had already colonised the whole plant in these accessions (Fig. 3). By contrast, *Fop* colonies were recovered only from root and basal stem segments of the other resistant and partially resistant *Pisum* accessions monitored (Fig. 3). While *Fop* colonies were detected on each extremity of the basal segment in nine resistant and partially resistant genotypes including P23, P42, P614, P615, P627, P632, P633, P650 and JI 1760, they were detected only at the lowest extremity in the other four resistant accessions including P638, P639, P656 and P669 (Fig. 3).

Detection of external and internal symptoms

At the end of the experiment, plants from the selected accessions were removed from vermiculite and visually compared. Interestingly, the external area of the upper root system, the crown and the basal shoot region of resistant and partially resistant genotypes were black- brown in colour. The extent of this discoloration varied according to the genotype, the largest colored area being observed in the highly resistant accession P42 (Fig. 4). This black-brown external discoloration was not detected on the susceptible accessions suggesting that it might play a role in the resistance to *Fop* (Fig. 4). Observation under a binocular microscope of longitudinal sections of resistant accessions showed that the discolored root and shoot tissues was still healthy albeit brown suggesting that this discoloration is due to an accumulation of pigmented substances within cells rather than due to cell death (Fig. 5). These sections also

revealed the extent of differences in the typical vascular discoloration between accessions. As it is characteristic for *Fop* race 2 infection, the vascular tissue of the whole plant of susceptible accessions such as P21 turned dark red (Fig. 5). By contrast, this vascular discoloration did not extend further than the plant crown in the resistant and partially resistant accessions as shown for P633 in Fig. 5.

Staining with the commercial Parker blue Quink ink at 30 dpi supported this observation. With this staining method, the whole shoot of the susceptible accessions, such as P21, appeared blue (Fig. 6a and b), indicating the presence of fungi in the entire shoot of susceptible plants. In these genotypes, we could detect some patches of more intense staining within the surface of the shoot that corresponded to fungal colonies growing out of the susceptible plant tissues (Fig. 6a). By contrast, the blue staining was only observed in the crown and basal shoot section of most resistant entries while the rest of the shoot remained clear (Fig. 6c and d). Altogether, these findings indicated that in most cases the fungal progression was efficiently stopped at crown level and suggested that the most discriminating defence mechanism may be acting in the crown (Figs. 3, 4, 5 and 6).

Discussion

F. oxysporum f. sp. *pisi* is a recurring problem causing important yield losses wherever pea is grown. In this study, different methods to accurately evaluate fusarium wilt disease in a controlled environment were assessed and used to screen a *Pisum* spp. collection to identify new sources of resistance to *Fop* race 2. The different methods of disease evaluation (DI, DR and AUDPC) revealed large variability in the response of the different accessions to *Fop* race 2 ranging from resistant to susceptible and

including many partially resistant accessions. Such range of responses suggests that resistance to this *Fop* isolate in this *Pisum* spp. collection is mainly of quantitative nature. As a result of this study, we identified and confirmed 11 new sources of resistance (JI1412, JI1559, JI1760, P23, P42, P614, P627, P633, P639, P650 and P656) showing a very high level of resistance to *Fop* race 2 that may be useful for a breeding program.

Fusarium wilt disease causes a series of external symptoms including vein clearing, leaf epinasty, wilting, chlorosis, necrosis, and abscission leading to the complete plant wilting and death (MacHardy & Beckman, 1983). Under the conditions of this study, the *Fop* race 2 isolate used was highly pathogenic causing fusarium wilt symptoms on susceptible and partially resistant accessions (Fig. 1 and Table 2) which allowed the development of a methodology based on leaf symptoms to evaluate the *Pisum* spp. collection.

Previous studies described pea lines as resistant (no symptoms) or susceptible (dead plants) to specific *Fop* isolates and ignored any variation in their symptom severity (Haglund & Kraft, 1979; Bodker *et al.*, 1993). As a consequence, most previous studies on the identification of resistance sources to this pathogen were based on the sole evaluation of DI (Haglund & Kraft, 1979; Haglund, 1989; McPhee *et al.*, 1999; Sharma *et al.*, 2010). In case of *Fop* race 2, variation in the DI value of pea accessions have often been detected hampering their classification within the resistant or susceptible group and the establishment of clear segregation ratios within populations (Hare *et al.*, 1949; McPhee *et al.*, 1999). In the present study, similar variation for DI was detected between experiments (Table 3). In addition, DI values did not always agree with the

resistance reactions in our collection, since we observed accessions such as P11 and P670 with severe fusarium wilt symptoms, but DI values of only 40% (Table 2). This highlights the need to evaluate additional parameters to accurately estimate disease reaction to *Fop* race 2 in pea. The evaluation of disease severity is often a good method to assess quantitative resistance mechanisms (Russell, 1978), but only a limited number of studies have used a disease severity index to evaluate pea resistance to fusarium wilt (Charchar & Kraft, 1989; Lebeda & Svabova, 1997; Neumann & Xue, 2003; Lebeda *et al.*, 2010). In these studies, the disease scoring was based on a 0–5 (Charchar & Kraft, 1989; Neumann & Xue, 2003) or 0-3 (Lebeda & Svabova, 1997; Lebeda *et al.*, 2010) rating system of the whole plant which appeared inadequate in our hand to accurately assign plant symptoms to a specific disease index. Instead two different methods to assess DR considering leaf symptoms of the whole plant was tested on a series of differential lines and used to evaluate disease reactions of a *Pisum* spp. germplasm collection. Results obtained from both methods were highly similar clearly discriminating between the resistant and susceptible genotypes and detecting intermediate reactions (Tables 1 and 3). The high correlation between both methods indicates that only one of them is require to determine the disease reaction of pea accessions (Table 4). The proportion of symptomatic leaves is the fastest method and would therefore be the method of choice to evaluate DR in future screening of pea germplasm to *Fop* race 2. Fusarium wilt disease development requires several weeks from plant infection to plant death, thus, AUDPC (Teng & James, 2002) that consider severity over time was also calculated. Although more time consuming, this method appears more reliable and reproducible to estimate with accuracy disease response of pea accessions which is supported by the high correlation existing between both AUDPC measurements and between AUDPC and the DR values (Tables 3 and 4). By

contrast, the low correlation coefficient obtained when comparing DI measurements with any other disease parameters confirmed that this parameter alone is not adequate to describe disease response to *Fop* race 2 in pea (Table 4).

Despite the limited number of accessions used in this study, this *Pisum* spp. collection contained sufficient genetic variation to detect a wide range of responses to *Fop* race 2 from highly resistant to susceptible genotypes (Tables 2 and 3). Among the 73 accessions screened, 59% of the collection showed some resistance with 24.7% genotypes presenting high level of resistance to this isolate, even more than the resistant differential cultivars (Tables 1, 2 and 3). Nevertheless it should be noted that only phenotypes of the selected accessions have repeatedly shown consistent reaction to the pathogen and that other accessions may need repeated testing to confirm their relative resistance. Of the 11 highly resistant accessions, only three belong to *P. sativum* ssp. *sativum* whereas eight belong to other subspecies of *P. sativum* including two *P. sativum* ssp. *elatius*, four *P. sativum* ssp. *arvense* and one *P. sativum* ssp. *jormadi* accessions and one belong to *P. fulvum* (Table 3). While screening for resistant to all four races of *Fop* have been extensively performed, few studies described very high level of resistance in collection of *Pisum* spp. (Lebeda & Svabova, 1997). Indeed, only one previous study specifically dealt with wild species and subspecies and although authors reported a wide variety of responses to *Fop*, they failed to identify complete resistance (Lebeda & Svabova, 1997). On the other hand, the screening of 452 pea accessions from the USDA core collection revealed that 14% of the whole collection (62 accessions) were resistant to races 1 or 2 of *Fop* (McPhee *et al.*, 1999). Interestingly, one *P. sativum* ssp. *abyssinicum* and one *P. sativum* ssp. *elatius* accessions were also identified as resistant in this study (McPhee *et al.*, 1999). The *P.*

sativum ssp. *elatius* accession PI344012 was also included in this study (P24), however, under these experimental conditions, this accession was highly susceptible to our *Fop* race 2 isolate showing a disease incidence of 80% (Table 2).

Resistance to all of the *Fop* races in pea has been considered qualitative with a monogenic inheritance (Infantino *et al.*, 2006). For race 2 of *Fop,* most previous studies identified only one genetic factor controlling resistance to this race (Hare *et al.*, 1949; Haglund, 1989; McPhee *et al.*, 1999). However, mixed phenotypes were often observed within accessions even when considering the set of differential lines used to characterise *Fop* isolates (Haglund, 1989; McPhee *et al.*, 1999). In these studies, these authors attributed the variability to heterogeneity of the seed population excessive root trimming prior to inoculation or the presence of another fungal pathogen (McPhee *et al.*, 1999). Interestingly, subsequent studies by the same authors reported intermediate resistance reactions in response to *Fop* race 2 questioning the reaction of these lines to this race (McPhee *et al.*, 2004). In the present study we detected a continuity of responses from highly resistant to susceptible (Fig. 2). A previous screening of another collection of *Pisum* spp. for resistance to *Fop* race 2 also detected large variation in the responses of individual accessions (Lebeda & Svabova, 1997). Such continuity would support the existence of quantitative resistance mechanisms in the *Pisum* spp. collection, which also can be seen in the differential lines in which small variation was detected (Table 1). Thus in accordance with the observation of McPhee et al. (2004), our results suggest the existence of additional genetic factors that control *Fop* race 2 resistance, although genotypic analysis of progenies of these accessions would be needed to confirm this hypothesis.

As a result of the present study, 43 accessions with quantitative resistance were identified of which 11 developed only very mild symptoms. As a next step it will be important to characterise the resistance mechanisms responsible for their phenotype to ease selection in breeding programs. Many studies in *Solanum lycopersicum*, *Arabidopsis thaliana* and *Pisum sativum* indicated that resistant plants to *F. oxysporum* displayed a wide and complex array of anatomical and biochemical responses to counteract pathogen infection (Beckman, 1987; Kraft, 1994; Michielse & Rep, 2009). However, the actual resistance mechanisms acting in a specific resistant accession are still unclear (Zvirin *et al.*, 2010). As a starting point to characterise the resistance mechanisms acting in the resistant accessions, a detailed observation of internal and external symptoms was undertaken.,These initial studies suggested that the strongest resistance response acted at the crown level. Indeed, fungal colonies were only isolated from roots and the basal part of the stem in resistant and most partially resistant accessions while *Fop* was present in the whole plant in susceptible accessions as early as 7 dpi (Fig. 3). In addition, observation of external and internal symptoms indicated clear differences between susceptible and resistant genotypes. Pea infection by *Fop* is usually associated with a discoloration of vascular tissue which turns orange or dark red (Kraft & Pfleger, 2001). In agreement with these observations, vascular tissue of most accessions screened in the present study turned dark red (Fig. 5). However, this discoloration was restricted to the root and basal stem section in the resistant and partially resistant accessions while it reached shoot apex in susceptible accessions (Fig. 5). This agreed with the observation that *F. oxysporum* colonization of resistant host and non-host was limited to the root and basal part of the stem of different plant species (Beckman, 1987; Charchar & Kraft, 1989). Interestingly, the restriction of the extent of vascular discoloration observed in resistant and partially resistant accessions was

accompanied by a blackening of the cortical and epidemical cells around the crown region (Figs 4 and 5). Previous studies indicated that pea infection by *Fop* race 2 was often associated with secondary cortical decay (Hagedorn, 1984; Kraft & Pfleger, 2001). However, our observations on the blackened regions did not suggest any decay of these tissues, but rather a cortical hardening as the blackened cells appeared to be still alive (Fig. 5). Further studies are now underway to determine the mechanisms acting in these resistant accessions at the cellular and molecular levels. In the meantime, the incorporation of these resistant accessions in breeding programs of elite pea cultivars together with the application of a simplified scoring method derived from the present study is expected to improve the resistance status of pea to *Fop* race 2 in the near future.

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Tables

Table 1. Disease Ratings of differential lines of *Pisum sativum* to *Fop 2*.

Accession	Fop 2	Disease Incidence (%)	Disease Ratings[1]			
			Plant Mean Disease Index		Symptomatic Leaves (%)	
			MVI	AUDPC	PSL	AUDPC
Little Marvel	S	100	5.0 ± 0.00b	84.6 ± 5.40b	100.0 ± 0.00b	2250 ± 0.0b
Dark Skin Perfection	S	100	5.0 ± 0.00b	57.9 ± 8.92b	100.0 ± 0.00b	1519 ± 206.6b
New Season	R	0	2.3 ± 0.24a	13.2 ± 2.56a	43.4 ± 6.18a	514 ± 74.7a
Mini	R	20	3.1 ± 0.52a	12.1 ± 4.79a	61.3 ± 10.57a	438 ± 137.1a
Mini 93	R	40	3.4 ± 0.73a	23.3 ± 8.42a	65.0 ± 15.00a	741 ± 184.9a
Wsu31	R	20	3.0 ± 0.52a	24.7 ± 4.31a	59.9 ± 10.78a	846 ± 215.7a
74SN5	R	20	3.0 ± 0.62a	18.6 ± 2.73a	66.9 ± 12.52a	622 ± 97.1a

[1] Data are means of 5 replicates, different letters indicates significant difference between value according to Duncan Multiple Range Test at $\alpha = 0.05$.

Table 2. Disease Ratings of the *Pisum* spp. accessions to *Fop* 2

Accession	Species	Disease Incidence (%)	Disease Ratings[1]			
			Plant Mean Disease Index		Symptomatic Leaves (%)	
			MVI	AUDPC	PSL	AUDPC
New Season (R)	*P. sativum*	0	$2.3 \pm 0.24^{a-h}$	$13.2 \pm 2.56^{a-e}$	$43.4 \pm 6.18^{a-f}$	$514 \pm 74.7^{a-c}$
P627	*P. sativum* ssp. *arvense*	0	$1.8 \pm 0.79^{a-d}$	4.0 ± 3.51^{a}	24.0 ± 19.39^{ab}	118 ± 84.5^{a}
P656	*P. fulvum*	40	$2.9 \pm 0.79^{b-j}$	$5.2 \pm 1.96^{a-c}$	$53.3 \pm 18.50^{b-h}$	244 ± 82.9^{ab}
P23	*P. sativum* ssp. *elatius*	0	$2.12 \pm 0.72^{a-f}$	$5.4 \pm 3.16^{a-c}$	$48.8 \pm 13.25^{a-g}$	279 ± 75.8^{ab}
JI1760	*P. sativum* (cv. Consort-*af*)	20	$1.9 \pm 0.40^{a-d}$	5.0 ± 2.54^{ab}	$37.0 \pm 12.93^{a-e}$	309 ± 141.0^{ab}
P633	*P. sativum* ssp. *arvense*	0	1.4 ± 0.09^{ab}	$6.5 \pm 1.85^{a-c}$	$22.3 \pm 1.72^{a-c}$	311 ± 63.9^{ab}
JI1559	*P. sativum* (cv. Mexique 4)	0	1.8 ± 0.14^{ac}	$6.9 \pm 1.91^{a-c}$	$30.4 \pm 3.88^{a-d}$	$475 \pm 124.4^{a-c}$
JI1412	*P. sativum* (cv. Marlin)	0	$2.1 \pm 0.19^{a-e}$	$12.5 \pm 0.67^{a-g}$	$35.3 \pm 4.38^{a-e}$	$485 \pm 57.2^{a-c}$
P669	*P. fulvum*	20	$2.5 \pm 0.45^{a-g}$	$8.6 \pm 1.68^{a-d}$	$67.3 \pm 13.01^{d-l}$	$529 \pm 84.0^{a-d}$
P42	*P. sativum* ssp. *arvense*	0	1.3 ± 0.18^{a}	$8.1 \pm 1.95^{a-d}$	20.0 ± 9.35^{a}	$536 \pm 106.7^{a-e}$
P638	*P. sativum* ssp. *arvense*	0	$2.8 \pm 0.09^{a-h}$	$10.7 \pm 1.30^{a-f}$	$53.9 \pm 4.82^{a-g}$	$577 \pm 67.8^{a-f}$
P632	*P. sativum* ssp. *arvense*	0	$2.5 \pm 0.26^{a-g}$	$17.0 \pm 3.45^{a-j}$	$43.4 \pm 9.00^{a-e}$	$649 \pm 103.8^{a-g}$
P613	*P. sativum* ssp. *tibetanicum*	20	$2.2 \pm 0.64^{a-f}$	$21.0 \pm 17.26^{a-k}$	$48.2 \pm 11.90^{a-g}$	$687 \pm 393.2^{a-h}$
P650	*P. sativum* ssp. *jormadi*	0	$1.9 \pm 0.32^{a-d}$	$12.6 \pm 4.16^{a-g}$	$34.1 \pm 10.18^{a-f}$	$712 \pm 149.3^{a-i}$
P615	*P. sativum* ssp. *elatius*	40	$3.3 \pm 0.75^{c-l}$	$14.1 \pm 4.96^{a-i}$	$84.0 \pm 16.00^{h-n}$	$716 \pm 215.0^{a-i}$
P614	*P. sativum* ssp. *elatius*	0	$2.0 \pm 0.18^{a-d}$	$13.1 \pm 2.10^{a-h}$	$38.0 \pm 4.18^{a-e}$	$731 \pm 87.3^{a-j}$
JI1766	*P. sativum* (cv. Barton-*af,st)*	100	5.0 ± 0.00^{m}	$30 \pm 0.00^{a-o}$	100.0 ± 0.00^{n}	$750 \pm 0.0^{a-j}$
P639	*P. sativum* ssp. *arvense*	0	$1.9 \pm 0.78^{a-d}$	$19.4 \pm 14.02^{a-k}$	$28.0 \pm 19.60^{a-c}$	$752 \pm 395.7^{a-j}$
JI1566	*P. sativum* (cv. Almota)	0	$3.1 \pm 0.26^{c-k}$	$25.1 \pm 2.23^{a-n}$	$61.4 \pm 8.09^{b-j}$	$840 \pm 56.1^{b-k}$
P634	*P. sativum* ssp. *arvense*	20	$2.4 \pm 0.70^{a-g}$	$19.6 \pm 10.18^{a-k}$	$37.3 \pm 16.81^{a-e}$	$841 \pm 275.7^{b-k}$
JI1747	*P. sativum* (cv. Almires)	40	$3.6 \pm 0.61^{f-m}$	$26.0 \pm 4.24^{a-n}$	$70.7 \pm 14.39^{e-n}$	$888 \pm 98.8^{b-l}$
P641	*P. sativum* ssp. *arvense*	0	$3.0 \pm 0.14^{c-j}$	$21.5 \pm 1.44^{a-l}$	$53.9 \pm 3.05^{a-g}$	$890 \pm 41.9^{b-l}$
P640	*P. sativum* ssp. *arvense*	0	$2.9 \pm 0.26^{b-j}$	$23.5 \pm 3.61^{a-m}$	$62.8 \pm 6.97^{c-k}$	$996 \pm 126.8^{c-m}$
P18	*P. sativum* ssp. *elatius*	40	$3.3 \pm 0.83^{c-l}$	$36.4 \pm 20.12^{d-p}$	$57.6 \pm 20.95^{c-j}$	$998 \pm 514.5^{c-m}$
P628	*P. sativum* ssp. *arvense*	40	$3.6 \pm 0.51^{f-m}$	$30.4 \pm 6.69^{a-o}$	$72.2 \pm 10.37^{e-n}$	$1090 \pm 136.5^{c-n}$
JI502	*P. sativum* (cv. Rondo)	80	$4.7 \pm 0.31^{m-o}$	$37.1 \pm 4.33^{e-p}$	$92.5 \pm 6.71^{l-n}$	$1165 \pm 114.9^{d-o}$
P621	*P. sativum* ssp. *jormadi*	20	$2.7 \pm 0.55^{a-h}$	$33.8 \pm 7.66^{c-o}$	$55.4 \pm 10.48^{b-j}$	$1181 \pm 181.6^{e-o}$
P637	*P. sativum* ssp. *arvense*	40	$3.6 \pm 0.59^{e-m}$	$31.7 \pm 6.62^{a-o}$	$71.1 \pm 10.59^{e-n}$	$1186 \pm 124.6^{f-o}$
P645	*P. sativum* ssp. *arvense*	20	$2.9 \pm 0.50^{b-i}$	$33.3 \pm 8.77^{b-o}$	$55.6 \pm 11.86^{b-i}$	$1193 \pm 229.5^{f-o}$
P636	*P. sativum* ssp. *arvense*	40	$3.3 \pm 0.71^{d-l}$	$32.5 \pm 12.97^{a-o}$	$73.4 \pm 9.81^{e-n}$	$1200 \pm 316.5^{f-o}$
JI2480	*P. sativum* (CGN 3352)	40	$3.8 \pm 0.44^{g-m}$	$28.3 \pm 5.22^{a-o}$	$83.3 \pm 9.43^{f-n}$	$1223 \pm 165.1^{f-o}$
JI2302	*P. sativum* (B76-197)	60	$4.1 \pm 0.56^{h-m}$	$36.2 \pm 8.48^{d-p}$	$84.3 \pm 10.20^{g-n}$	$1225 \pm 189.1^{f-o}$
P619	*P. fulvum*	80	$4.4 \pm 0.40^{i-m}$	$41.3 \pm 14.80^{h-r}$	$93.3 \pm 6.67^{l-n}$	$1232 \pm 334.5^{g-o}$
P316	*P. fulvum*	60	$4.4 \pm 0.60^{i-m}$	$39.0 \pm 10.26^{f-q}$	$88.0 \pm 12.00^{j-n}$	$1296 \pm 201.1^{g-p}$
P731	*P. sativum* ssp. *arvense*	80	$3.8 \pm 0.62^{g-m}$	$41.8 \pm 15.66^{i-r}$	$96.7 \pm 3.33^{l-n}$	$1331 \pm 334.7^{h-p}$
JI210	*P. sativum* (cv. Lucknow Boniya)	100	5.0 ± 0.00^{m}	$45.7 \pm 13.99^{k-s}$	100.0 ± 0.00^{n}	$1352 \pm 315.2^{i-q}$
P649	*P. sativum* ssp. *jormadi*	40	$3.0 \pm 0.74^{c-j}$	$38.0 \pm 16.01^{e-p}$	$63.0 \pm 13.97^{d-l}$	$1378 \pm 361.6^{j-r}$
P635	*P. sativum* ssp. *arvense*	60	$4.6 \pm 0.34^{k-m}$	$39.4 \pm 9.32^{g-q}$	$93.3 \pm 6.67^{l-m}$	$1418 \pm 279.4^{k-s}$
P11	*P. sativum*	40	$3.7 \pm 0.62^{g-m}$	$43.5 \pm 11.02^{j-s}$	$72.0 \pm 11.38^{e-n}$	$1429 \pm 281.6^{k-s}$
P68	*P. sativum* ssp. *elatius*	40	$2.8 \pm 0.92^{a-h}$	$40.7 \pm 15.44^{g-r}$	$44.0 \pm 23.15^{a-e}$	$1443 \pm 329.8^{k-s}$
P630	*P. sativum* ssp. *arvense*	80	$4.5 \pm 0.47^{k-m}$	$47.2 \pm 9.44^{k-u}$	$93.3 \pm 6.67^{l-n}$	$1471 \pm 235.2^{k-s}$
P626	*P. sativum* ssp. *arvense*	100	5.0 ± 0.00^{m}	$50.2 \pm 3.19^{l-w}$	100.0 ± 0.00^{n}	$1532 \pm 106.3^{l-t}$
P54	*P. sativum* ssp. *arvense*	80	4.6 ± 0.40^{lm}	$44.4 \pm 4.19^{j-s}$	$93.3 \pm 6.67^{l-m}$	$1563 \pm 177.0^{m-u}$
P631	*P. sativum* ssp. *arvense*	80	4.7 ± 0.33^{lm}	$50.2 \pm 6.84^{m-w}$	$93.3 \pm 6.67^{l-n}$	$1601 \pm 103.6^{m-v}$
P617	*P. sativum* ssp. *thebaicum*	100	5.0 ± 0.00^{m}	$55.71 \pm 11.47^{o-y}$	100.0 ± 0.00^{n}	$1656 \pm 189.8^{n-v}$
P643	*P. sativum* ssp. *arvense*	80	$3.6 \pm 0.34^{e-m}$	$53.0 \pm 5.63^{n-x}$	$71.1 \pm 7.00^{d-m}$	$1659 \pm 123.8^{n-v}$
P19	*P. sativum* ssp. *elatius*	100	5.0 ± 0.00^{m}	$47.8 \pm 5.04^{k-v}$	100.0 ± 0.00^{n}	$1684 \pm 136.8^{n-v}$
P670	*P. fulvum*	40	$3.3 \pm 0.69^{c-l}$	$46.2 \pm 12.26^{k-t}$	$65.4 \pm 14.49^{d-l}$	$1686 \pm 177.9^{n-v}$
P642	*P. sativum* ssp. *arvense*	80	4.6 ± 0.40^{lm}	$63.4 \pm 16.45^{p-z}$	$91.1 \pm 8.89^{l-n}$	$1748 \pm 310.9^{n-v}$
P647	*P. sativum* ssp. *arvense*	100	5.0 ± 0.00^{m}	$66.1 \pm 9.42^{q-z}$	100.0 ± 0.00^{n}	$1778 \pm 194.1^{o-v}$

Table 2. (Continued)

Accession	Species	Disease Incidence (%)	Disease Ratings[1]			
			Plant Mean Disease Index		Symptomatic Leaves (%)	
			MVI	AUDPC	PSL	AUDPC
P648	*P. sativum* ssp. *arvense*	100	5.0 ± 0.00^{m}	$63.9 \pm 7.07^{p-z}$	100.0 ± 0.00^{n}	$1920 \pm 105.1^{p-v}$
P651	*P. sativum* ssp. *elatius*	80	$4.4 \pm 0.57^{i-m}$	$73.3 \pm 16.66^{t-z}$	$86.7 \pm 11.93^{i-n}$	$1942 \pm 308.0^{p-v}$
JI82	*P. sativum*	100	5.0 ± 0.00^{m}	$67.9 \pm 11.42^{r-z}$	100.0 ± 0.00^{n}	$1991 \pm 216.7^{q-v}$
P24	*P. sativum* ssp. *elatius*	80	$4.4 \pm 0.51^{j-m}$	$74.2 \pm 12.37^{u-z}$	$90.0 \pm 8.94^{k-n}$	$1999 \pm 251.0^{q-v}$
JI1951	*P. sativum*	100	5.0 ± 0.00^{m}	$74.5 \pm 3.00^{u-z}$	100.0 ± 0.00^{n}	$2021 \pm 71.2^{r-v}$
P691	*P. sativum* ssp. *elatius*	100	5.0 ± 0.00^{m}	$69.8 \pm 6.44^{s-z}$	100.0 ± 0.00^{n}	$2022 \pm 140.0^{r-v}$
P14	*P. sativum* ssp. *abysinicum*	100	5.0 ± 0.00^{m}	$75.2 \pm 6.37^{v-z}$	100.0 ± 0.00^{n}	$2063 \pm 145.2^{s-v}$
P629	*P. sativum* ssp. *arvense*	100	5.0 ± 0.00^{m}	$75.2 \pm 3.94^{v-z}$	100.0 ± 0.00^{n}	$2152 \pm 70.1^{t-v}$
JI1213	*P. sativum* (cv. erylis)	100	5.0 ± 0.00^{m}	$79.0 \pm 5.15^{x-z}$	100.0 ± 0.00^{n}	$2153 \pm 97.5^{t-v}$
JI1210	*P. sativum* (cv. Erygel)	100	5.0 ± 0.00^{m}	$77.7 \pm 3.11^{w-z}$	100.0 ± 0.00^{n}	2184 ± 40.7^{uv}
P667	*P. fulvum*	100	5.0 ± 0.00^{m}	86.5 ± 3.50^{z}	100.0 ± 0.00^{n}	2190 ± 60.0^{uv}
P666	*P. fulvum*	100	5.0 ± 0.00^{m}	86.0 ± 4.00^{z}	100.0 ± 0.00^{n}	2200 ± 50.0^{uv}
JI2840	*P. sativum* (RIL 15x399_68)	100	5.0 ± 0.00^{m}	82.5 ± 4.69^{yz}	100.0 ± 0.00^{n}	2250 ± 0.0^{v}
P21	*P. sativum* ssp. *elatius*	100	5.0 ± 0.00^{m}	90.0 ± 0.00^{z}	100.0 ± 0.00^{n}	2250 ± 0.0^{v}
P312	*P. sativum* ssp. *elatius*	100	5.0 ± 0.00^{m}	90.0 ± 0.00^{z}	100.0 ± 0.00^{n}	2250 ± 0.0^{v}
P623	*P.sativum* ssp. *transcaucasicum*	100	5.0 ± 0.00^{m}	90.0 ± 0.00^{z}	100.0 ± 0.00^{q}	2250 ± 0.0^{v}
P657	*P. fulvum*	100	5.0 ± 0.00^{m}	90.0 ± 0.00^{z}	100.0 ± 0.00^{n}	2250 ± 0.0^{v}
P659	*P. fulvum*	100	5.0 ± 0.00^{m}	90.0 ± 0.00^{z}	100.0 ± 0.00^{n}	2250 ± 0.0^{v}
P661	*P. fulvum*	100	5.0 ± 0.00^{m}	90.0 ± 0.00^{z}	100.0 ± 0.00^{n}	2250 ± 0.0^{v}
P662	*P. fulvum*	100	5.0 ± 0.00^{m}	90.0 ± 0.00^{z}	100.0 ± 0.00^{n}	2250 ± 0.0^{v}
P671	*P. sativum* ssp. *elatius*	100	5.0 ± 0.00^{m}	90.0 ± 0.00^{z}	100.0 ± 0.00^{n}	2250 ± 0.0^{v}
P675	*P. sativum* ssp. *elatius*	100	5.0 ± 0.00^{m}	90.0 ± 0.00^{z}	100.0 ± 0.00^{n}	2250 ± 0.0^{v}
Little Marvel (S)	*P. sativum*	100	5.0 ± 0.00^{m}	84.6 ± 5.40^{z}	100.0 ± 0.00^{n}	2250 ± 0.0^{v}

[1] Data are means of 5 replicates, different letters indicates significant difference between value according to Duncan Multiple Range Test at $\alpha = 0.05$.

Table 3. Disease Ratings of selected accessions to *Fop* 2

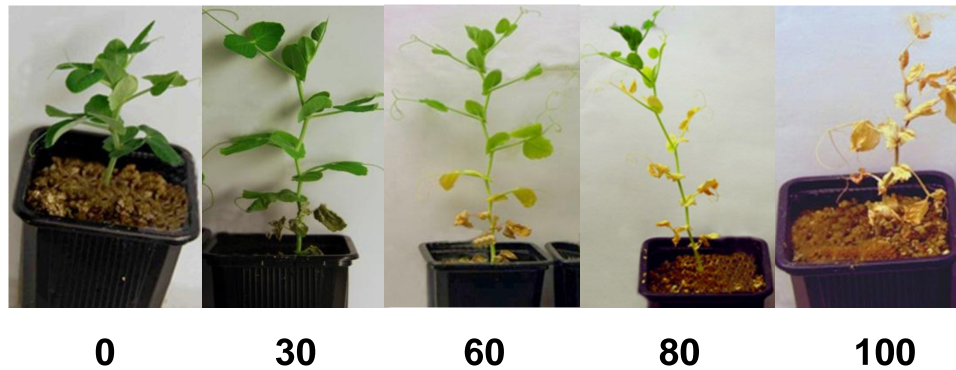
Accession	Species	Disease Incidence (%)	Disease Ratings[1]			
			Plant Mean Disease Index		Symptomatic Leaves (%)	
			MVI	AUDPC	PSL	AUDPC
P23	*P. sativum* ssp. *elatius*	20 ± 0.0[bc]	2.3 ± 0.42[ab]	9.7 ± 4.65[a]	45.8 ± 8.45[a]	343 ± 106.4[a]
JI1412	*P. sativum* ssp. *sativum*	0 ± 0.0 [a]	2.1 ± 0.08[ab]	12.2 ± 1.03[a]	34.6 ± 2.83[a]	440 ± 20.6[ab]
JI1760	*P. sativum* ssp. *sativum*	7 ± 6.7[ab]	2.4 ± 0.30[ab]	10.4 ± 1.86[a]	43.9 ± 7.48[a]	477 ± 62.4[ab]
P627	*P. sativum* ssp. *arvense*	7 ± 6.7[ab]	2.1 ± 0.36[ab]	15.2 ± 3.69[a]	31.3 ± 8.93[a]	479 ± 113.9[ab]
P656	*P. fulvum*	33 ± 6.7[cd]	3.6 ± 0.50[cd]	12.8 ± 3.72[a]	68.3 ± 11.76[b-d]	505 ± 109.1[ab]
P633	*P. sativum* ssp. *arvense*	0 ± 0.0 [a]	1.8 ± 0.12[a]	11.7 ± 1.85[a]	30.0 ± 2.78[a]	507 ± 60.4[ab]
P42	*P. sativum* ssp. *arvense*	0 ± 0.0 [a]	1.8 ± 0.14[a]	12.2 ± 1.61[a]	32.3 ± 3.33[a]	571 ± 66.4[ab]
JI1559	*P. sativum* ssp. *sativum*	0 ± 0.0 [a]	2.1 ± 0.12[ab]	15.1 ± 2.69[a]	32.8 ± 2.30[a]	634 ± 79.9[a-c]
P614	*P. sativum* ssp. *elatius*	0 ± 0.0 [a]	2.1 ± 0.12[ab]	14.1 ± 1.30[a]	35.6 ± 2.84[a]	660 ± 49.0[a-c]
P639	*P. sativum* ssp. *arvense*	7 ± 6.7[ab]	2.1 ± 0.35[ab]	18.4 ± 6.07[ab]	34.3 ± 8.82[a]	684 ± 172.1[a-d]
P650	*P. sativum* ssp. *jormadi*	0 ± 0.0 [a]	1.9 ± 0.22[a]	12.9 ± 2.29[a]	34.0 ± 6.86[a]	694 ± 97.4[a-d]
P632	*P. sativum* ssp. *arvense*	0 ± 0.0[a]	2.5 ± 0.11[ab]	20.8 ± 1.89[ab]	42.2 ± 4.06[a]	715 ± 50.5[b-d]
P638	*P. sativum* ssp. *arvense*	13 ± 6.7[a-c]	2.9 ± 0.22[bc]	19.3 ± 3.38[ab]	54.5 ± 5.44[ab]	755 ± 67.7[b-d]
P669	*P. sativum* ssp. *elatius*	40 ± 20.0[cd]	3.4 ± 0.40[cd]	20.3 ± 4.06[ab]	76.5 ± 7.63[cd]	756 ± 84.6[b-d]
P18	*P. sativum* ssp. *elatius*	53 ± 6.7[de]	3.5 ± 0.50[cd]	34.3 ± 9.78[c]	68.9 ± 11.24[bc]	965 ± 245.3[c-e]
P615	*P. sativum* ssp. *elatius*	53 ± 6.7[de]	3.8 ± 0.45[cd]	29.9 ± 6.41[bc]	83.8 ± 9.03[c-e]	1010 ± 163.2[de]
P316	*P. sativum* ssp. *arvense*	73 ± 0.7[e]	4.1 ± 0.41[d]	37.1 ± 6.25[c]	88.6 ± 6.68[de]	1265 ± 129.0[e]
P662	*P. fulvum*	100 ± 0.0[f]	5.0 ± 0.00[e]	73.9 ± 0.43[d]	100.0 ± 0.00[e]	2028 ± 64.2[f]
P21	*P. sativum* ssp. *elatius*	100 ± 0.0[f]	5.0 ± 0.00[e]	83.5 ± 2.91[d]	100.0 ± 0.00[e]	2274 ± 23.0[f]

[1] Data are means of 15 replicates, different letters indicates significant difference between value according to Duncan Multiple Range Test at $\alpha = 0.05$.

Table 4. Correlation of the different parameter assessed calculated according to Spearman's Rank Correlation. * and ** after the correlation coefficient indicate statistical significance a p< 0.01 and p< 0.001 respectively.

	DI	MVI	AUDPC (MVI)	PSL	AUDPC (PSL)
DI	1.000				
MVI	0.483**	1.000			
AUDPC (MVI)	0.312*	0.803**	1.000		
PSL	0.524**	0.925**	0.757**	1.000	
AUDPC (PSL)	0.298*	0.727**	0.942**	0.716**	1.000

a



b

Figure 1 Evolution of disease symptoms induced by *F. oxysporum* f. sp. *pisi* race 2 on susceptible pea accessions. **(a)** The photographs represent the evolution of fusarium wilt symptoms at whole plant level of susceptible pea accessions inoculated with one isolate of *Fop* race 2. Number below each photograph represent their respective Disease Ratings estimated as the percentage of symptomatic leaves. **(b)** Typical progression of fusarium wilt disease of susceptible pea accessions inoculated with *Fop* race2 at leaf level. Numbers under each leaf indicate its respective Disease Ratings value based on a disease index scale ranging from 1 (healthy leaf) to 5 (dead leaf).

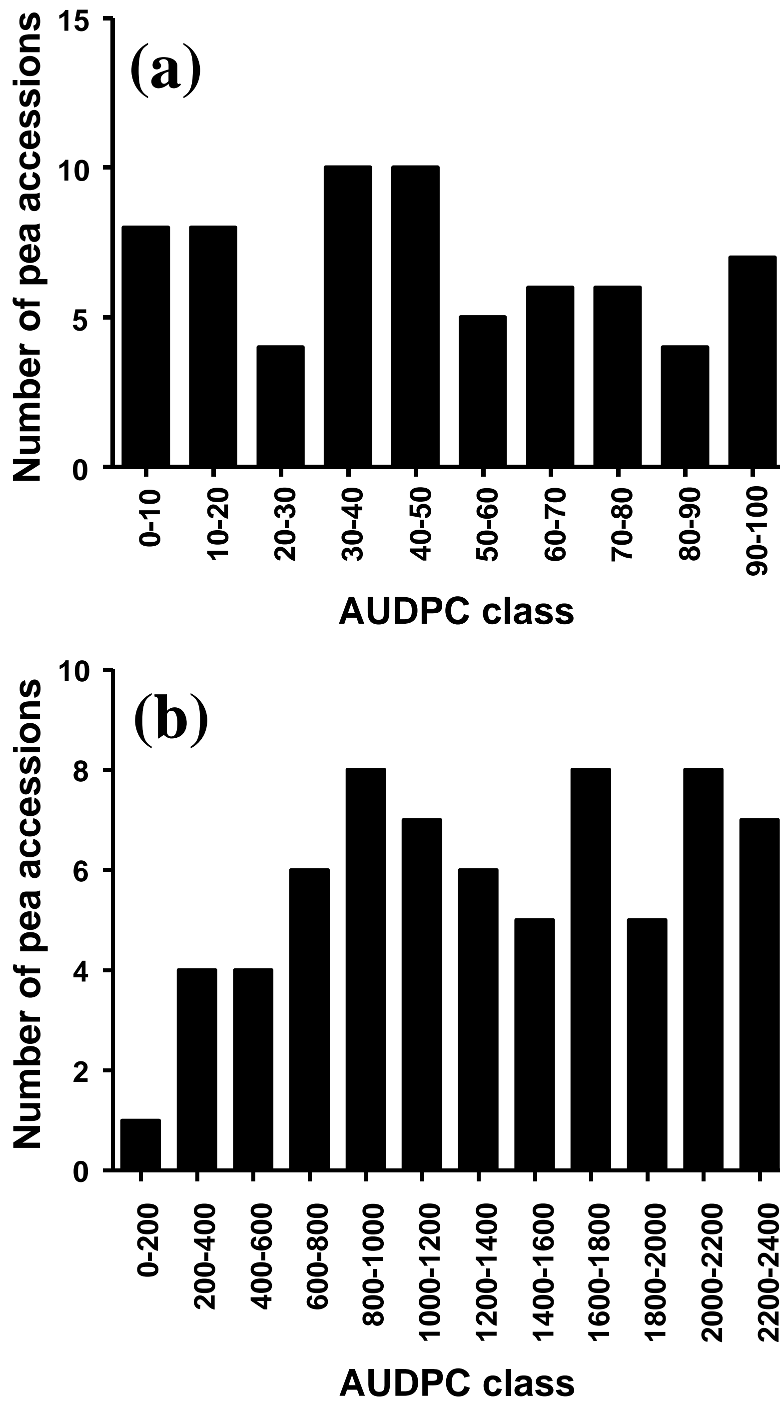


Figure 2 Distribution of the pea accessions upon inoculation with one isolate of *Fusarium oxysporum* f. sp. *pisi* race 2 according to AUDPC values calculated from the evaluation of Disease Ratings for MVI (**a**) and PSL (**b**).

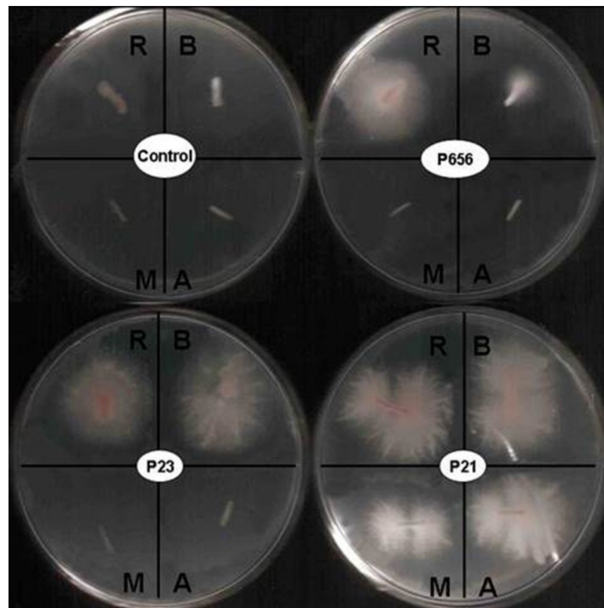


Figure 3 Isolation of *F. oxysporum* f. sp. *pisi* (*Fop*) race 2 colonies from inoculated plants. Photographs compare the extension of *Fop* race 2 colonies out of plant tissue from control non-inoculated plants, and 7 days inoculated plants of the susceptible accession P21, the partially resistant accession P656 and the resistant accession P23. R, B, M and A stand for root section, basal stem section, middle stem section and apex section respectively.

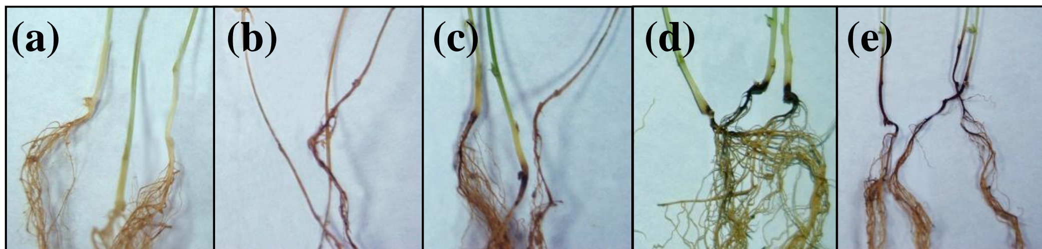


Figure 4 Comparison of *Fop*-induced superficial crown darkening on different susceptible and resistant accessions. (**a**) Control plants of the susceptible accession P21 kept non-inoculated. (**b**) susceptible accession P21 at 30 dpi. (**c**) partially resistant accession P656 at 30 dpi. (**d**) Resistant accession P23 at 30 dpi. (**e**) Resistant accession P42 at 30 dpi.

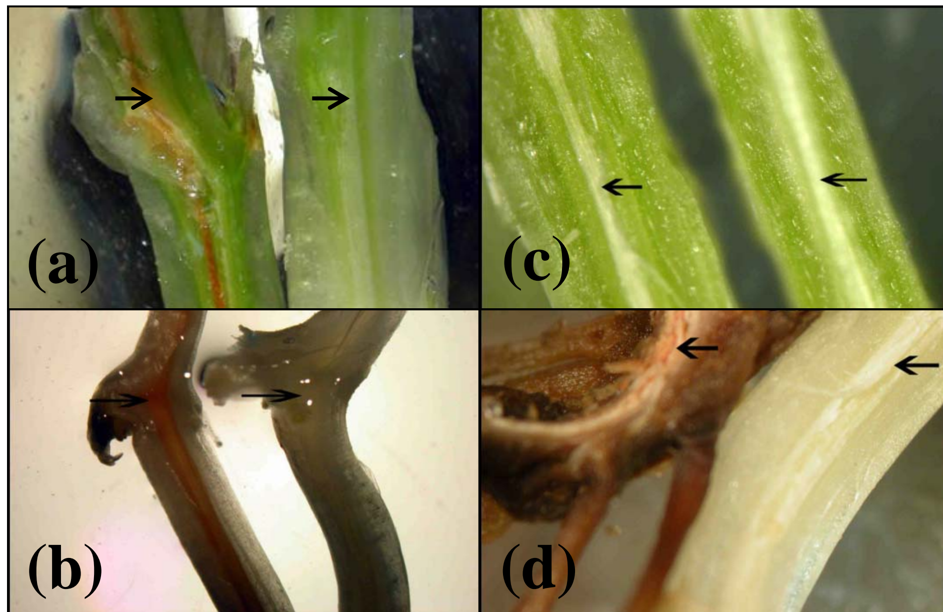


Figure 5 Comparison of the extension of the typical *Fop*-induced vascular discoloration between susceptible and resistant pea accessions. Each picture show hand-made longitudinal sections of stem (**a, c**) and crown (**b, d**) for inoculated (left) and non-inoculated control (right) plants. (**a, b**) Susceptible accession P21 showing the typical dark red vascular discoloration in crown and stem 30 dpi with *Fop* race 2. (**c, d**) Resistant accession P633 showing the typical dark red vascular discoloration only within crown section. Black arrow indicate vascular tissue.

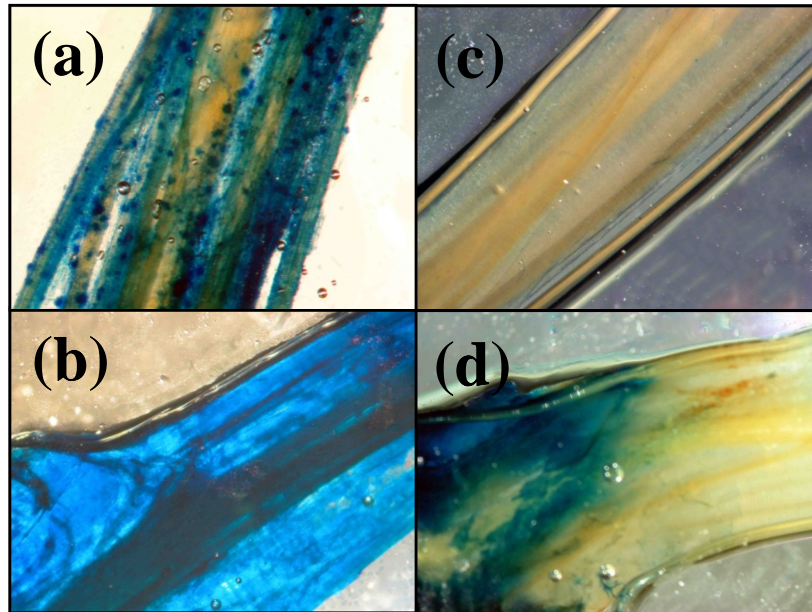


Figure 6 Comparison of the extension of *F. oxysporum* f. sp. *pisi* race 2 colonisation of plant tissues between susceptible and resistant pea accessions. Each picture show superficially stained sections of stem (**a, c**) and crown (**b, d**) of 30 days inoculated plants with the commercial ink Parker Blue Quinck indicating fungal presence as a blue coloration. (**a, b**) Susceptible accession P21 showing fungal presence over the whole crown and stem surface. (**c, d**) Resistant accession P23 showing fungal presence only within crown section.