

Study of Powdery Mildew Resistance in seven Lines of Snow Pea, *Pisumsativum*

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Abstract

Snow pea is one of the important crops grown on highland of Thailand. However, it is very susceptible to powdery mildew. Thus, resistant cultivar increases the production of this crop at a reduced costs. Seven pea lines/cultivars, namely P117, P185, P309, No.3, No.4, No.5 and Fang No.7, were evaluated for phenotypic powdery mildew resistance at Pang Da Royal Agricultural Station, Samoeng district, Chiang Mai during the period of November, 2007 – March, 2008. They were divided into 3 groups: highly resistant group (R) *i.e.* P117, P185 and P309, moderately resistant group (M) *i.e.* No.3, No.4 and 5, and susceptible group (S) *i.e.* Fang No.7. In addition, DNA marker for powdery mildew resistance, *er*, was tested using PCR technique with 3 primers, ScOPD10, OPU17 and OPO02. It was found that only ScOPD10 could yield an 850-bp marker specific to resistant group. DNA sequencing of this 850 bp marker showed 618-624 bases. The sequence was compared

with databases in NCBI GenBank. After BLAST searches, the 618-624 base sequences had 90-91% homology to the DNA sequence from clone JICPSV-598E15, complete sequence of *P. sativum*.

Keywords: Pea, Powdery mildew resistance, PCR Technique

1. Introduction

Snow pea, *Pisumsativum* L.(Fabaceae), is an important source of protein which has continuously increased market demand and becomes popular for the Thai consumers. Fresh frozen pea pod has been exported to Japan as much as fresh consumption in Thailand. Because snow pea is a major winter annual pulse crop which needs cool condition for their growth and yield, the major production area is located on the highland especially in the northern part of Thailand such as Chiang Mai, Chiang Rai and on the highland of Petchaboon and Tak Provinces (Pongphal *et al.*, 2003). In Thailand, powdery mildew is a common disease of snow pea, and the cause of this disease is *Oidium* spp. (Wanasiri, 2007). It is prevalent in many areas of Thailand where pea is cultivated in every season (Khumpai *et al.*, 2001). The commercial snow pea cultivars, widely used by farmers, produce high yield but are very susceptible to powdery mildew disease. The disease infection can rapidly spread when the growing climates are favorable to the disease. Severe damage about 25-50% to the production could occur (Warkentin *et al.*, 1998). Moreover, the number of pods per plant, seeds per pod, heights and the number of nodes could drastically decrease (Fondevilla *et al.*, 2007). Therefore, chemical control of the disease is needed, but it increases cost of production. Moreover, it is not safe to the producers, consumers and environment. Thus, the use of resistant varieties to powdery mildew is more efficient, economical and ecologically sound for controlling disease (Keller *et al.*, 2000). There is a report that the resistance to the powdery mildew disease in pea is controlled by recessive gene *er-1* and (or) *er-2*. (Tiwari *et al.*, 1998). In addition, the resistant *er-3* gene has been found in *P. fulvum* which originated in eastern Mediterranean and Near East areas. The presence of resistant gene showed the dominant specific character and also depended on the climate (Fondevilla *et al.*, 2007). Marker-assisted selection (MAS) may be used to shorten time needed to fix resistance to powdery mildew in segregating populations. Markers tightly linked to gene of interest increase the efficiency and accuracy of selection (Sanchez *et al.*, 2000). DNA maker is a tool that has been widely used to identify and separate resistant pea cultivar from the susceptible cultivar by using polymorphic band of *er* gene linkage such as ScOPO18₁₂₀₀ and ScOPE16₁₆₀₀ in Canadian resistant cultivar (Tiwari *et al.*, 1998), OPU17₁₀₀₀ and ScOPD10₆₅₀ in Indian resistant cultivar whereas OPO02₁₄₀₀ in Indian susceptible cultivars (Janila and Sharma, 2004). Moreover, the presence of DNA marker, *er* gene, has specific linkage to landrace cultivar. The objective of this study was to identify the *er* gene-linked molecular marker in snow peas which were developed in Thailand by using three primers, OPU17, OPO02 and ScOPD10 which were reported by Janila and Sharma (2004).

2. Materials and Methods

2.1 Phenotypic Evaluation of Powdery Mildew Resistance

This research was conducted in the winter season from November 2006 to March 2007 at Pang Da Royal Agricultural Station, Samoeng District, Chiang Mai. Seven pea lines/cultivars, P117, P185, P309, No.3, No.4, No.5 and Fang No.7, were used. Randomized Complete Block Design with 6 replications was used. Fourteen-day prior to transplanting, Fang No.7, the susceptible cultivar, was planted as source of *Oidium* sp. inoculum. Evaluation was done according to the presence of disease on the 4th, 7th, 10th nodes and a whole plant at 45, 55, 65 and 75 days, respectively. Disease severity scoring was based on the amount of disease on each leaf and calculated for the percentage of infected area ranging from 0 to 100% (Ondrej *et al.*, 2005; Young *et al.*, 1993) and the scores were analyzed by Statistix version 8.0.

2.2 DNA Marker Linked to Powdery Mildew Resistance in Snow Pea

Genomic DNA of 7 pea lines/cultivars was extracted from young leaf using Plant DNAzol Reagent (Invitrogen Co., Ltd.). The 20 µl reaction mixture was composed of 15 ng DNA template, 1 x PCR buffer (10mM Tris-HCl pH 8.3, 50 mM KCl, 0.2 mM MgCl₂, Enhancer solution), 0.5 mM dNTPs, 80 ng primer, 1 unit i-Taq[™] DNA polymerase (iNtRON biotechnology, Inc.) and distilled water. Three primers, one SCAR primer, ScOPD10 and two RAPD primers, OPU17 and OPO02, obtained from Operon Technologies Inc., Alameda, California, USA), were used for PCR reaction. The amplification was done following the method of Janila and Shama (2004), 1 cycle of 92°C for 120 sec. then 92°C for 30 sec, 42°C 30 sec, and 72°C 60 sec. for 44 cycles and 72°C 300 sec. for 1 cycle. PCR product was kept overnight at 4°C before checking the quality and quantity by 1.7% agarose gel electrophoresis. The amplified DNA bands were stained in 0.1 µl/ml ethidium bromide and photographed under UV light using BIODOC-It[™] M-20 System (Gibthai Co., Ltd.).

2.3 DNA Sequence Analysis

The 850-bp PCR products derived from two plants each of three resistant lines, P117, P185 and P309, were purified and sequenced at Pacific Science Co., Ltd.

3. Results

3.1 Phenotypic Evaluation of Powdery Mildew Resistance

The percentage of powdery mildew infection on the leaf surface areas of 7 pea lines/cultivars was measured. The results showed that in all stages of growth of cultivar Fang No.7, the highest severity on infection (100%) was observed on the leaves. Based on the disease severity ratings of the study, the tested materials were classified into 3 groups as follows; highly resistant (R), lines P117, P185 and P309; moderately resistant (M), lines No.3, No.4 and No.5 and susceptible (S), cultivar Fang No.7. At 45 days after transplanting, evaluation was carried out on the 4th node stage of growth, and it was found that the resistant group showed only 0.3-1.9% of leaf area infection, whereas the moderately resistant and susceptible groups showed 62.1-85.1 and 100% of leaf infections, respectively (Figure 1). At 55 days after transplanting, evaluation was done again on the 7th node, and it was noted that there was no infection on resistant groups, whereas the moderately resistant and susceptible groups showed 18.9-47.7 and 73.9% of infections, respectively. Then at 11th node or at 65 days after transplanting, disease rating was recorded and it was observed that only light infection of 1.6-2.3% was seen of leaves of the resistant lines. Meanwhile the moderately resistant and susceptible groups showed 37.8-60.4 and 90.0% of infections, respectively. Finally, powdery mildew disease scoring of the whole plant was carried out at 75 days after transplanting, and it was found that the resistant group showed only 4.5-10.9% disease infections. The moderately resistant and susceptible groups showed 32.4-56.6 and 96.0% disease infections, respectively.

3.2 DNA Marker Linked to Powdery Mildew Resistance in Snow Pea

Three primers, ScOPD10, OPU17 and OPO02 were used to amplify DNA in PCR reaction. It was found that all 3 primers generated the multiple sizes of DNA bands of each sample. However, primer SCOPD10 produced a total of 13 DNA bands and this primer could yield polymorphic DNA band at 850 bp. only in the resistant lines, P117 P185 and P309 (Figure 2A), while the powdery mildew susceptible lines/cultivars, lines No.3, No.4, No.5 and cultivar Fang No.7, showed no band of this size. The primer OPU17 and OPO02 produced a total of 6 and 8 DNA bands, respectively (Figure 2B-C). These two primers could not produce polymorphic band in either resistant or susceptible cultivars as reported in Indian cultivars according to Janila and Sharma (2004).

3.3 DNA Sequence Analysis

ScOPD10 primer was used to detect resistant DNA marker in 3 resistant pea lines, P117, P185 and P309. The 850-bp PCR products of 3 resistant lines were sequenced by Pacific Science CO., Ltd. The result showed that the DNA sequences for each line were 618, 621 and 624, respectively, and they were compared with DNA sequence databases at National Center for Biotechnology Information (NCBI) GenBank. After BLAST searches of sequence, DNA sequence showed 91, 90 and 91% homology to the DNA sequence from clone JICPSV-598E15, complete sequence of *P. sativum*. (Table 1, Figure 3-5).

4. Discussions

4.1 Phenotypic Evaluation of Powdery Mildew Resistance

Based on the result of this study, 7 lines/cultivars of snow pea were identified for the resistant levels to powdery mildew disease in the field conditions. The disease evaluation showed that it started on all tested materials at flowering stage. The greater amount of spore and mycelium fully covered the leaves of three lines, No.3, No.4, No.5 and cultivar Fang No.7. However, it was found that at every growth stages of cultivar Fang No.7, the commercial cultivar which is widely used in Thailand, was infected by the disease at the highest percentage level. The differential resistant level in each cultivar was influenced by many factors such as pathogen races, environmental conditions for pathogen and plant (Vaid and Tyagi, 1997). According to the statistical analysis of the observed disease percentages tested, snow pea lines/cultivar in the study could be divided into 3 groups as follows; highly resistant cultivar group (R) i.e. lines P117, P185 and P309, moderately cultivar group (M) i.e. lines No.3, No.4 and No.5 and susceptible group (S) i.e. cultivar Fang No.7. Based on to the result of phenotypic assessment, it could be stated that only three lines of P117, P185 and P309 were resistant to powdery mildew, due to the lowest percentages of disease infection on the leaves and the whole plants. Since the 7 snow pea lines/cultivars in the experiment were tested under the same conditions, the occurrence of the resistance must have been the result of the resistant gene. Tiwari *et al.* (1998) stated that the resistance to the powdery mildew disease in pea (*P. sativum*) is controlled by recessive gene *er-1* and (or) *er-2* and the resistance gene *er-1* may be

present in many resistant lines from around the world. However, the powdery mildew resistance which was found in this study might not have been the same genes as previously reported.

4.2 DNA Marker Linked to Powdery Mildew Resistance in Snow Pea

In this study, the resistant DNA marker in 7 lines/cultivars of snow pea was identified using three primers, ScOPD10, OPU17 and OPO02. None of them could yield band that had the same size as reported by Janila and Sharma (2004). That might be due to the difference of the origin of germplasm. Although, the SCAR marker, ScOPD10, was developed to be used in identifying powdery mildew disease resistance in Canadian germplasm by Timmerman *et al.* (1994) and Tiwari *et al.* (1998) whereas this germplasm has been less employed in Indian pea breeding programme. Only 80% of the result was reliable when this primer was used with Indian cultivars. Meanwhile, one of the two markers developed by Tiwari *et al.* (1998) could not differentiate resistant and susceptible lines of the Indian origin (Janila and Sharma, 2004). Since the origin of snow pea lines/cultivar used in this study is not known and they might have contained different genetic origin from those of Canadian and Indian origins. Thus, when primer, ScOPD10, was used, it could not yield band that has the same size of 650 bp as reported in Canadian cultivars (Timmerman *et al.*, 1994) and Indian cultivars (Janila and Sharma, 2004). However, band at 850 bp was found in all the resistant lines, lines P117, P185 and P309. The difference in size may occur according to the difference of pea genetic background (Fondevilla *et al.*, 2008). On the other hand, other two primers, OPU17 and OPO02, failed to detect any marker. That might occur due to the markers of OPU17 and OPO02, were RAPD primers, which were not specific and there were many limitations of this method, especially, high sensitivity to reaction conditions that reduces reproducibility of the results obtained in different laboratories (Ek *et al.*, 2005). However, the specific DNA band at 850 bp. could be used to confirm the powdery mildew resistance in snow pea cultivar in Thailand.

4.3 DNA Sequence Analysis

The 850-bp PCR products of 3 resistant lines, P117, P185 and P309, were sequenced and the result showed 618-624 of the base number. After comparing with DNA sequence databases at National Center for Biotechnology Information (NCBI) GenBank. DNA sequence showed 90-91% homology to the DNA sequence from clone JICPSV-598E15, complete sequence of *P. sativum*. In addition, this DNA sequence was also similar to DNA sequence of BARE-1 gene in barley and RIRE-1 gene in rice (Smykal, 2009). BARE-1 gene in barley was resistant gene to powdery mildew caused by *E. graminis* f. sp. *Hordei* (Wei, 1999). Thus, it might be stated that resistant pea lines in this study contained the same resistant gene as other powdery mildew resistant pea lines.

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Table 1. Description of three specific bands comparing with NCBI database

Lines	Bases	Accession	Description	Query Coverage
P117	618	CU655881.10	<i>P. sativum</i> DNA sequence from clone JICPSV-598E15, complete sequence	91%
P185	621	CU655881.10	<i>P. sativum</i> DNA sequence from clone JICPSV-598E15, complete sequence	90%
P309	624	CU655881.10	<i>P. sativum</i> DNA sequence from clone JICPSV-598E15, complete sequence	91%

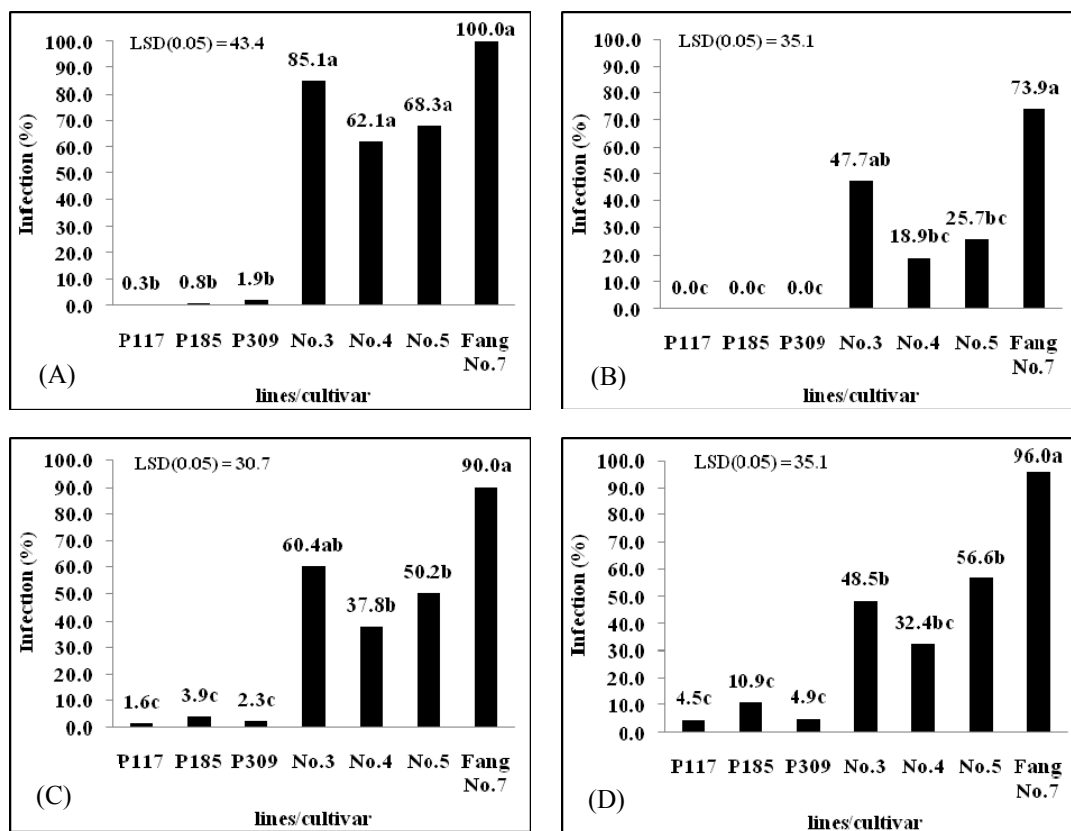


Figure 1. Percentage of powdery mildew infection on leaf surface area at the 4th node (A), 7th node (B), 11th node (C) and whole plant (D) in 7 pea lines/cultivars

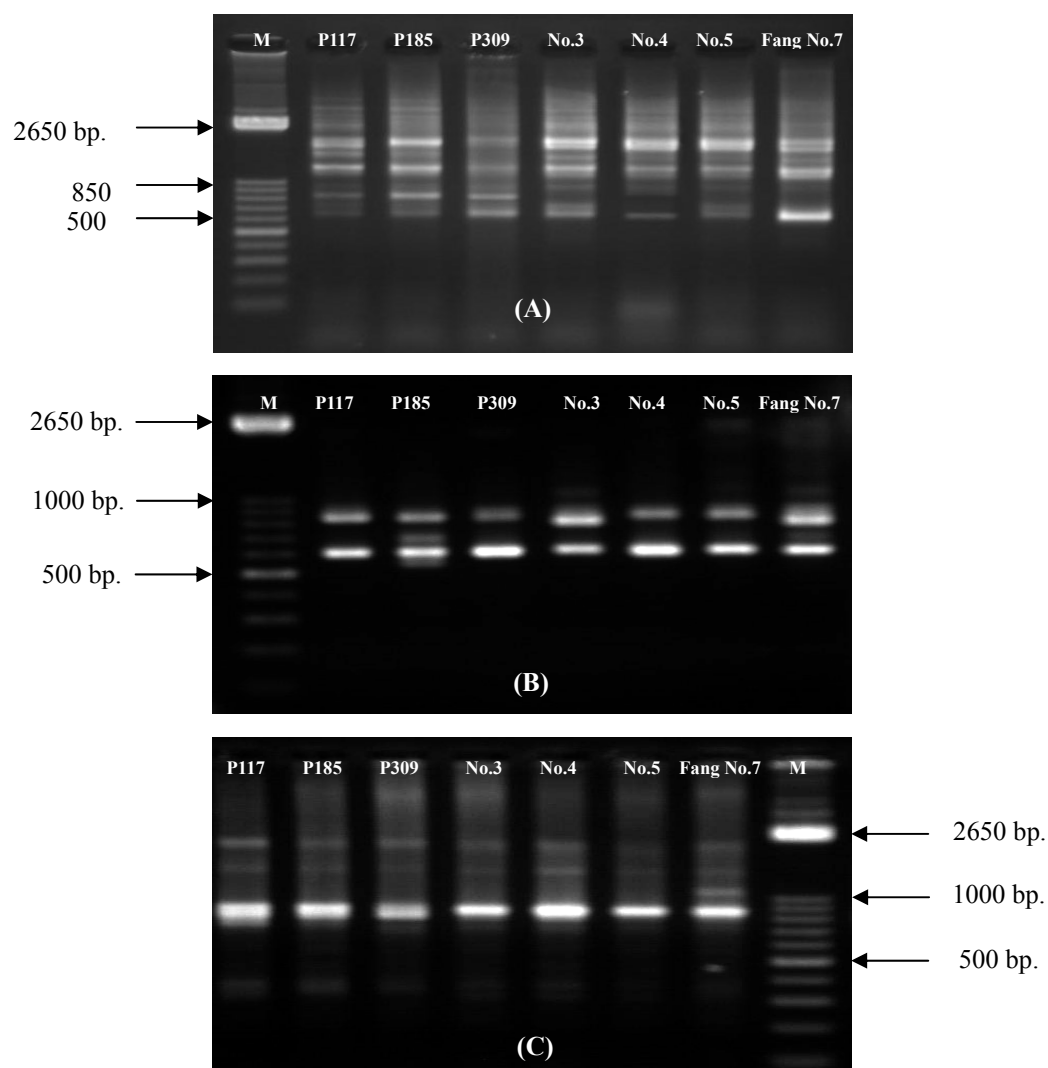


Figure 2. DNA fingerprinting generated by primer ScOPD10 (A), primer OPU17 (B) and primer OPO02 (C) of 7 pea lines/cultivars which were divided into 3 groups: 1) resistance (R), P117, P185 and P309; 2) moderate resistance (MR), No.3, No.4 and No.5 and 3) susceptible (S), Fang No.7. The 850-bp DNA marker could be yielded only in R group

TAAAAATTGAAATACTCAACAGCTCAATATGCATGAATGATGAATTAAAGAACTTCCCTTGATTGT
 ATTTGAGCAAAGGTTGAGGTTTCTTCATGAGCAAGGCACAATTGATACATAGATGAATTAGGGTTT
 CCTTGGGAAACAAGCCTCAAACCCTTTGGTTTGGTTTGATCAAAACGATGAATTGAGATACTTGGG
 AGGCATATTTGATGGATTAGAGATTTGGGAACCATTGCCATGCTTCTTTTCATCTTCTCTTGGCTAT
 ATCAATGCACATAGGATCTCCTAGAAGCTTTGGACCTTATGACTGTTCAAGCTACAAACAAAAGAT
 GTTAGTGACATATTTTTATGCTTTTGGTTAGTGAATAAAAAATGAGAAAAGAAATAATATACAATT
 TAAGCATGCTTTGTGATCTCAAACCAACTCTCAAAGATCCCACCCTAGGGTAAGAGCCAAGATGC
 TTATGATCCTTAAGGCAAGATGCAAATGCAATGTTATGATGCCATGAGGGATCTTAGGGTCAAATA
 GGGGTCTTACAGTATGTTTCGAGCATGCGATATGTTTCGGGTATATTTTCGCTAGCTTCACGGACATAG
 TACGGACACTGTTTAGGTGTAGACCATAT

Figure 3. DNA sequence of the specific band of line P117

CTTACAATTATTCGAATCGAGAGCTCCAACCTTCTGCAATTCTAGGCTCTGGGTTTGGGTTGGGGGTG
 CTCCTGTCGTGCAACGGATATACATGAGCAAGGCACAATTGATACATAGATGAATTAAGGTTTCCC
 TGGGAAACAAGCCTCAAACCCTTTGGTTTGGTTTGATCAAAACGATGAATTGAGATACTTGGGAGG
 CATATTTGATGGATTAGAGATTTGGGAACCATTGCCATGCTTCTTTTCATCTTCTCTTGGCTATATCA
 ATGCACATAGGATCTCCTAGAAGCTTTGGACCTTATGACTGTTCAAGCTACAAACAAAAGATGTTA
 GTGACATATTTTTATGCTTTTGGTTAGTGAATAAAAAATGAGAAAAGAAATAATATACAATTTAAG
 CATGCTTTGTGATCTCAAACCAACTCTCAAAGATCCCACCCTAGGGTAAGAGCCAAGATGCTTAT
 GATCCTTAAGGCAAGATGCAAATGCAATGTTATGATGCCATGAGGGATCTTAGGGTCAAATAGGG
 GTCTTACAGTATGTTTCGAGCATGCGATATGTTTCGGGTATATTTTCGCTAGCTTCACGGACATAGTAC
 GGACACTGTTTAGGTGTAGACCA

Figure 4. DNA sequence of the specific band of line P185

CGTAAAACACGAGTCTCAACAGTTCAATATGCATGAAATGATGAATTAAAGAACTTCCCTTGATTG
 TATTTGAGCAAAGGTTGAGGTTTCTTCATGAGCAAGGCACAATTGATACATAGATGAATTAGGGTT
 TCCTTGGGAAACAAGCCTCAAACCCTTTGGTTTGGTTTGATCAAAACGATGAATTGAGATACTTGG
 GAGGCATATTTGATGGATTAGAGATTTGGGAACCATTGCCATGCTTCTTTTCATCTTCTCTTGGCTA
 TATCAATGCACATAGGATCTCCTAGAAGCTTTGGACCTTATGACTGTTCAAGCTACAAACAAAAGA
 TGTTAGTGACATATTTTTATGCTTTTGGTTAGTGAATAAAAAATGAGAAAAGAAATAATATACAAT
 TTAAGCATGCTTTGTGATCTCAAACCAACTCTCAAAGATCCCACCCTAGGGTAAGAGCCAAGATG
 CTTATGATCCTTAAGGCAAGATGCAAATGCAATGTTATGATGCCATGAGGGATCTTAGGGTCAAAT
 AGGGGTCTTACAGTATGTTTCGAGCATGCGATATGTTTCGGGTATATTTTCGCTAGCTTCACGGACATA
 GTACGGACACTGTTTAGGTGTAGACCA

Figure 5. DNA sequence of the specific band of line P309