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Occurrence of Bacterial Wilt in Potato in aeroponics

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Aeroponics technology was established at Central Potato Research Station, Shillong to produce disease free mini tubers for seed potato. The system was under standardization in the research station and performance of the system was satisfactory in summer as well as autumn planting in 2013 with the total production of 360 Kg (72,000 numbers) of mini tubers in six major notified varieties of the region. Likewise, in 2014 the production system was at par in the summer planting. However, unusual and unexpected disease symptoms appeared on each aeroponic panel on each of six potato varieties. Symptoms were sudden wilting, drooping of leaves and toppling of entire green plant on the aeroponic panel. Wilted plants were taken out and ooze test was done. There was clear oozing out from cut stems and mini tuber which made turbid water. The bacterium was also isolated and purified with irregular shape colonies with pinkish centre. The bacterium was identified as Ralstonia solanacearum. Presuming, natural rain fall water may be the probable source of bacterial inoculum. Further, entire aeroponic system was cleaned and used water from different source. Water was taken from Municipality Water Supply Corporation; Shillong through water tanker.Then bacterial wilt infection was not detected in any potato cultivars in the operation of aeroponics during 2015 and 2016.

Key words: Aeroponics, Bacterial wilt, *Ralstonia solanacearum*, seed potato, disease free mini tubers

INTRODUCTION

Supplying of good quality potato seed tubers to the farmers at right time is a real challenge in the national level. Biotechnological interventions along with tissue culture and suitably engineered growing structure called aeroponics can provide a future solution to the rapid production of truthful quality as well as breeder seed of potato. With this aim, CPRI has developed and standardized aeroponics system in India.

MATERIALS AND METHODS

Our team in CRPI Regional station, Shillong, Meghalaya has initiated a prototype of this aeroponic structure suitable for the NEH region. Aeroponics technology for mini tuber production was established at CPRS Shillong (25°54' N latitude and 91°84' E longitude and an altitude of 1742 meters above mean sea level), Meghalaya in the summer 2013 to enhance the production of truthful quality seed potato in the North eastern Himalayan region of India (Bag et al. 2015).

RESULTS AND DISCUSSION

The system was under standardization in the research station and performance of the system was satisfactory in summer as well as autumn planting in 2013 with the total production of 360 Kg (ca. H" 3.0-5.0g each, 72,000 numbers) of mini tubers in 6 major notified varieties (Kufri Giridhari, Kufri Giriraj, Kufri Himalini, Kufri Himsona, Kufri Jyoti, Kufri Kanchan and Kufri Megha) of the region. In 2014 the production system was at par in the summer planting. However, unusual and unexpected disease symptoms appeared on each aeroponic panel on each of six potato varieties (Fig. 1a, b, c, d, e and f) in autumn planting in 2014.

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Table 1: Autumn planting of potato micro plant in aeroponics and their harvest in different picking

Varieties	Date of Planting	Picking (harvesting)				Total harvest	
		1st picking		2nd picking		Numbers of mini tubers	Weight (kg)
		Numbers of mini tubers	Weight (kg)	Numbers of mini tubers	Weight (kg)		
K.Megha	05.09.14	1533	0.778	1829	2.084	3362	2.862
K.Himalini	05.09.14	1237	0.526	826	0.954	2063	1.480
K.Megha	06.09.14	580	1.148	*	*	580	1.148
K.Kanchan	06.09.14	345	0.944	*	*	345	0.944
K.Giriraj	06.09.14	298	0.562	*	*	298	0.562
K.Himalini	06.09.14	144	0.358	*	*	144	0.358
K.Himsona	06.09.14	44	0.076	*	*	44	0.076
K.Girdhari	11.09.14	410	1.375	*	*	410	1.375
K.Megha	17.09.14	53	0.076	*	*	53	0.076
		7299	8.881				

*no harvest was possible and entire crop was abundant on 30.10.2014 due to heavy infection of *Ralstonia solanacearum*

Symptoms were sudden wilting, drooping of leaves and toppling of entire green plant on the aeroponic panel. Aerial parts of the plants were normal having no visible symptom of inflicting injuries except wilting. Root systems were checked carefully in all the aeroponic panels and apparently there were no symptoms on the roots. Roots were completely white and healthy. Wilted plants were splited longitudinally and vascular discolouration was distinctly visible near color region and main roots. Wilted plants were taken out, roots were trimmed and cut stems were put under ooze testing with distilled water (Fig. 2 a & b). There was clear oozing out from cut stems which made distilled water turbid indicating the presence of the bacteria.

After the appearance of suspected bacterial wilt disease in potato plants on aeroponic panels, crop history was judged carefully and it was found that a total of 2800 virus free tissue culturally raised micro plants were planted in aeroponics system during autumn season in the month of September on 5th September, 6th September, 11th September and 17th September 2014 (Table 1). Crop stand inside aeroponics was very good up to 30 days with no signs of disease or infection. After 30-35 days, symptoms of drooping of plants started appearing in the cv. K. Girdhari; subsequently, similar symptoms were also observed in other potato varieties. Initially one or two infected plants were picked up with entire roots and discarded. On subsequent days, number of wilted plants increased progressively. Percent wilting was varies from 4-5% in each potato varieties. As more number of plants was found to be infected, we felt very serious and at 45 days of planting, plants were tested for bacterial wilt symptoms. Plants were tested positive for the bacterial wilt disease. All plants from the aeroponics were abundant and removed on 30 October, 2014. Only 2 picking each for K. Megha and K. Himalini were possible. Only one picking was done for the remaining varieties (Table 1).The picked up tubers were kept under drying and greening process inside laboratory.

Fig. 1 : Wilting of different cultivars in aeroponic panels a. Kufri Girdhari,b. Kufri Himsona, c.Kufri Giriraj, d. Kufri Kanchan, e. Kufri Himalini, f.Kufri Megha

Based on the confirmation of presence of the bacterium in infected plants, early harvested mini tubers which were kept in diffused light for drying and greening process in the laboratory were randomly selected and cut in the middle with sharp

scalpel with the assumption that bacterial inoculum may be present in the early harvested minitubers. Surprisingly, on an average 4-5 tubers (out of 50 randomly selected minitubers) were found infected which were similar to brown rot symptom in the vascular region of the mini tubers (Fig.2d). Finally, entire quantity (ca. H" 8.89Kg, 7300 number minitubers) of early one/two harvests of autumn crop in 2014 was discarded and burnt in pit.

An attempt was made to isolate the pathogen on artificial media from infected potato plant and mini

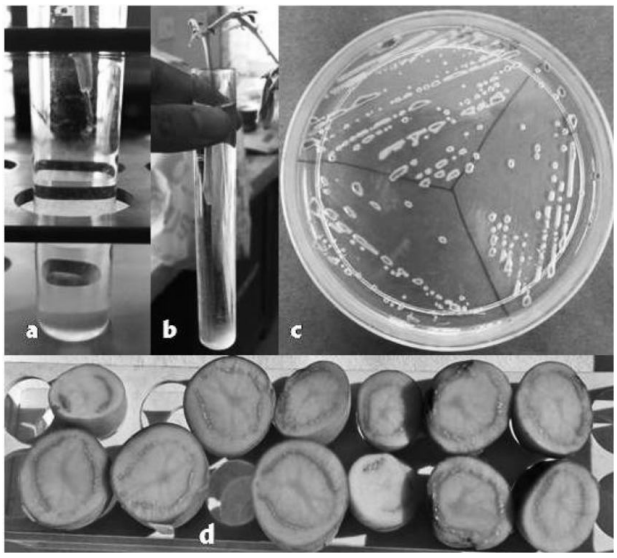

Fig. 2 : Owze test of bacteria from infected potato (a.slimy mass of bacteria coming out from cut stem b. Turbidity of water after nozing) c. Ralstomia setanacearum on artificial medium showing irregular colonies with pinkish centre d. Sign of presence of ralstom in solanacearum on vascular bundles of cut mini tubers harvested from aeroponics

tubers collected from aeroponics. The infected samples (stem pieces and mini tubers) were surface-disinfected with 70% ethanol, macerated in sterile distilled water. Macerates were streaked on Kelman's Triphenyl Tetrazolium Chloride (TZC) agar medium and plates were incubated at 28 ± 2^{O}C for 48-72 h. Typical pink coloured colony with watery white margin (Fig.2c) appeared on the medium after 48 hours of incubation. Bacterial colonies developing the typical irregular mucoid colonies were again transferred to fresh TZC medium for further purification. One full loop of bacterial culture transferred in 1 ml of double distilled sterile water and stored at 20±2^{O}C in BOD (incubator). Pathogenicity was tested on potted plants of potato, by stem stab inoculation method. The inoculated plants were incubated in poly house conditions and were observed for wilt appearance till 21 days. The bacterium was identified as Ralstonia solanacearum which is known for causing bacterial wilt in Solanaceous crops including potato. Ralstonia solanacearum was reported to cause bacterial wilt of potato/ brown rot of potato in the north eastern Himalayan region of India (Chakraborty, 2011) including mid hills of Meghalaya (Gurjar *et al*. 2013). Bacterial wilt is a major constraint for potato cultivation in this region. The annual rainfall is very high (ca. 2850 mm) in the state of Meghalaya and potatoes were being cultivated as rainfed crop in the state. Runoff water collected and used as source of water for running aeroponics may be the probable source of bacterial inoculum which aggravated in aeroponics system.

Basically aeroponic system was conceptualized and established in the NEH Region of India (Shillong, Meghalaya) to produce disease free basic seed of potato tubers. Facing with the new challenge/problem, our entire team became very much concerned with the successful operation of the system in the future and started to analyze the primary source of the inoculum. Virus free tissue cultured plantlets were taken from ICAR-Central Potato Research Institute, Shimla (Himachal Pradesh) which never showed any disease symptom in culture tubes. Millipore filtered water was used in the tissue culture media to raise micro plants in our station at Shillong and we were confirmed that this water was not definitely a source of bacterial inoculum.

Further we concentrated our concern on second source of water used in preparation of nutritional solution in the aeroponics. Large amount of water is required for preparation and injection of nutrition in the form of mist in the root zone. Entire quantity of water used in the aeroponics has been pumped from the natural fountain which is renewed by frequent rainfall in the hill. Our observation in the station was that usually frequency of wilt of potato in the field crop was higher in the autumn crop. This is because, after severe winter in the region, bacterium get first host in the summer potato crop where bacterium multiply and get washed away with runoff water of rain. This runoff water flowed down and accumulated in lower valley where artificial water reservoir was created at the end of our farm. Recently, phylotype IV of Ralstonia solanacearum causing bacterial wilt of potato was also reported from Meghalaya (Gurjar *et al*., 2015).

In this concern of bacterial wilt, first we targeted to make our aeroponics system complete free from bacterial inoculum which was presumed to come from runoff water. Infected growth panels where entire growth chambers were aggravated with bacterial wilt pathogen in 2014 were treated with bleaching powder solution (ca. H" 250g/1000L water tank) operating the system as normal for 1 hr every day for one week without planting any micro plants to eradicate the bacteria survived (if any) on the wall of growth chambers and solution tanks. The growth panels were then sun dried completely and entire left over bleached solution was pumped out from solution tanks before operation in the coming season in 2015. Presuming that natural fountain water may be the probable source of bacterial inoculum, we planned to use water entirely from different source. We purchased water from Municipality Water Supply Corporation, Shillong through tanker. That water was regularly stored in black plastic tanks (Syntax) with treatment with chlorine tablet (10 tables/1000L water) for 10 days. This treated water was strictly used for preparation of nutritional solution in the subsequent operation of aeroponics in 2015 and 2016 both in summer and autumn planting. We did not get any bacterial wilt infection on any cultivars in the operation of aeroponics during 2015 and 2016. Therefore, use of highly purified water (if possible either RO water or sterilized distilled water) can successfully keep the aeroponic system free from bacterial pathogen. Earlier it has been reported that *R. solanacearum* can survive many years in moist soils or water (Alvarez *et al*. 2008).

In India, bacterial wilt /brown rot caused by Ralstonia solanacearum was reported to be endemic on potato crop in Andaman and Nicobar Islands, Chhota Nagpur plateau, Karnataka, Kerala, Madhya Pradesh, Nilgiris, Annamalai and Palani hills of Tamil Nadu, North-Western Kumaon hills, Orissa, West Bengal, Western Maharashtra and North Eastern states of India (Chakrabarti, 2011). In Meghalaya, the disease is known to appear in potato crop in both the seasons in summer as well as in autumn crop (Gurjar *et al*., 2013). In the present communication, we report the occurrence of bacterial wilt for the first time on potatoes grown in aeroponics which is considered as an innovative protected cultivation system. We confirmed the disease based on the symptomatology, ooze test, sign of the pathogen on infected mini tubers and typical colony characteristics on artificial medium which are most widely used laboratory detection techniques worldwide. Here, we also report the probable source of bacterial inoculum and successful management of bacterial wilt in aeroponics in the successive years.

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