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Detection and quantification of *Ralstonia solanacearum* through conventional and molecular techniques and spoilage in stored potato at different temperatures during storage

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Abstract
Bacterial wilt (*Ralstonia solanacearum*) infected potato samples collected from Odisha (cv. Kufri Pukhraj) and Uttarakhand (cv. Kufri Jalandhar), to study decay of potato tubers, other pathogens associated with potato tubers and survivability of *R. solanacearum* during storage at different temperatures (5, 15, 25 and 35 ºC) up to 60 days. Minimum decay of stored potato of Kufri Jalandhar (5.82%) and Kufri Pukhraj (7.53%) was found at 5ºC followed by 15ºC after 60 days of storage. *R. solanacearum, Erwinia* spp. and *Bacillus* spp. were found to be common in rotted potato of both the potato cultivars Kufri Pukhraj and Kufri Jalandhar. In case of fungi, *Fusarium oxysporum*, *Mucor genevensis* and *Penicillium* sp. were common in both potato cultivars while *Macrophomina phaseolina* was recorded only on Kufri Pukhraj cultivar. Study on survivability of *R. solanacearum* in stored potato tubers cvs. Kufri Pukhraj and Kufri Jalandhar at 5, 15, 25 and 35ºC temperatures up to 60 days by dilution plate technique, conventional-PCR and RT- PCR methods. The population of *R. solanacearum* was found maximum at 5 ºC (6.27 cfu log/g tissue and 6.20 cfu log/g tissue) followed by 15 ºC and minimum at 25 and 35 ºC stored potato samples in both the potato cultivars Kufri Pukhraj and Kufri Jalandhar by serial dilution plating techniques respectively. Conventional PCR results also confirmed the detection of *R. solanacearum* at 5 and 15ºC stored potato which was gradually increased up to 60 days of storage in both the cultivars, whereas the samples stored at 25 and 35ºC the detection of *R. solanacearum* was gradually declined after 20 days of storage. The detection and quantification *R. solanacearum* population by RT –PCR, maximum population of *R. solanacearum* was recorded in 5ºC stored potato followed by 15 ºC. The C_T value of *R. solanacearum* was 26.90 at 5ºC and 34.67 at 35ºC in Kufri Pukhraj whereas 25.70 at 5 ºC and 31.70 at 35ºC in Kufri Jalandhar after 60 days of storage.

Keywords: Potato, *Ralstonia solanacearum*, PCR, RT-PCR, Detection

Introduction
The bacterial wilt caused by *Ralstonia solanacearum* damage the potato in field as well as under storage. *R. solanacearum* is capable of survive as latent infection in the tubers produced from wilt affected plants. During post harvest period these infected tubers is likely to rot in stores. Brown rot of potato has been estimated to affect 3.75 million acres in approximately 80 countries with global damage estimates exceeding $950 million per year (Floyd, 2007) [4]. In India, *R. solanacearum* causes 50% crop loss in potato in a regular manner (Mukherjee and Dasgupta, 1989) [11] and up to 75% losses as reported in some areas of Karnataka (Gadewar *et al.*, 1991) [5]. In India, bacterial wilt has become a limiting factor in potato cultivation, and cause losses in yield to the tune of 30 to 70% (Somani *et al.*, 2010) [17].

The ability of *R. solanacearum*, the causal agent of bacterial wilt of many important crops (Kelman, 1953) [9] to survive long-term in soil under natural conditions remains poorly understood. *R. solanacearum* also survive in the infected tubers as a latent infection and cause wilting of the plant in the field and spoilage of tubers in storage. This is largely because of a lack of sensitive detection protocols for studying low residual pathogen populations amongst high numbers of saprophytic bacteria in the soil environment.

Seal & Elphinstone, (1994) [15] have reviewed the advances in identification and detection of *R. solanacearum*. Detection of *R. solanacearum* has previously relied on the use of selective media and indicator plants. Several semi selective media were developed, principally for the detection of *R. solanacearum* in soil (Okabe, 1971; Karganilla & Buddenhagen, 1972; Graham & Lloyd, 1979; Granada & Sequeira, 1983) [12, 8, 6, 7]. None of these has gained wide acceptance, although detection of as few as 10^2 - 10^4 CFU per g dry soil was often possible.

The major disadvantage was that pathogen growth was often inhibited by overgrowth or competition in the presence of high populations of antagonistic saprophytic bacteria, resulting in false negative diagnoses.

In recent years, studies on improved identification and detection of plant pathogens have mostly concentrated on molecular approaches because of their potential advantages of increased specificity and sensitivity. With the development of *R. solanacearum* specific PCR methods, Seal *et al*. (1993) [16] were able to detect 16S rDNA sequences from a single cell grown in culture.

The most commonly used method worldwide for detection and identification of *R. solanacearum* has been isolation on TTC medium (Kelman, 1954) [10] because of the relatively low cost, simplicity of use and consistency of results between different laboratories. Increasingly, other methods of detection involving immunofluorescence (IF), SMSA medium and PCR, and identification using fatty acid profiling and rep-PCR are being used in commercial laboratories, where speed and accuracy of diagnoses are often more important than cost and ease of use.

Therefore, the aim of present study was to investigate the ideal temperature for survival of *R. solanacearum* in stored potato tubers, compare the specificity and sensitivity of techniques for routine detection of *R. solanacearum* thereby increase the shelf life of potato in storage and maintaining the quality of potato tubers during storage.

Materials and Method

Survivability of *R. solanacearum,* and quality of stored potato: To study the survivability of *R. solanacearum* in stored potato, the samples were collected from the infested field by *R. solanacearum* causing bacterial wilt/brown rot disease of potato from two locations Odisha (village - Bermuda, district – Bhubneshwar) (cv. Kufri Pukhraj) and Uttarakhand (Village – Ghingrani, district – Nainital) (cv. Kufri Jalandhar). Potato tubers were packed in cloth bag and stored at 4 different temperatures *i.e.* 5, 15, 25 & 35ºC in the incubators with 3 replications. The tubers were stored up to 60 days. Survivability of *R. solanacearum* in stored potato, decay, association of fungi and bacteria and quality of stored potato were studied at 0, 20, 40 and 60 days.

Decay of potato tubers during storage at different temperatures: Decay percentage of stored potato tubers were recorded at 20, 40 and 60 days of storage, samples stored at different temperature collected from Odisha and Uttarakhand.

Occurrence of bacteria and fungi on potato tubers during storage: The potato samples cv. Kufri Pukhraj and Kufri Jalandhar, rotted during storage were taken to observe the microbes associated with rotted potato. 1.0g rotted potato tissue from both the cultivars was taken in 10 ml of sterilized distilled water kept for 10 minutes. 100 µl of microbial suspension from 10^{-3} and 10^{-7} serial dilution were poured on to nutrient agar, potato dextrose agar and TTC medium and spread by sterile glass spreader. Then the petriplates were incubated at 28±1° to grow the microbes in the medium. The microbes grown on medium were identified based on morphological characters of colony.

Detection and quantification of *R. solanacearum* from stored potato through dilution plate techniques: Potato tubers cvs. Kufri Pukhraj and Kufri Jalandhar stored at 5, 15, 25 and 35 ºC were taken randomly at 0, 20, 40 and 60 days of storage from each storage temperature with 3 replications. From each tuber 1.0g core tissue from vascular region at stolen end of potato was taken in 10 ml of sterilized distilled water. The 100 µl of suspension was taken from 10^{-1} to 10^{-7} serial dilution and poured onto TTC medium and spread by sterile L-shaped glass spreader. The Petriplates were incubated at 28±1°C for 72 h to grow the *R. solanacearum* on the medium. The colonies were counted and converted into log value for statistical analysis and further interpretation of data.

Detection of *R. solanacearum* from potato tubers through Conventional – PCR: The same bacterial suspensions were used as template for PCR. The detection of *R. solanacearum* through conventional PCR was done by using *R. solanacearum* specific primer OLI 1 and Y2 (Seal *et al*. 1993) [16]. PCR amplification was performed in a total volume of 25 µl. 5.0 µl of 5X Taq buffer, 1.5 µl of 25 mM $MgCl_2$, 0.4 µl of 10 mM dNTPs,0.5 µl each 10 pmoles of the primers OLI 1 and Y2, 1 unit Taq polymerase (Promega) and 5 µl of bacterial ooze suspension was used as templates. Amplification was performed in a BIO-RAD C1000 thermo cycler, with an initial denaturation step at 95 °C for 2 min; followed by 35 cycles of denaturation at 94 °C for 20 s, annealing at 68 °C for 20 s, and extension at 72 °C for 30 s; and a final extension step at 72 °C for 10 min. The PCR products were resolved using a 1.2% agarose gels with ethidium bromide at 0.5 µg/ml and photographed under UV light.

Detection and quantification of *R. solanacearum* from potato tubers through RT-PCR: Further, the same bacterial suspensions were used as template for RT-PCR, as the samples were prepared earlier. for detection and quantitative analysis by Real–Time PCR assay, the reaction mixture prepared in 20 µl volume. 10.0 µl of 2X Syber green master mix, 1.0 µl each 10 pmoles of the primers OLI 1 and Y2 and 2 µl of bacterial ooze suspension was used as templates. RT-PCR cycling parameters consisted of an initial denaturation at 95 ºC for 3 min followed by 40 cycles of 95 ºC for 10 sec, 68 ºC for 10 sec and 55ºC for 20 sec. Reactions were run on an IQ5 Real-Time PCR System (BIO-RAD). The PCR cycle at which fluorescence exceeded the threshold was defined as the cycle threshold (Ct). The, data from molecular analyses were recorded as Ct value. PCR inhibition was determined by comparing Ct values of negative controls without template with Ct values of potato samples stored at different temperatures. All tests were run in duplicate and each run contained a negative (water) and positive (DNA of *R. solanacearum*) with known DNA concentration as control.

Statistical analysis: All the analyses were performed in triplicate. The data obtained with respect to different parameters under different treatments during storage at different temperature were analysed by factorial analysis at 5% level of significance ($p< 0.05$).

Results

Decay of potato tubers during storage

The results of potato samples collected from Odisha and Uttarakhand revealed that decay of stored potato was found minimum7.53 and 5.82% in Kufri Pukhraj and Kufri Jalandhar at 5 ºC after 60 days of storage respectively. Maximum decay of potato tuber was 95.09 and 87.37% in Kufri Pukhraj and Kufri Jalandhar was recorded at 35ºC

followed by 25 ºC of storage after 60 days of storage respectively (Table 1).

Occurrence of bacteria and fungi on potato tubers during storage:

The results presented in the Table 2 revealed that different type of fungi and bacteria were recorded during storage of potato. Three genera of bacteria *i.e. R. solanacearum, Erwinia* spp. and *Bacillus* spp. and four genera of fungi such as *Fusarium oxysporum*, *Mucor genevensis*, *Macrophomina phaseolina* and *Penicillium* spp; were recorded on potato cv. Kufri Pukhraj collected from Odisha and three genera of each fungi (*F. oxysporum*, *Mucor genevensis* and *Penicillium* spp.) and bacteria (*R. solanacearum, Erwinia* spp. and *Bacillus* spp.) were recorded on potato cv. Kufri Jalandhar during storage. Occurrence of bacterial genera in both the cultivars was common. In case of occurrence of fungi *i.e. F. oxysporum*, *M. genevensis* were common, while *M. phaseolina* occurred on Kufri Pukhraj cultivar but it did not occur on Kufri Jalandhar during storage.

Detection and quantification of *R. solanacearum* from stored potato tubers:

To study the survivability of *R. solanacearum* in stored potato, collected from Odisha cv. Kufri Pukhraj and from Uttarakhand cv. Kufri Jalandhar, were stored at 5, 15, 25 and 35ºC temperatures. The population of *R. solanacearum* was recorded at initial and at 20 days intervals up to 60 days by using dilution plate technique, conventional-PCR and RT-PCR.

Dilution plate technique: The observation in case of serial dilution plating technique, the population of *R. solanacearum* was found 6.27 cfu log/g tissue and 6.20 cfu log/g tissue in stored potato at 5 ºC Kufri Pukhraj and Kufri Jalandhar collected from Odisha and Uttarakhand during storage after 60 days of storage (Table 3).

The population of *R. solanacearum* in stored potato tuber of both the cultivars was gradually decreased during storage at 25 and 35ºC. However, the population of *R. solanacearum* in potato cv. Kufri Pukhraj collected from Odisha was not found at 25 and 35ºC temperature after 60 days of storage, whereas Kufri Jalandhar collected from Uttarakhand, *R. solanacearum* was detected and population was 5.38 and 4.65 cfu log / g of tissue at 25 and 35ºC after 60 days of storage respectively. Detection level of *R. solanacearum* in potato tuber was decreased by increasing the storage temperature in both the cultivars. In Kufri Pukhraj, *R. solanacearum* was detected from 100% to 50% at temperature from 5^0C to 35^0C. In case of Kufri Jalandhar, *R. solanacearum* was detected 66.75% at 5^0C to 50% at 35^0C temperature.

Storage period of potato was also affected the survivability of *R. solanacearum*. The population of *R. solanacearum* increased up to 40 days 5.13 cfu log value at initial level to 5.29 cfu log value at 40 days and after that it was declined. 3.00 cfu log value/ g of tissue in cultivars Kufri Pukhraj, whereas in Kufri Jalandhar initial population of *R. solanacearum* low (4.96 cfu log value/g of tissue) and after that it was increased 5.23 cfu log value/g of tissue, at the 20 days to 5.46 cfu log value/g of tissue) after 60 days of storage. Whereas, the population of *R. solanacearum* in cv. Kufri Pukhraj and Kufri Jalandhar potato stored at 5 and 15 ºC was increased by increasing the storage period to 60 days, whereas the potato samples stored at 25 and 35ºC, population of the bacteria was decreased after 20 days storage. However, the survivability of *R. solanacearum* at 35ºC decreased more as compare to 25 ºC (Table 3&4).

Conventional-PCR. The advanced detection technique such as conventional-PCR and RT-PCR was used to detect *R. solanacearum* in stored potato at different temperature. *R. solanacearum* was detected by conventional PCR using the same bacterial sap from stored potato tuber. The results of the conventional PCR revealed that the *R. solanacearum* was detected at 5 ºC and 15 ºC is more as compare to 25 ºC and 35 ºC (Table 4). This also confirms the survivability of *R. solanacearum* at 5 and 15 ºC was increased it was decreased at 25 ºC and 35 ºC. *R. solanacearum* was not detected in cv. Kufri Pukhraj at 35 ºC stored potato tubers after 60 days of storage (Table 4 and Fig. 1). In case of Kufri Jalandhar *R. solanacearum* was detected up to 60 days of storage at all the temperatures (Table 4 and Fig. 2).

RT-PCR: The same tuber extracts were also used for detection and quantification of *R. solanacearum* by using RT –PCR. The result showed that population of *R. solanacearum* in both the cultivars of potato tubers stored at 5 and 15ºC was increased gradually up to after 60 days of storage (Table 5). Initially C_T value of *R. solanacearum* population in both the cultivars *i.e.* Kufri Pukhraj (Odisha) and Kufri Jalandhar (Uttarakhand) varied 26.40 to 30.50 and 26.30 to 34.70 C_T value respectively. It showed that variation in population of *R. solanacearum* was more in individual tubers. Maximum population was recorded at 5ºC in both the cultivars Kufri Pukhraj (26.90 C_T value) and Kufri Jalandhar (25.70 C_T value), which was significantly higher than the other storage temperature. The *R. solanacearum* was detected 100 percent at 5 ^{0}C. Minimum population of *R. solanacearum* (34.67 C_T value) was found in cv. Kufri Pukhraj collected from Odisha at 35 ºC after 60 days of storage followed by at 25 ºC temperature in same cultivar (32.70 C_T value) and detection level was also lower at 35 ºC of storage. By increasing the storage temperature, the population of *R. solanacearum* was decreased significantly and detection level was also decreased in due course of storage time (Table 5).

For detection of *R. solanacearum*, three technique *i.e.* dilution plate technique, conventional PCR and RT – PCR methods were used. In comparative study, detection test of *R. solanacearum* was highest in RT-PCR. 83.33% as compared to conventional PCR (50%) at 35^0C temperature. After 60 days of storage, detection level of *R. Solanacearum* was also found in RT-PCR. 75% in both the cultivars as compared to conventional PCR 58% and dilution plate technique 50% in Kufri Pukhraj and 58% in Kufri Jalandhar.

Discussion

In the present investigation, we have attempted to study the decay of potato at different storage temperatures, pathogen associated with rotted potato, ideal temperature for survival of *R. solanacearum* and detection and quantification of *R. solanacearum* in two stored potato cultivars at different temperatures. Further, compare the specificity and sensitivity of techniques for detection of *R. solanacearum*.

In the study of the decay of two potatoes cultivars *i.e.* Kufri Pukhraj and Kufri Jalandhar collected from the infested field by *R. solanacearum* causing brown rot disease from Odisha and Uttarakhand respectively and stored the mat 5, 15, 25 and 35 ºC for 60 days. The potato stored at 5 and 15 ºC recorded minimum rotting where as the potato stored at 25 and 35 ºC showed maximum rotting. The almost all the stored potato of

both the cultivars were rotted up to 60 days of storage. However, potato cv. Kufri Pukhraj causes more rotting than Kufri Jalandhar at all the storage temperature during storage. The rotting was higher, when the potato was stored at 25 and 35ºC, it may be due to increase in respiration, favourable environment for bacterial multiplication to increase population. A significant variation in decay in both the cultivars was found and Kufri Pukhraj showed higher decay than Kufri Jalandhar during storage at all the storage temperatures. The decay was increased in both the cultivars at all the temperature level by increasing the storage period significantly.

Cheftel and Cheftel, (1992) [2] reported that the temperature above 21ºC increases respiration rate and prevent the accumulation of reducing sugars but cause spoilage. This confirms the finding that potato stored at 25 and 35 ºC causes more spoilage as compare to potato stored at 5 and 15 ºC.

The associated micro-organism with rotting of potato during storage was isolated and identified. Bacterial genera, *R. solanacearum, Erwinia* spp. and *Bacillus* spp. were found to be common Kufri Pukhraj and Kufri Jalandhar. In case of fungi, *Fusarium oxysporum*, *Mucor genevensis* and *Penicillium* sp. were common in both potato cultivars while *Macrophomina phaseolina* was recorded on Kufri Pukhraj cultivar.

In relation to this study, Abiodun and Olumide, (2007) [1] isolated five pathogenic fungi *i.e. Botryodiplodia theobromae*, *Fusarium redolens*, *F. oxysporum*, *Penicillium* sp. and *Rhizopus oryzae* from rotted potato tubers during post harvest storage. Clark and Moyer, (1988) [3] also reported pathogenic fungi associated with post-harvest Irish potato tuber rot are *Alternaria solani* (early blight). *Rhizogospora subtranea* (Powdery Scab), *Fusarium roseum* and *Fusarium solani* (fusarium rot disease), *Helminthosporium solani* (skin blemishes disease), *Colletotrichum atramentarium* (black rot disease), *Aspergillus niger*, *Pythium ultimum* (Pythium tuber rot disease), *Phytophthora erythroseptica* (Pink rot disease), and *Phytophthora infestans* (late blight disease). In this study, *Fusarium oxysporum* had the highest frequency and it occurred on all potatoes types. It has been observed in this study again that pathogenic organisms establish their contacts with their hosts by utilising enzymatic substances. Also discovered in this study is the fact that infection occurs as a result of wound created on the tuber, this is because wounded tuber rotted easily more than the unwounded tuber (Rabinorich *et al*., 2002) [13]. Thus, tubers should be handled carefully during harvest to prevent wounding. However, these fungi were not recorded under this study.

Potato tuber is a main source of primary inoculums of the bacterial wilt disease pathogen and also transmitted the pathogen to distant places. For survivability study of *R. solanacearum*, infected potato were stored at 5, 15, 25 and 35ºC temperatures up to 60 days of storage. The detection and quantification of *R. solanacearum* population; three techniques such as dilution plate technique, conventional-PCR and RT-PCR were used.

In dilution plate technique the population of *R. solanacearum* was found maximum at 5 ºC (6.27 cfu log/g tissue and 6.20 cfu log/g tissue) followed by 15ºC and minimum at 25 and 35 ºC stored potato samples in both the potato cultivars Kufri Pukhraj and Kufri Jalandhar respectively. The population of *R. solanacearum* was gradually increased up to 60 days of storage at 5 and 15ºC, but it was gradually decreased after 20 days of storage at 25 and 35ºC in both the cultivars. The significant interaction was found between storage period and storage temperature and cultivars and storage temperature. However no significant variation was found between storage period and cultivars. No significant interaction was recorded among storage period, storage temperature and cultivars.

Conventional-PCR results also confirms the detection level of *R. solanacearum* at 5 and 15ºC stored potato which was gradually increased up to 60 days of storage in both the cultivars. Whereas the samples stored at 25 and 35ºC detection level of *R. solanacearum* was gradually decreased after 20 days of storage in both the cultivars. The detection results of *R. solanacearum* further confirmed that population of *R. solanacearum* was more in potato stored at 5 and 15ºC as compare to potato stored at 25 and 35ºC in both the cultivars.

The detection and quantification *R. solanacearum* population was further confirmed by RT –PCR. The result of RT-PCR clearly showed that the C_T value gradually decreased, that means population of *R. solanacearum* gradually increased in potato samples stored at 5 and 15ºC up to 60 days of storage. The C_T value of potato samples stored at 25 and 35ºCwas gradually increased after 20 days of storage, which indicates the population of *R. solanacearum* gradually was decreased up to 60 days of storage. No significant variation in population of *R. solanacearum* was found, after 60 days of storage. However, the maximum population of *R. solanacearum* was recorded in 5ºC stored potato followed by 15ºC and minimum population of *R. solanacearum* recorded at 35ºC stored potato samples in both the cultivars Kufri Pukhraj and Kufri Jalandhar followed by 25ºC after 60 days of storage. The result obtained from these three methods the survivability of *R. solanacearum* was affected by temperature and similar result was reported earlier by Scherf *et al*., (2010) [14] that survival of R3bv2 longer at 4^0C in potato tuber. Later Tomlinson *et al.*, (2011) [18] reported *R. solanacearum* was longer at 15 ^{0}C as compare to 28 and 35 ^{0}C.

Scherf *et al*., (2010) [14] studied the effect of temperature on *R. solanacearum* and reported that in water at 4 ºC R3bv2 does not survive as long as native U.S. strains, R3bv2 remain viable longer than U.S. strains in potato tuber at 4 ºC.

However, no significant interaction on survivability of *R. solanacearum* was found between cultivars, location and duration of storage, cultivars and storage temperature, duration and storage temperature and cultivars duration and temperature. But survivability of *R. solanacearum* was significantly affected by storage temperature and by increasing the temperature up to 35 ºC, population of *R. solanacearum* was decreased.

Table 1: Decay of potato tubers during storage at different temperatures collected from Odisha and Uttarakhand.

Storage Temperature (ºC)	Decay (%)					
	Kufri Pukhraj (Odisha)			Kufri Jalandhar (Uttarakhand)		
	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS
5	2.44^c	5.47^c	7.53^b	2.00^c	4.71^c	5.82^b
15	2.78^c	6.95^c	9.62^b	2.22^c	5.96^c	7.92^b
25	17.22^b	47.22^b	84.19^a	10.44^b	32.93^b	78.54^a
35	24.89^a	67.00^a	95.09^a	13.67^a	44.292^a	87.37^a

DAS: Days after storage.

Means followed by the same letter within a column are not significantly different as determined by LSD test ($\alpha = 0.05$), data present means of the experiment within 3 replication each.

Table 2: Occurrence of bacteria and fungi on potato tubers collected from brown rot prone areas of Odisha and Uttarakhand states during storage.

Location	Cultivar	Microbes associated with rotted potato	
		Bacteria	Fungi
Odisha	Kufri Pukhraj	*Ralstonia solanacearum*, *Erwinia* spp. *Bacillus* spp.	*Fusarium oxysporum* (I.D. No. 9441.14)* *Mucor genevensis* (I.D. No. 9442.14)* *Macrophomina phaseolina* (I.D. No. 9443.14)* *Penicillim* spp.
Uttarakhand	Kufri Jalandhar	*Ralstonia solanacearum*, *Erwinia* spp. *Bacillus* spp.	*Fusarium oxysporum* (I.D. No. 9441.14)* *Mucor genevensis* (I.D. No. 9442.14)* *Penicillim* spp.

(*) Identified by Indian Type Culture Collection, Indian Agricultural Research Institute, New Delhi.

Table 3: Detection and quantification of *R. solanacearum* from stored potato tubers collected from bacterial wilt infested field of Odisha and Uttarakhand during storage at different temperatures through dilution plate techniques.

Storage Temperature (ºC)	Population of *R. solanacearum* (cfu log value /g of tuber tissue)**									
	Kufri Pukhraj (Odisha)					Kufri Jalandhar (Uttarakhand)				
	Initial	20 DAS	40 DAS	60 DAS	Mean	Initial	20 DAS	40 DAS	60 DAS	Mean
5	5.66 (100.00)*	5.27 (100.00)	5.73 (100.00)	6.27 (100.00)	5.73 (100.00)	5.90 (33.33)	5.86 (66.66)	6.12 (66.66)	6.20 (100.00)	6.02 (66.75)
15	5.06 (33.33)	4.74 (66.66)	5.65 (66.66)	5.72 (100.00)	5.29 (66.75)	4.04 (33.33)	4.73 (66.66)	5.25 (66.66)	5.61 (66.66)	4.90 (58.33)
25	4.19 (66.66)	5.14 (66.66)	4.27 (66.66)	0.0 (0.0)	3.40 (50.00)	4.25 (33.33)	5.52 (100.00)	5.48 (66.66)	5.38 (33.33)	5.16 (58.25)
35	5.61 (100.00)	5.55 (66.66)	5.49 (33.33)	0.0 (0.0)	4.16 (50.00)	5.66 (66.66)	4.81 (66.66)	4.70 (33.33)	4.65 (33.333)	4.96 (50.00)
Mean	5.13 (75.00)	5.18 (75.00)	5.29 (66.75)	3.00 (50.00)		4.96 (41.67)	5.23 (75.00)	5.39 (58.25)	5.46 (58.25)	

Statistical analysis		
Factor	CD(Value)	SE (d)
A(Period)	NS	0.76
B (Cultivars)	NS	0.54
C (Temp)	1.52	0.76
AXB	NS	1.08
AXC	1.52	1.52
BXC	1.08	1.08
AXBXC	NS	2.16

******(Average of 3 samples); DAS (Days after storage).
Value in parenthesis is showing detection (percentage) of *R. solanacearum*.

Table 4: Detection of *R. solanacearum* from stored potato tubers collected from bacterial wilt infested field of Odisha and Uttarakhand at different temperature through conventional - PCR.

Storage Temperature (ºC)	Detection of *R. solanacearum* (%)*									
	Kufri Pukhraj (Odisha)					Kufri Jalandhar (Uttarakhand)				
	Initial	20 DAS	40 DAS	60 DAS	Mean	Initial	20 DAS	40 DAS	60 DAS	Mean
5	100.00	100.00	100.00	100.00	100.00	33.33	66.66	66.66	100.00	66.50
15	33.33	66.66	66.66	100.00	66.5	33.33	66.66	66.66	66.66	58.25
25	66.66	66.66	66.66	33.33	58.33	33.33	100.00	66.66	33.33	58.25
35	100.00	66.66	33.33	0.0	50.00	66.66	66.66	33.33	33.33	50.00
Mean	75.00	75.00	66.5	58.25		41.67	75.00	58.25	58.25	

Statistical analysis								
Factor	CD(value)	SE (d)	CD(value)	SE (d)	CD(value)	SE (d)	CD(value)	SE (d)
A (Location)	NS	20.41	NS	20.41	NS	22.04	NS	16.66
B (Period)	NS	28.86	NS	28.68	NS	31.18	50.39	23.57
Tempratre (AXB)	NS	40.82	NS	40.82	NS	44.09	NS	33.33

*****(Average of 3 samples); DAS (Days after storage).

Table 5: Detection and quantification of *R. solanacearum* from stored potato tubers collected from bacterial wilt infested plot of Odisha and Uttarakhand at different temperature during storage through RT-PCR.

Storage Temperature (ºC)	C_T value of *R. solanacearum* in stored potato**									
	Kufri Pukhraj (Odisha)					Kufri Jalandhar (Uttarakhand)				
	Initial	20 DAS	40 DAS	60 DAS	Mean	Initial	20 DAS	40 DAS	60 DAS	Mean
5	26.40 (100.00)*	31.80 (100.00)	27.60 (100.00)	26.90 (100.00)	28.18 (100.00)	30.30 (66.66)	29.50 (100.00)	26.98 (100.00)	25.70 (100.00)	28.12 (91.66)
15	30.50 (100.00)	32.90 (100.00)	30.00 (100.00)	29.90 (100.00)	30.82 (100.00)	34.70 (66.66)	31.80 (66.66)	30.75 (66.66)	28.10 (66.66)	31.33 (66.66)

25	28.60 (100.00)	34.10 (100.00)	31.10 (100.00)	32.70 (66.66)	31.62 (91.66)	33.30 (100.00)	26.00 (100.00)	29.56 (100.00)	30.15 (66.66)	29.75 (91.66)
35	29.50 (100.00)	29.30 (100.00)	31.90 (100.00)	34.67 (33.33)	31.34 (83.33)	26.30 (100.00)	30.60 (100.00)	31.50 (66.66)	31.70 (66.66)	30.02 (83.33)
Mean	28.75 (100.00)	32.02 (100.00)	30.15 (100.00)	31.04 (75.00)		31.15 (83.33)	29.47 (91.66)	29.69 (83.33)	28.91 (75.00)	

Statistical analysis		
Factor	**CD(Value)**	**SE (d)**
A (Period)	NS	1.05
B (Location)	NS	0.74
C (Temp)	2.09	1.05
AXB	NS	1.48
AXC	NS	2.10
BXC	NS	1.48
AXBXC	NS	2.97

******(Average of 3 samples); DAS (Days after storage).

*Value in parenthesis is sowing detection of *R. solanacearum* in percentage.

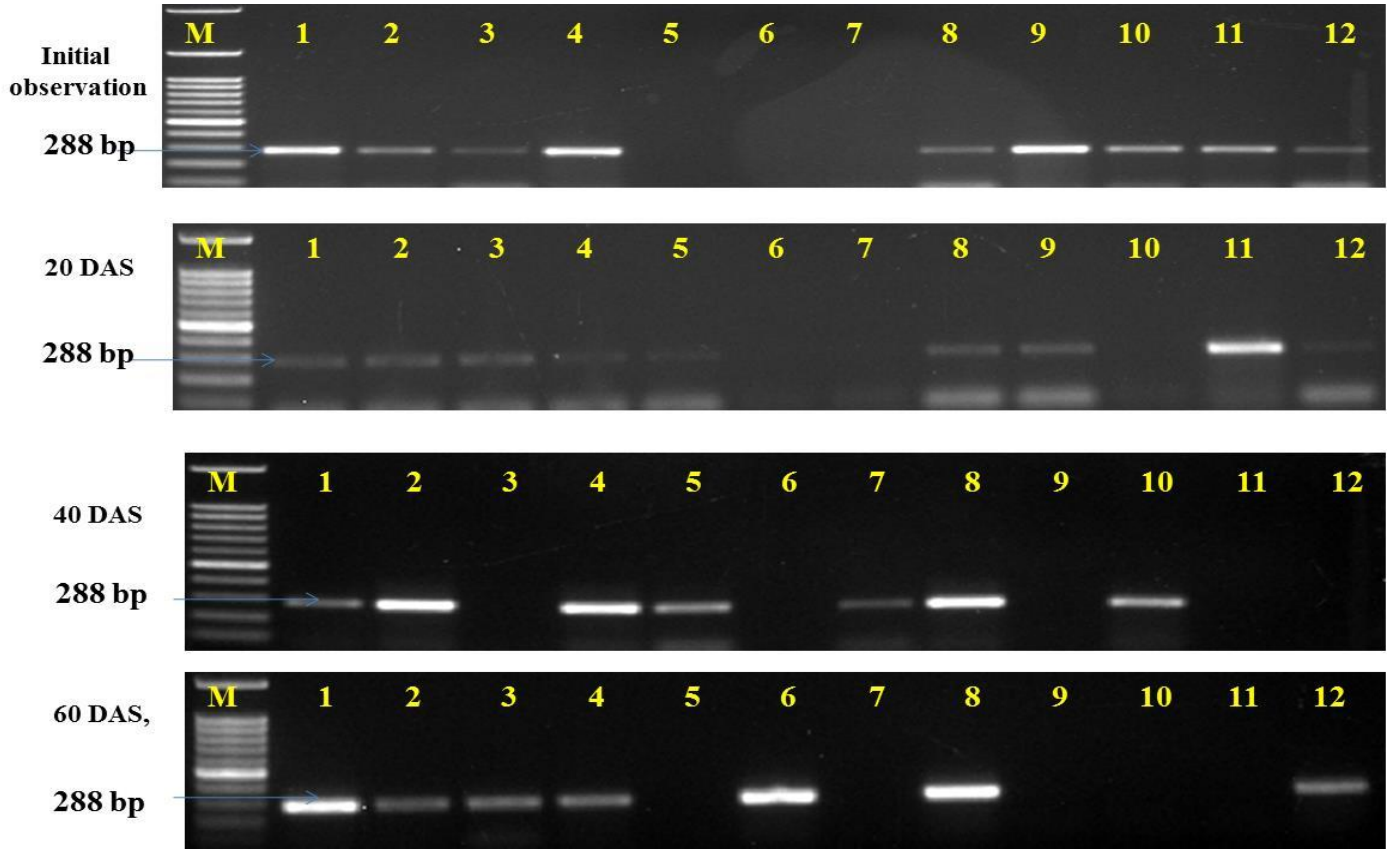
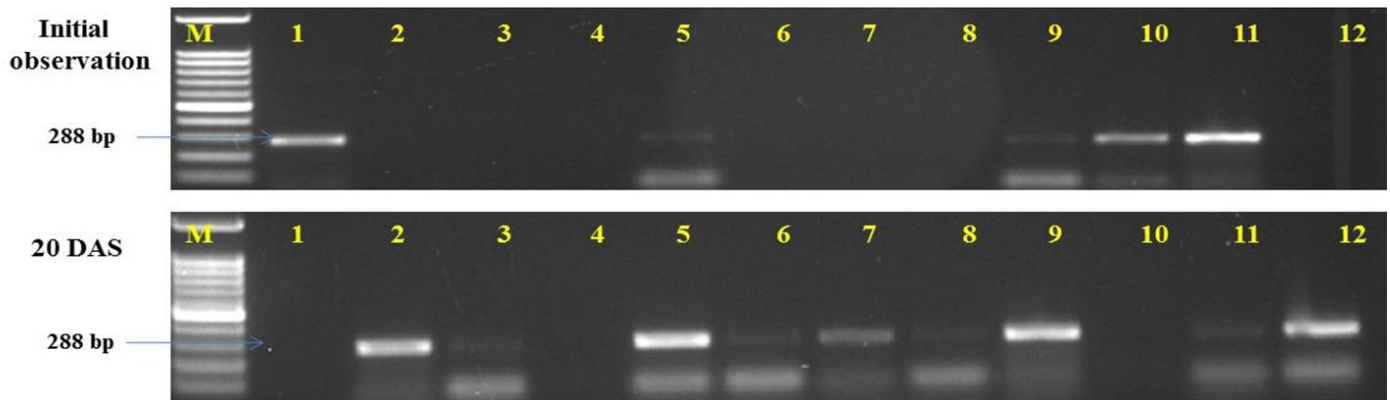


Fig 1: Detection of *R. solanacearum* through conventional – PCR from stored potato tubers collected from Odisha (Kufri Pukhraj) Lane M: 100 bp DNA ladder lanes, 1-3: samples at 5 ºC, 4- 6: samples at 15 ºC, 7-9 samples at 25 ºC, 10-12: samples at 35 ºC



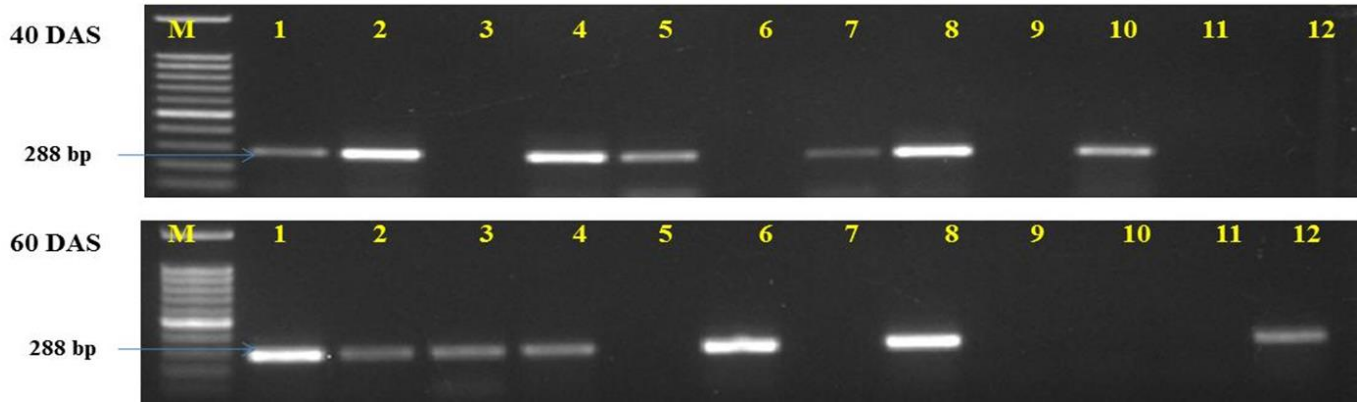


Fig 2: Detection of R. solanacearum through conventional – PCR from stored potato tubers collected from Uttarakhand (Kufri Jalandhar) Lane M: 100 bp DNA ladder lanes, 1-3: samples at 5 ^{0}C, 4- 6: samples at 15 ^{0}C, 7-9 samples at 25 ^{0}C, 10-12: samples at 35 ^{0}C.

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