EFFICIENT RESISTANCE TO *POTATO VIRUS Y* INFECTION CONFERRED BY CYTOSOLIC EXPRESSION OF ANTI-VIRAL PROTEASE SINGLE-CHAIN VARIABLE FRAGMENT ANTIBODY IN TRANSGENIC POTATO PLANTS

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SUMMARY

The production of transgenic potato plants that express anti-*Potato virus Y* (PVY) single chain variable fragment (scFv) antibodies directed against a viral protease is reported. Complementary DNA encoding the recombinant anti-PVY NIa protease antibody was transferred to potato plants (cvs Belle de Fontenay, Claustar and Nicola) and inserted downstream of the *Tobacco mosaic virus* (TMV) Ω leader sequence in order to increase translation efficiency. Protein expression in transgenic plants was verified by Western blot and its subcellular localization by immunocytochemical analyses. The intracellular accumulation of scFv in protoplasts from transgenic potato leaves was assessed. Transgenic potato plants were transferred to a greenhouse and mechanically inoculated with PVY^{O}. Transgenic potato plants expressing anti-PVY protease scFv antibodies in the cytosol led to complete resistance against the target virus.

Key words: PVY^{O}, PVY NIa protease, immunocytochemistry, recombinant antibody

INTRODUCTION

The expression of recombinant antibodies in transgenic plants has become a valuable approach and an important tool in plant science that can be used for different purposes. Among the various engineered antibody species tested, the molecule corresponding to the single-chain variable fragment (scFv) has been widely used to modify plant biochemistry (Owen *et al.*, 1992), to induce disease resistance (Tavladoraki *et al.*, 1993; Conrad and Fiedler, 1998; Zimmerman *et al.*, 1998; Bonrood *et al.*, 2004; Schillberg *et al.*, 2000; Goldbach *et al.*, 2003; Orecchia *et al.*, 2008; Safarnejad *et al.*, 2011) and to produce therapeutic molecules against human diseases (Galeffi *et al.*, 2005; Almquist *et al.*, 2006; Stöger *et al.*, 2000; Ko and Koprowski, 2005). Although the expression levels of scFv obtained can be disappointing, especially when scFvs are targeted to the cytosol (Vine *et al.*, 2001; Firek *et al.*, 1993; Conrad and Fiedler, 1998), they can be improved by targeting the endoplasmic reticulum (ER) (Schouten *et al.*, 1996; Gil *et al.*, 2011) or the apoplastic space (Fischer *et al.*, 2001; Malembic-Maher *et al.*, 2005; Li *et al.*, 2008).

The first successful use of plantibodies against virus infection was described in *Artichoke mottle crinkle virus* (AMCV) by means of an scFv directed against the viral coat protein (CP) (Tavladoraki *et al.*, 1993). Later, different reports have confirmed the efficiency of this method in plant protection against different viruses (reviewed in Schilberg *et al.*, 2000; Prins *et al.*, 2008; Safarnejad *et al.*, 2011). Nevertheless, the main problem of this approach has been the instability of many scFvs in plants cells (Prins *et al.*, 2008). Indeed, since most viruses replicate in the cytosol, it is necessary to direct scFvs to this compartment, but in some cases lack of correct folding of the scFv was observed. The fusion of these antibodies into the ER retention signal (KDEL) stabilized them (Conrad and Fiedler, 1998; Schouten *et al.*, 1996). Thus, the use of a bacterial pectate lyase signal peptide (PelB) (Lei *et al.*, 1987) led to quite a high expression level of the recombinant antibodies in the apoplastic space.

Nevertheless, Zimmerman *et al.* (1998) showed that despite the low cytosolic expression of scFv antibodies directed against the *Tobacco mosaic virus* (TMV) CP, resistance to the virus was obtained. A high protection level nearing immunity was also reached upon expression of plantibodies (Bajrovic *et al.*, 2001). More recently, Villani *et al.* (2008) were able to immunomodulate the ectopic expression of intracellular scFv antibodies directed against *Cucumber mosaic virus* (CMV). These authors developed a synthetic scFv phage display library and performed an *in vitro* screening using immobilized virions. After this *in vitro* 'biopanning' the authors selected two scFvs which were then biochemically characterized, showing high affinity toward the antigen. These scFv antibody fragments were subsequently expressed in the cytosol of transgenic tomato plants. Challenge

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with high viral dose showed that both scFvs were able to elicit resistance to infection. Such large scale selection of functional intrabodies from a human scFv library was also described by Philibert *et al.* (2007).

The expression of scFv directed against viral non-structural proteins that play an important role in virus multiplication, such as the viral replicase, was achieved in some cases (Esteban *et al.*, 2003; Boonrod *et al.*, 2004; Gil *et al.*, 2011). This approach has led to the selection of transgenic lines fully resistant to the target virus. Similarly, scFv molecules specific for a tospovirus movement protein were shown to accumulate in the cytosol of tobacco cells. Their accumulation restricted infection in transgenic plants resulting in delayed symptoms (Zhang *et al.*, 2008). More recently, the first successful application of an scFv-mediated resistance approach was described against a plant DNA virus (Safarnejad *et al.*, 2009) and these scFvs interacted with the viral multifunctional replication initiator protein.

In this context, we have shown in a previous report that efficient apoplastic expression of scFv antibodies directed against the NIa protease of *Potato virus Y* (PVY) can protect transgenic potato plants against this virus (Gargouri-Bouzid *et al.*, 2006). These scFv antibodies were able to recognize the most common PVY strains (PVY^O and PVY^N).

PVY, the type member of *Potyvirus* genus, is one of the most important plant viruses causing damage on potato and other solanaceous plants such as tomato and pepper (Scholthof *et al.*, 2011). Infection by this virus is responsible for important yield losses in a number of economically important solanaceous crops (Kerlan, 2006). PVY possesses a positive-sense RNA genome that encodes a polyprotein of 350 kDa that is cleaved autocatalytically leading to different functional viral proteins. The nuclear inclusion a (NIa) protein corresponds to one of the proteases involved in cleavage of the polyprotein. It is responsible for the release of the VPg protein and the viral RNA-dependent RNA polymerase involved in genomic RNA replication.

The aims of this study were: (i) to determine if the absence of any signal peptide can allow efficient expression of the anti-PVY-NIa protein scFv in the cytosol, (ii) to examine the subcellular localisation of these antibodies in comparison to those targeted to the apoplast and (iii) to determine the effect of cytosolic accumulation of scFv on the protection of the host against the target virus.

MATERIALS AND METHODS

DNA cloning. We have previously produced and characterized monoclonal antibodies directed against different PVY^O proteins (Rouis *et al.*, 2001). The cDNA encoding the anti-PVY NIa scFv antibody fused to the c-myc peptide was then cloned into the pHEN vector (Rouis *et al.*, 2006). This PelB-scFv-c-myc cDNA was transferred into potato leading to apoplastic antibody expression and high PVY resistance of transgenic plants (Gargouri-Bouzid *et al.*, 2006). Moreover, the VH fragment originating from the same scFv antibody was also over-expressed in transgenic potato plants, leading to partial protection against the virus (Bouaziz *et al.*, 2009). In this report, the same scFv-c-myc cDNA was ligated into the Bluescript vector (Stratagene, USA) containing the TMV 5'-translation enhancer (Hassaïri *et al.*, 1998). The resulting construct was transferred to a constitutive plant expression vector (PKYLX35S2; J. Albouy, INRA Versailles, France) containing the 35S CaMV promoter sequence. The recombinant vector (Fig. 1A), was transferred to *Agrobacterium tumefaciens* (LBA4404) as described by Gargouri-Bouzid *et al.* (2006).

Transformation and regeneration of potato plants. Internodes and leaf tissues from *Solanum tuberosum* (cvs Belle de Fonteney, Claustar and Nicola) were transformed as described by Hmida-Sayari *et al.* (2005). The tissues were incubated on regeneration medium supplemented with kanamycin (100 µg/ml) and cefotaxime (250 µg/ml). The *de novo*-induced shoots were then transferred to MS (Murashige and Skoog, 1962) medium supplemented with kanamycin (100 µg/ml) for elongation and rooting. Plants that rooted in the presence of kanamycin were selected as putative transgenic plants.

Analysis of DNA from transformed plants. Genomic DNA was isolated from both transformed and non-trans-

Table 1. Plant lines used in this work. a: lines used for DNA analysis; b: lines used for Western blot analysis; c: lines used for protoplast analysis; d: lines used for immunocytochemical analysis; e: lines used for virus challenging analysis.

Untransformed lines and potato cultivars used for transformation	Transgenic lines	
Cl: Clauster BF: Belle de Fontenay Ni: Nicola	Produced in this work (cytosolic expression)	Characterized by Gargouri-Bouzid *et al*. (2006) and used as positive control (apoplastic expression)
	Ni-Mi7 [a,b]	BF1312 [b,c]
	BF-MI7 [a,b,c,d,e]	BF1335 [b,e]
	BF-MI76 [a,b,e]	C113 [b]
	CI-MI7 [b,e]	

formed potato plantlets as described by Dellaporta *et al.* (1983). The presence of the scFv cDNA was verified by PCR using two VH-chain-specific convergent primers: H4F: 5'-TGAGGAGACGGTGAGGTTCCTTGGCC-CC-3' and H4B: 5'-AGGTGCAGCTTCAGGAG TCAGG-3' and 1 unit of GO Taq DNA polymerase (Promega, USA) for 30 ng of genomic DNA. The PCR reaction was performed in a thermocycler 2700 (Applied Biosystems, USA). The amplification program started by one denaturation cycle at 94°C for 4 min, followed by 35 cycles of denaturation for 1 min at 94°C, annealing at 52°C for 1 min and elongation at 72°C for 1 min. A final elongation for 7 min at 72°C was performed. Amplification products were analysed by agarose (1.5%) gel electrophoresis in TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA, pH 8).

Analysis of the scFv protein expression. The scFv protein expression in transgenic potato plants was analysed by Western blot and ELISA using anti-c-myc antibodies (Invitrogen, USA). Proteins were extracted from fresh leaves (1 g) of transgenic and untransformed control plants by grinding in a phosphate-buffered saline (PBS) solution (5 ml) containing 137 mM NaCl, 2.6 mM KCl, 10 mM Na_2HPO_4, 1.7 mM KH_2PO_4, pH 7.4 and 2 mM PMSF (phenylmethylsulphonyl fluoride), followed by centrifugation at 12,000 rpm for 10 min at 4°C. The protein concentration was determined by the Bradford (1976) method. The same protocol was used for protein extraction from plants cultivated in the greenhouse and inoculated with PVY^O strain.

For Western blot analyses, the proteins contained in crude extracts (20 µg) were separated by electrophoresis on denaturing 12% polyacrylamide slab gels (SDS-PAGE; Laemmli, 1970) and blotted onto a nitrocellulose Hybond-C membrane (Amersham, USA) by electrotransfer. They were then incubated with anti-c-myc antibodies as described (Rouis *et al.*, 2006). ELISA tests were performed as reported (Gargouri-Bouzid *et al.*, 2006) using proteins from protoplasts of transgenic plant leaves. Briefly, microtiter wells were coated with crude protein extracts (20 µg) and incubated overnight at 4°C; 100 µl of a diluted (1/5000) anti-c-myc monoclonal antibody (Invitrogen, USA) were then loaded followed by the addition of 100 µl of peroxidase-conjugated anti-mouse antibodies. After each step, the plates were washed three times with PBS containing 0.05% Tween 20. The absorbance was measured at 490 nm after addition of O-Phenylenediamine Dihydrochloride (OPD).

Protoplast isolation and analysis. Sterile potato leaf sections from *in vitro* cultivated transgenic plants were used for protoplast purification as described by Nouri-Ellouz *et al.* (2006). Protoplasts were isolated from transgenic plants harbouring the pelB-scFv-c-myc construct (BF1312 transgenic potato line; Gargouri-Bouzid *et al.*, 2006) and the pelB-scFv-c-myc construct (BFM17). The protoplasts obtained were resuspended in liquid VKM culture medium (Binding *et al.*, 1978) and their density was adjusted to 10^5 cells/ml. They were then cultured at 24°C and samples were taken at 6 and 20 h of culture to assay protein expression.

Each sample (1 ml) was centrifuged at 4,000 rpm for 5 min, the supernatant was dialysed against water, concentrated by speed vac to a final volume of 100 µl and the protein content was measured. The extraction of proteins from the cell pellets was performed by grinding in a mortar in 100 µl of 0.5X PBS buffer and 2% PMSF. After centrifugation at 8,000 rpm for 10 min at 4°C, the supernatant was recovered and protein concentration was measured by the Bradford (1976) method.

Immunocytochemical analysis. Transgenic plants and untransforrmed control plants were cultivated in a greenhouse for 6 weeks, then used for immunocytochemical analyses as described by Ternynck and Avrameas (1987). The leaf epidermis was removed from transgenic and control plants and 0.5 cm sections were cut, soaked in water and mounted on slides coated with 0.5% gelatin. Specimens were fixed overnight at 4°C in 1X PBS and 4% formaldehyde. Tissues were permeabilized by dehydration through 25, 50 and 75% methanol dilutions in PBS supplemented with 0.1% Tween 20 (PBT) followed by two bath treatments in 100% methanol for 20 min each. Samples were rehydrated through methanol bath treatments used in the reverse order, rinsed twice with PBT, then incubated at room temperature for 45 min with anti-c-myc antibodies diluted 1:100 in PBS. The slides were washed three times with PBS, and Texas Red fluorescent-conjugated anti-mouse immunoglobulin diluted 1:250 in PBS was applied to the sections and incubated at room temperature for 30 min. After washing as above, the slides were examined by a confocal microscope (LSM510 Zeiss) with 25 fold magnification. The emission of Texas red was measured at 560 nm.

Greenhouse planting and inoculation with PVY. Ten plants from each transgenic line (T_0) and from untransformed controls, cultivated *in vitro* for 2 to 3 weeks, were transferred to a greenhouse. Three weeks later, seven plants from each line were inoculated with 1:10 dilution of PVY^O crude extract after dusting two lower leaves with carborundum (Gargouri-Bouzid *et al.*, 2006). A second inoculation was performed one week later. Leaf samples were taken weekly for crude protein extraction from PVY-inoculated plants and the presence of the virus was assessed by ELISA on 20 µg of proteins, using an anti-PVY CP monoclonal antibody diluted to 1:1000 (Rouis *et al.*, 2001) as described above. Protein extraction was performed as described above. Leaves intermediate between inoculated and up-

per leaves were used for ELISA testing.

Table 1 summarizes the different non-transgenic and transgenic plant lines used in this work, including those expressing the anti-PVY NIa scFv in the apoplastic space (Gargouri-Bouzid *et al*., 2006).

RESULTS

Generation of scFv-expressing potato plants. Transgenic plants were produced by *Agrobacterium*-mediated transformation of leaves and internodes of three potato cultivars (Claustar, Belle de Fonteney and Nicola). The recombinant *A. tumefaciens* strain used for plant transformation harboured a binary vector containing the expression construct depicted in Fig. 1A with the kanamycin-resistance gene (nptII gene) as selection marker. Kanamycin-resistant regenerated plants were selected and subcultured. The genomic DNA was then purified from these plants and used as template in PCR analyses using VH-specific primers leading to amplification of a band of 300 bp (Fig. 1B). All plant lines that rooted on kanamycin-containing medium were positive by PCR analysis of the genomic DNA. The transformation efficiency was 40%. Thirty transgenic lines were obtained, 12 of which were selected for further analyses.

Selected PCR-positive lines were further screened for the expression of the scFv protein by Western blot analysis of crude protein extracts using the anti-c-myc antibodies (Fig. 1C). The scFv-c-myc proteins were detected in all the tested transgenic lines. The scFv expression level observed here was similar to that obtained in a previous report in which scFv accumulation was targeted to the apoplastic compartment (Gargouri-Bouzid *et al.*, 2006) by addition of a bacterial PelB signal peptide (Lei *et al.*, 1987) upstream of the scFv cDNA. These data differ from those of other reports indicating very low expression levels of cytosolic scFv antibodies in transgenic plants (Tavladoraki *et al.*, 1993).

Analysis of subcellular localisation of the expressed scFv protein. Immunohistochemical analysis of one transgenic potato plant (BFM17) (Fig. 2) confirmed the intracellular accumulation of scFv protein. However, the presence of the PelB allowed the detection of the recombinant scFv protein (BF1312 plant line) mainly at

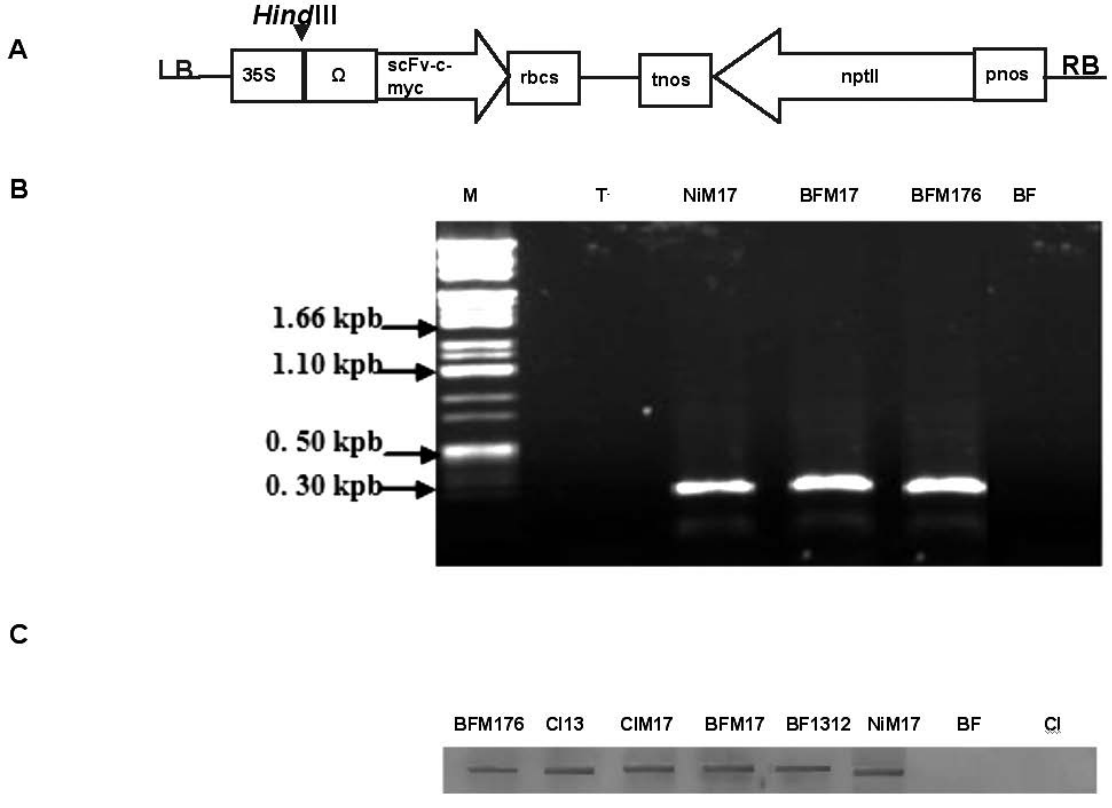

Fig. 1. A. Construct used for the expression of anti-NIa scFv antibodies in the cytosol of transgenic potato plants. RB: right border; LB: left border; nptII: neomycin phosphotransferase II; 35S: *Cauliflower mosaic virus* 35S RNA promoter; pnos: nopaline synthase gene promoter; tnos: nopaline synthase gene terminator; Ω : 5'untranslated region of TMV genomic RNA; rbcs: ribulose 1,5-bisphosphate carboxylate synthetase terminator; scFv-c-myc: fusion protein between scFv and a mouse myc epitope tag. B. PCR amplification profile of the VH cDNA fragment from genomic DNA of different kanamycin-resistant plants (NiM17, BFM17, BFM176). BF: DNA from untransformed control plant. M: λ DNA marker digested by *Hinc*II; T-: negative control amplification without DNA. C. Western blot analysis of the anti-NIa scFv protein from crude extracts of transgenic lines: BFM176, Cl13, ClM17, BFM17, BF1312, and NiM17. BF, Cl: non-transgenic plant protein extracts from cv BF and Cl, respectively.

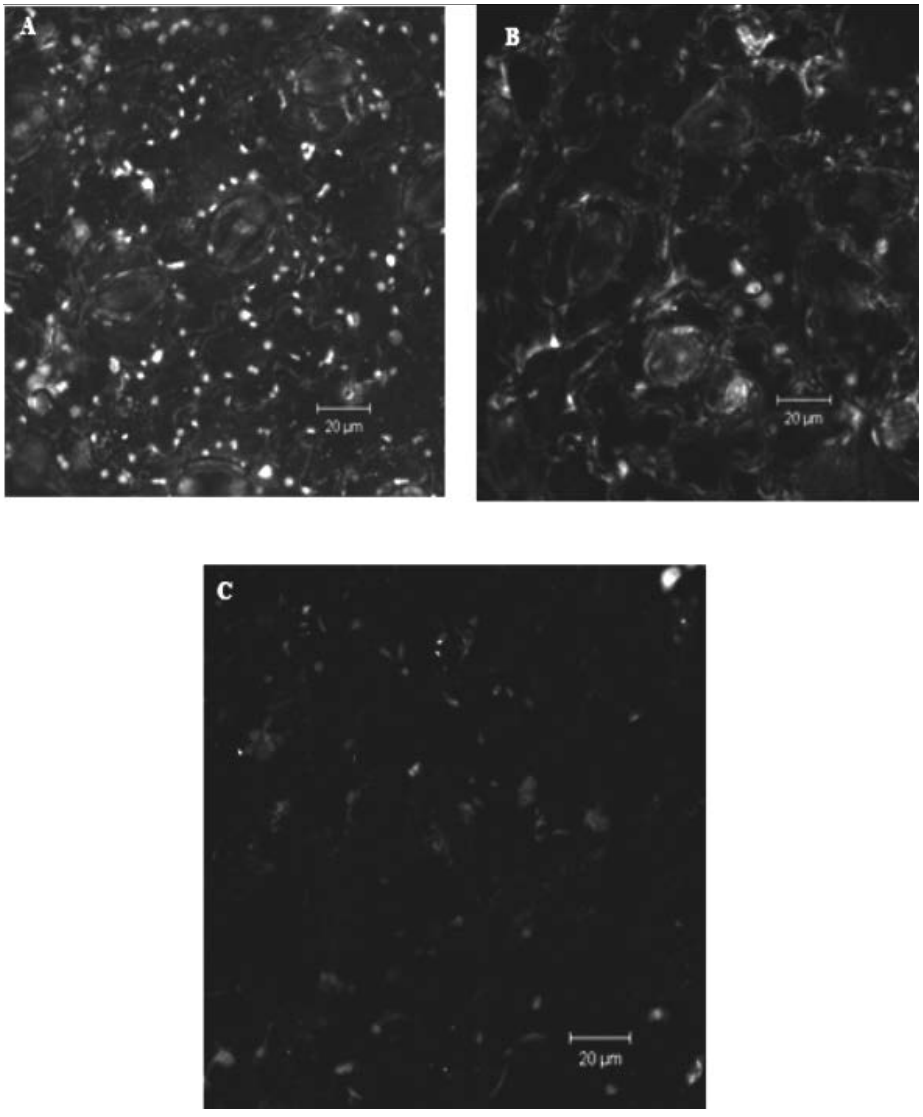

Fig. 2. Immunohistochemical analysis of the expression of the scFv antibodies in transgenic plants. A. BFM176 transgenic plant expressing the scFv fragment in the cytosol. B. BF1312 transgenic plant expressing the scFv fragment in the apoplast. C: untransformed control plant, in which only non-specific autofluorescence is observed.

the cell periphery. No specific antibody reactivity was observed in leaf samples from untransformed plants used as negative controls. To further confirm the intracellular accumulation of scFv antibodies in transgenic plants, protoplasts were cultured for 6 and 20 h and the scFv-c-myc protein expressed was analysed in the culture supernatant as well as in the cell pellet. The data revealed that this protein was retained within the cells in the BFM17 transgenic line whereas it was mainly in the supernatant of the BF1312 positive control line (Fig. 3). These results confirmed that a stable accumulation of scFv antibodies was obtained in both transgenic lines. However, the expressed protein was secreted into the culture medium of protoplasts from transgenic lines containing PelB, while the absence of this signal peptide in the BFM17 line allowed efficient expression and accumulation of the scFv in the cytosol.

Investigation of virus resistance of transgenic plants expressing cytosolic scFv. Transgenic plants expressing scFv in the cytosol as well as a control plant expressing the same scFv in the apoplastic space (BF1312) were transferred to a greenhouse and mechanically inoculated with crude extract from PVY^O-infected tobacco (*Nicotiana tabacum*). Symptom development and virus accumulation (Fig. 4) were analyzed weekly. Untransformed plants were treated similarly and used as controls. Three transgenic plant lines were tested here for their response to PVY^O infection (ClM17, BFM17, and BFM176). Two of them (ClM17 and BFM176) showed efficient resistance against the target virus. Indeed, neither symptoms appeared nor virus accumulation was detected in the leaves of these plants following inoculation with PVY^O (Fig. 4) while the control untransformed plants (BF, Cl) rapidly showed symptoms and

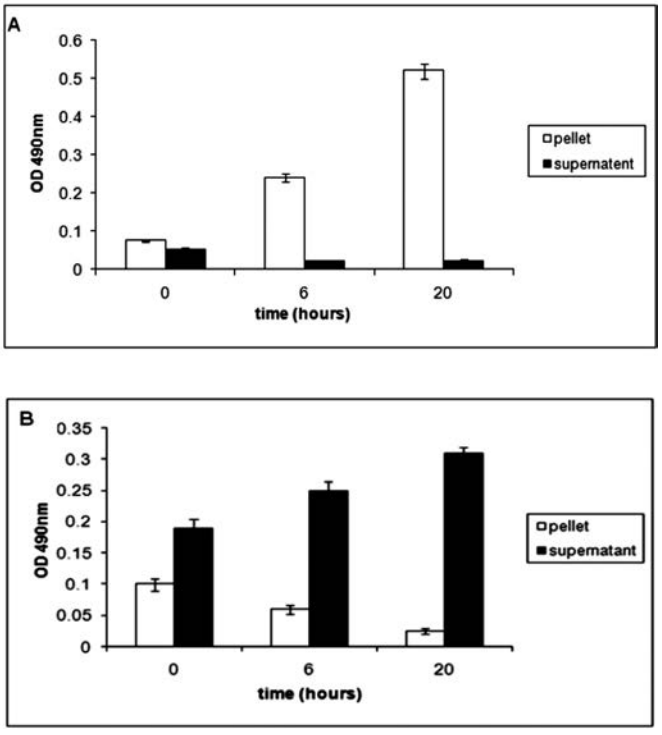

Fig. 3. ELISA analysis of the recombinant scFv antibodies in protoplast culture isolated from transgenic plants. A. transgenic line BFM176 expressing the scFv fragment in the cytosol. B. transgenic line BF1335 expressing the scFv fragment in the apoplast. Means of 3 independent replicates are presented here and standard deviations are indicated.

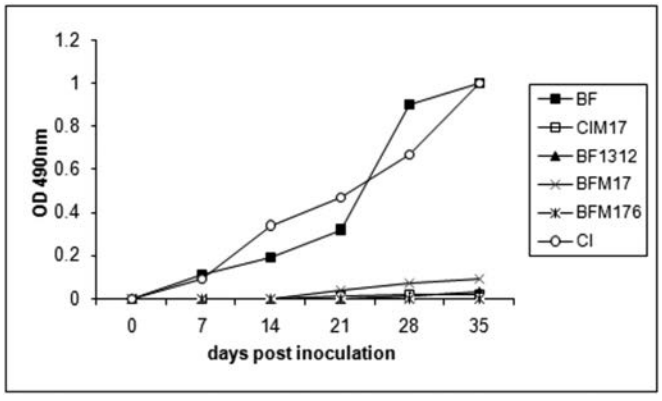

Fig. 4. Virus accumulation measured by ELISA using anti-PVY monoclonal antibodies. Transgenic lines (ClM17, BFM17, BF1335 and BFM176) and untransformed lines (BF and Cl) were mechanically inoculated by the PVY^O strain. Virus accumulation was followed weekly on leaf crude protein extracts (20 µg) using PVY^O CP scFv antibody fragment. PVY inoculated untransformed plants were used as negative control.

virus accumulation. However, the BFM17 transgenic line showed a low level infection at 35 days post inoculation which suggests that the expression of the scFv confers significant resistance against PVY multiplication, but not absolute resistance to systemic infection. The resistance rate of ClM17 and BFM176 transgenic plants expressing the pelB-scFv-c-myc construct was similar to that of the positive control plant (BF1312) expressing the same scFv in the apoplastic space. These results suggest that the expression either in the cytosol (this study) or in the apoplast (Gargouri-Bouzid *et al.*, 2006) of scFv recombinant antibodies directed against a functional viral protease can protect against PVY infection.

DISCUSSION

The objective of this study was to determine the effect of cytosolic expression of recombinant scFvs directed against the PVY NIa protease. The data showed that an efficient and detectable expression of anti-NIa scFv occurred in transgenic potato plants. The over-expression of cytosolic scFv was quite similar to that obtained in the apoplastic space (Gargouri-Bouzid *et al.*, 2006). However, such cytosolic localization of scFv plantibodies was frequently reported to be lower than that observed in the apoplastic space (Fischer *et al.*, 2001). Moreover, two transgenic potato lines exhibited full resistance to the virus since no virus multiplication was detected. This report provides additional data supporting the fact that expression of recombinant antibodies can neutralize virus multiplication in plants by disturbing replication, assembly or movement of the virus (Prins *et al.*, 2008; Turturo *et al.*, 2008). The impact of virus specific recombinant antibodies depends on three main factors: yield and stability, localization and intracellular targeting and binding efficiency (Safarnejad *et al.*, 2011). In this context, different viral proteins such as the structural CP, RNA-dependant RNA polymerase, movement protein or viral protease, have been targeted by plantibodies. This study has focused on the cytosolic expression of scFv antibodies directed against the main protease of PVY. The resulting transgenic plants displayed efficient inhibition of virus multiplication. Such virus resistance was also observed when the same scFv protein was expressed in the apoplastic compartment (Gargouri-Bouzid *et al.*, 2006). These plantibodies can bind to viral protein products and thus interfere with steps of the virus life cycle resulting in either low titre infection or aborted infection.

Similar data were described in a number of reports that emphasized that antibody-based resistance can be effective even with low expression levels of cytolosic scFv (Tavladoraki *et al.*, 1993; Zimmermann *et al.*, 1998). Schillberg *et al.* (2000) claimed that recombinant antibody cannot fold properly in the plant cytosol because of the reducing conditions that inhibit the formation of disulfide bonds. Nevertheless, suppression of viral infection was achieved in transgenic plants harbouring cytosolic anti-viral scFv (Zimmermann *et al.*, 1998;

Bajrovic *et al.*, 2001; Villani *et al.,* 2005*,* 2008; Philibert *et al.*, 2007). The advantage of scFv antibodies is that they do not require assembly and thus can be directly expressed in the cytosol. More recently**,** Nölke *et al.* (2009) showed that complete resistance against *Grapevine fanleaf virus* (GFLV) was achieved despite the very low level of expressed antibodies, and resistance to *Plum pox virus* (PPV) in plants, expressing cytosolic, ER and nuclear single-chain antibodies against the viral RNA NIb replicase, was described (Esteban *et al.*, 2003; Gil *et al.*, 2011). Nickel *et al.* (2008) expressed a scFv specific for *Potato leaf roll virus* (PLRV) P1 protein in tobacco (*Nicotiana tabacum*) and some of the resulting transgenic plants exhibited reduced PLRV accumulation.

Our data show that the levels of scFvs required to neutralize infection were reached in transgenic lines. The expression of antibodies directed against non structural proteins such as the replicase (Boonrod *et al.*, 2004; Nickel *et al.*, 2008) or the protease used in our case have the potential to increase the efficiency of the approach towards reaching immunity. The scFv antibodies directed towards the viral CP may block assembly or disassembly of the target virus, yielding the production of less susceptible plants that exhibit a delay in symptom development (Fecker *et al.*, 1997; Safarnejad *et al.*, 2011).

It was hypothesised that viral enzymes that are present in quite small amounts in plant cells may provide more suitable target proteins that would allow efficient suppression of viral infection because these proteins are necessary for virus multiplication and do not accumulate in large amounts.

Our data also suggest that cytosolic and apoplastic localization of the expressed scFv leads to highly efficient defense against the target virus. They confirm the usefulness and effectiveness of scFv expression in transgenic plants for protection against the virus. In contrast, the expression of the VH portion of the same full antibody (hybridoma line 22-1; Rouis *et al.*, 2001) and the same scFv antibody (ScFv clone number 22-19 deriving from hybridoma 22-1; Rouis *et al.*, 2006) were unable to protect transgenic potato plants against PVY infection despite their high expression level (Bouaziz *et al.*, 2009). Similarly, different subcellular compartments were targeted for the expression of recombinant antibodies such as the apoplasm which has led to an improved expression of different antibody species in transgenic plants (Voss *et al.*, 1995). The ER of plant cells provides an appropriate environment for antibody folding, assembly and retention (Fecker *et al.*, 1997). However, the ER is generally not the best cell compartment to achieve virus contact (Fecker *et al.*, 1997).

In conclusion, this report confirms that the expression of recombinant antibodies directed against the PVY protease can inhibit virus multiplication in transgenic potato plants, which express detectable level of anti-NIa scFv in the cytosol that seems sufficient to block the viral replication cycle.

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