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Bacillus vallismortis Strain EXTN-1 Mediated Systemic Resistance against *Potato virus Y* and *X* in the Field

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Efficacy of plant growth promoting rhizobacteria (PGPR) *Bacillus vallismortis* strain EXTN-1 has been proved in eliciting induced systemic resistance (ISR) in several crops. The present paper described the beneficial effects of EXTN-1 in potato as increase in yield and chlorophyll content, and plant protection against Potato Virus Y and X (PVY & PVX). EXTN-1 induced systemic resistance to the plants resulting in significant disease suppression in the field. Also the plants under treatment with EXTN-1 had higher chlorophyll content. The bacterized plants had significantly higher yields over the untreated control plants. The strain induced activation of defense genes, PR-1a and PDF 1.2 in transgenic tobacco model, which indicated the possible role of both SA, and JA pathways in EXTN-1 mediated plant protection against crop diseases.

Keywords **:** *Bacillus vallismortis*, Induced Systemic Resistance, Potato Virus

Potatoes are a vegetatively propagated crop, and many disease-causing organisms including several viruses and a viroid are disseminated in tubers (Zitter et al., 2002). *Potato virus Y* (PVY) is one of the most important viruses infecting potatoes and the symptoms include mottling or yellowing of leaflets, leaf crinkling, and sometimes leaf drop. *Potato Virus X* (PVX) is one of the most widely distributed viruses of potatoes and shows symptoms of latent mosaic, and the yield loss is 15% or more when compared to virus-free plants (Zitter et al., 2002). Because there are no direct control measures, management through induction of plant's natural defenses is important. The ability of plants to defend themselves against pathogens through a distinct signal transduction pathway is referred to as systemic acquired resistance (SAR) (Ryals et al., 1996). SAR by a chemical or biological agent provides an induced resistance to a broad spectrum of pathogens. SAR against viral diseases has been documented by biological and chemical inducing agents (Ryals et al., 1994). Plant defenses induced through the use of beneficial microorganisms is referred to induced systemic resistance (ISR) (van Loon et al., 1998). ISR using plant growth promoting rhizobacteria (PGPRs) have been established against CMV, TMV and *Tomato mottle virus* in cucumber, tobacco and tomato, respectively (Maurhofer et al., 1994; Murphy et al., 2000; Raupach et al., 1996).

The present study evaluated the efficacy of *Bacillus vallismortis* strain EXTN-1 to induce systemic resistance in potato against PVX and PVY. The activation of defense genes PR-1a and PDF 1.2 also was tested in transgenic tobacco upon treatment with EXTN-1. This study was also extended to understand the efficiency of EXTN-1 to increase the chlorophyll content of the treated potato plants and thereby increase in growth and vigor of the plant.

Materials and Methods

Microbial isolates and plant source. *B. vallismortis* strain EXTN-1, used in the present study has been demonstrated to induce systemic resistance against multiple pathogens in various crops (Park et al., 2001). This strain was maintained in tryptic soy broth (TSB) with 20% Glycerol. Potato (*Solanum tuberosum* L.) variety Daeseo was used for all purposes in this experiment. Transgenic tobacco plants expressing β-glucuronidase (GUS) gene fused to the PR-1a promoter or PDF 1.2 were used for studies on defense gene expression.

Field experiment. Seed coating of the potato seeds were carried out with the bacterial (EXTN-1) suspension (10^7 cfu /ml). Application of Benzothiadiazole (BTH) was carried out by seed soaking in a 1.0 mM solution for 60 min. The treated seeds were sowed in the field (40 plants per plot). Ten replicate plots were maintained for each treatment. Untreated control plots were also maintained. Natural disease incidence was estimated over a period of three consecutive years, 2003, 2004 and 2005 and the percentage of diseased plants in a plot was calculated. Upon 90 days of

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growth, the chlorophyll contents were estimated following standard protocols described by Hiscox and Israelstam (1979). Yield of the plants were also recorded for three consecutive years. Data were statistically analyzed with ANOVA and means were compared with LSD at P=0.05.

GUS assay for PR 1a and PDF 1.2 gene expression. Three-week old seedlings of tobacco were leaf-infiltrated with a cell suspension of EXTN-1 (1×10^5 cfu/ml) in sterile water. Two different transgenic tobacco plants were used, one with GUS gene fused to the PR-1a promoter and the other with GUS gene fused to the PDF 1.2 promoter. Infiltration of 0.1 mM of BTH (Lawton et al., 1996) and sterile water served as positive control and negative control respectively. The leaves from the plants were collected 12 h after infiltration. GUS activity was measured in leaflets by using a fluorometric assay described by Jefferson (1987) and Park and Kloepper (2000). The results were recorded as '+' that indicated low GUS activity (below 1,000 nM MU/10 mg fresh weight/hour), '++' that indicated medium GUS activity (between 10,000 nM MU/10 mg fresh weight/hour) and '+++' that indicated strong GUS activity (between 100,000 nM MU/10 mg fresh weight/hour). The experiment was repeated three times.

Results

Field Experiment. Even though the disease suppressive effects of the treatments were not steady over the years, the treatments with EXTN-1 or with BTH resulted in significant decrease of disease severity caused by PVY and PVX in potato plants compared to those in the untreated control (Table 1 and Fig. 1). There were 17-62% of the plants infected during 2003, 2004 and 2005 in the control plots, while only 0-29% of the plants showed disease symptoms in the EXTN-1 treated plots. Similar observations were recorded on the yield of the plants (Fig. 2), except in yr 2004 where there were no significantly higher yield. In the yrs, 2003 and 2005, the yield of EXTN-1 treated plants had significantly increased compared to that of the control. Over the three years, in 2003 and 2005, there was 35-45% increase in the yield in the EXTN-1 treated plants. It has been also observed that the EXTN-1 treated plants had higher chlorophyll content in the leaves compared to those of untreated control plants (Fig. 3).

GUS assay for gene expression of PR 1a and PDF 1.2. GUS activity of EXTN-1-infiltrated plants indicated that the defense genes PR-1a and PDF 1.2 were strongly activated by the bacterial cells (Table 2). Among the treatments, the maximum GUS activity for PR-1a was recorded in the BTH treatment (positive control), followed by EXTN-1, while there was no significant GUS activity in the plants infiltrated with sterile water. The maximum GUS activity in the tobacco-PDF 1.2 plants was recorded in EXTN-1 treatment. With BTH, tobacco-PDF1.2 exhibited

Table 1. Disease suppression [PMV (Y, X)] in potato by treatment with EXTN-1

	Year 2003	Year 2004	Year 2005
Treatments	% diseased plants per plot		
Control	17.5[a*]	62.0[a]	25.8[a]
BTH 0.1 mM	0.0[c]	ND**	0.0[c]
EXTN-1	0.0[c]	29.2[b]	0.0[c]

*The same letters in a column do not differ significantly at P=0.05
**Not Determined

Fig. 1. Better vigor in the EXTN-1 treated potato plants. The plants also had higher chlorophyll content compared to the control.

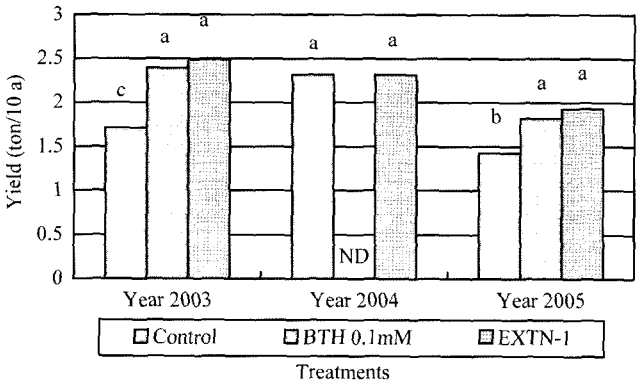

Fig. 2. Increase in yield of potato plants upon treatment with EXTN-1. ND: Not determined, Bars with same letters do not differ significantly at P=0.05

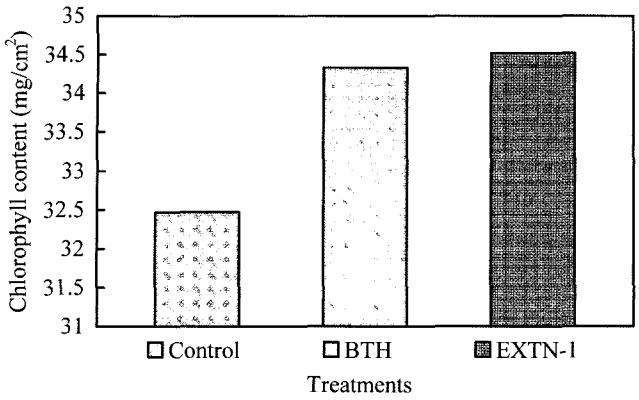

Fig. 3. Treatment with EXTN-1 resulted in higher chlorophyll content in the potato leaves.

Table 2. GUS activity in tobacco plants after infiltration with a cell suspension of EXTN-1

Treatment (Infiltration)	GUS activity in tobacco-PR-1a	GUS activity in tobacco-PDF-1.2
EXTN-1	++	++
Control (sterile water)	–	–
BTH (0.1 mM)	+++	+

'–' Indicated no significant GUS activity (below 100 nM MU/10 mg fresh weight/hour)
'+' Indicated low GUS activity (between 1,000 nM MU/10 mg fresh weight/hour)
'++' Indicated medium GUS activity (between 10,000 nM MU/10 mg fresh weight/hour)
'+++' Indicated strong GUS activity (between 100,000 nM MU/10 mg fresh weight/hour)

low GUS activity compared to tobacco-PR-1a. Consistent results were obtained upon repetition.

Discussion

Many researchers have reported PGPR mediated plant protection against viral diseases of crop plants. In the present study, EXTN-1 imparted beneficial properties in potato plants in terms of reduced disease severity and also resulted in significantly higher yield, when compared to the untreated plants. Treatment with EXTN-1 resulted in considerable disease suppression in potato plants. There were up to 62% of the plants infected during 2003, 2004 and 2005 in the control plots, while only a maximum of 29% of the plants showed disease symptoms in the EXTN-1 treated plots. The disease suppression observed in the bacterial treated plants is supposed to be by the induction of systemic resistance (ISR) by the strain as has been previously reported in many crops and also against many pathogen systems (Park et al., 2001). ISR by rhizobacteria has been proved against several bacterial, fungal and viral plant-diseases (Alstrom, 1991; Leeman et al., 1995).

Virus infection typically has a negative effect on photosynthesis and allocation of resources between organs, which leads to the characteristic chlorosis (Hull, 2002). The present study has revealed the effect of the bacterial strain EXTN-1 in bringing out higher chlorophyll content in the leaves of the treated potato plants. This could be one of the reasons why there is a significantly higher yield in the bacterial treated plants in the field. The present study had up to 45% increases in the yield in the bacterial treated plants, when compared to the untreated control. Enhanced chlorophyll content upon PGPR treatment has been reported in various crops (Idris et al., 2002; Ma et al., 2001).

The host resistance pathways involved in protection of crops from viral disease are unclear, even though there are various reports on systemic protection of plants from viral infection. Our present experiments with EXTN-1 in transgenic tobacco have proved that there was activation of PR-1a and PDF 1.2 defence genes upon treatment with EXTN-1. Ahn et al. (2001) also proved that there is simultaneous activation of both PR-1a and PDF 1.2 genes in tobacco and arabidopsis upon leaf-infiltration with EXTN-1. This indicated a salicylic acid (SA) and jasmonic acid (JA) dependant pathway getting activated in crops with EXTN-1 as PR-1a gene is commonly used as an indicator of SA signaling and PDF 1.2 of JA signaling (Reymond and Framer, 1998). It has also been suggested that, for the full expression of PDF 1.2, both ethylene and jasmonate are required, indicating that these hormonal signals act in concert (Penninckx et al., 1998). Malamy et al. (1990) suggested salicylic acid a likely endogenous signal in the resistance response of tobacco to viral infection. With application of BTH, in the present experiment, the expression of PR-1a was more prominent than PDF-1.2, may be that BTH-mediated ISR works mainly through an SA dependant pathway, not via JA. (Lawton et al., 1996; Görlach et al., 1996).

Chivasa et al. (1997) reported reduced accumulation of TMV- RNA in leaf tissues of TMV-susceptible-tobacco, *via* a mechanism involving SA-pathway. According to Naylor et al. (1998), SA treatment inhibited the replication of TMV in inoculated tobacco tissue. They have also reported the inhibition of long distance movement of CMV and also inhibition of TMV and PVX replication. SA independent SAR also has been demonstrated (Friedrich et al., 1995). We have demonstrated earlier that the up regulation pathways of phenyl alanine ammonia lyase (PAL) and 3-hydroxy, 3-methylglutaryl CoA reductase (HMGR) in EXTN-1 treated tobacco plants upon challenge inoculation with *Pepper mild mottle virus* (PMMoV) (Ahn et al., 2001). Coordinated reduction of viral genome accumulation was clearly detected in the leaves of tobacco pretreated with

EXTN-1.

Since the present study demonstrated the activation of defense genes in transgenic tobacco model with EXTN-1, it could be ascertained that EXTN-1 – potato system has an activated defense machinery that protected the crop from PVX and PVY. Also EXTN-1 significantly increased the growth, vigor and yield in the treated potato plants. The strain could be used as an effective biocontrol and plant growth promoting agent in potato cultivation.

Refernces

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