Prevalence and spread of potato cyst nematodes, *Globodera* spp. in northern hilly areas of India

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Potato cyst nematodes (PCNs), *Globodera rostochiensis* (Woll.) and *G. pallida* (Stone), are globally important pests of potato prevalent in the Nilgiri area of South India. PCNs were first intercepted from North India at Shimla, Himachal Pradesh (HP) in 2010. Subsequent surveys in the hilly areas of North India have revealed the occurrence of PCNs in several districts of HP, Uttarakhand and Jammu & Kashmir, despite domestic quarantine against this nematode. High populations of PCN were mainly encountered in the Potato Seed Multiplication Farms that supply seed potato to farmers. Here, a hypothesis on the dissemination of PCNs within India is proposed.

Keywords: Domestic quarantine, hilly areas, potato cyst nematodes, prevalence and spread.

POTATO cyst nematodes (PCNs), *Globodera rostochiensis* and *G. pallida* are globally considered as important pests of potato. These are known to be prevalent in several countries across continents and are subjected to stringent quarantine and/or regulatory procedures, wherever they occur. PCNs present a severe threat to domestic and international commerce in potato. It is believed that PCN had their origin in the Andes Mountains of South America, which is the original home for potato[1]. During the 1850s, PCNs were introduced into Europe along with the breeding materials brought for late blight resistance, from where they spread throughout the world through the introduction of improved varieties developed in Europe[2]. PCNs probably spread from Europe to other countries with exported seed tubers or breeding materials[2]. John Sullivan, the founder of present-day Udhagamandalam (the Nilgiris, Tamil Nadu (TN), India), initially introduced potatoes to TN in 1822 (ref. 3), where PCNs were first reported[4].

Subsequent surveys conducted in the Nilgiris revealed the widespread prevalence of PCNs in potato fields around Udhagamandalam[5]. In 1971, the Government of Madras, TN, enforced domestic quarantine in India to check further spread of PCNs within the country. Later, their occurrence was also reported from Kodaikanal hills (TN)[6], the adjoining hills of Karnataka[7], and Pazhathotam, Idukki district, Kerala[8] bordering TN, indicating the possibility of spread by infested seed materials from the nearby Kodaikanal hills.

It is interesting to note the first presence of PCNs in hilly areas of Pakistan in 1980 (ref. 9), since the hilly States/Union Territories (UTs) of North India are adjacent to this geography. PCNs were intercepted in North India in the farm area of ICAR-Central Potato Research Institute (CPRI), Shimla, Himachal Pradesh (HP)[10]. Since CPRI Shimla is a nodal institution for the development of potato varieties/hybrids, lot of germplasm and seed material exchange takes place between the institution and other Central/State Government agencies mandated to work on potato in the country. The basic seed produced at the farms of CPRI is multiplied at different Potato Seed Multiplication Farms (PSMFs) of States/UTs, Departments of Agriculture (HP, Uttarakhand, Jammu & Kashmir (J&K)) for further distribution to farmers in these and other states of the country. So it is apprehended that PCNs might have got introduced into some new areas in North India that are conducive for their establishment and multiplication. Therefore, district-wise surveys were initiated under the aegis of ICAR-All India Coordinated Research Project on Nematodes in the three hilly States/UTs, namely, HP, J&K and Uttarakhand to earmark the areas infested with PCNs, with the ultimate aim of regulation and management of this nematode. The results of these surveys conducted during 2011–19 are presented in this article.

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Materials and methods

Survey

Survey for PCNs was started in HP during 2011, immediately after their interception at CPRI, Shimla. The PSMFs were specifically targeted, since these are mandated to provide seed potato to farmers in their areas of jurisdiction. In J&K and Uttarakhand, the surveys were initiated during 2015 and 2016 respectively.

Surveys were mostly conducted during the *kharif* season, with the exception of some localities during autumn and *rabi* season in HP. Soil and root samples were collected randomly from the rhizosphere of the plants when the crop was nearing maturity, and cysts were easily visible on potato roots. The samples (200 cm^3 soil each) were processed in the laboratory of the respective institutions. The soil samples were processed by sieving method and the residue collected over 60 mesh sieve was examined under a stereo zoom microscope. All the cysts from a sample were collected in a vial using a forceps or brush and their numbers counted.

Identification

Ten randomly picked cysts were processed for the preparation of vulval cones for preliminary identification of the species according to the characters included in the *EPPO Bulletin*[11].

The molecular identification of PCNs was performed on three populations from J&K, by mechanical crushing of 10 cysts from each population together and lysing them in 25 µl of molecular-grade water. Next, 25 µl of lysis buffer (0.2M NaCl + 0.2M Tris-HCl (pH 8.0) + 1% (v/v) β-mercaptoethanol + 800 µg/ml proteinase K) was added to the crushed cysts[12] and incubated in a thermocycler at 65°C for 3 h with intermittent vortexing, followed by inactivation of proteinase K by incubation at 100°C for 5 min. Then 2 µl of the lysate (diluted 1 : 10) was used as DNA template in the PCR reaction using PCN species-specific primers (*G. rostochiensis*: forward 5-GCAAGCCCAGCGTCAGCAAC-3, reverse 5-GAACATCAACCTCCTATCGG-3, and *G. pallida*: forward 5-TGTCCATTCCTCTCCACCAG-3, reverse 5-CCGCTTCCCCATTGCTTTCG-3)[13]. The *G. pallida*-specific primers amplified a 798 bp DNA fragment, whereas *G. rostochiensis* amplified a 315 bp product[13]. The amplified PCR products were resolved on a 1.2% agarose gel and visualized in a gel-documentation system.

Results

Himachal Pradesh

Among 11 PSMFs surveyed in seven districts of HP, 9 were positive for the presence of PCN (Table 1 and Figure 1). In five of these farms, i.e. Umladwar, Kharapather (Shimla district), Khunna (Kullu district), Rajgunda (Kangra district), and Kamraha (Mandi district), the frequency of occurrence of PCNs in all the samples collected from each farm was 100% with mean density ranging from 23 to 516.6 cysts per 200 cm^3 soil. In rest of the PSMFs, i.e. Khadrala (Shimla district), Phuladhar and Kheradhar (Mandi district), and Ahla (Chamba district), the frequency of occurrence ranged from 10% to 91.7% with the mean population ranging from 10.3 to 377.8 cysts per 200 cm^3 of soil. In two PSMFs, i.e. Shilaroo (Shimla district) and Darang (Lahaul and Spiti district) from where 40 and 15 samples respectively, were analysed, all the samples were found to be free from cysts of PCN.

From the farmers' fields, PCN cysts were found in seven locations, all in Shimla district. The frequency of occurrence ranged between 75% and 100% and mean density ranged from 16 to 277.4 cysts/200 cm^3 of soil. It was observed that PCNs were prevalent only in locations at elevations more than 1950 m amsl. PCNs were not recorded from autumn or spring season crops both in the soil and in the roots. Autumn and spring season potato crops are grown in low and mid-hills of Una (less than 500 m amsl) and Kangra (mostly less than 1000 m amsl); perhaps PCNs do not establish in these areas due to relatively higher temperature.

Jammu & Kashmir

In Jammu division, a large and old PSMF at Nathatop (Udhampur district) was heavily infested (Table 2 and

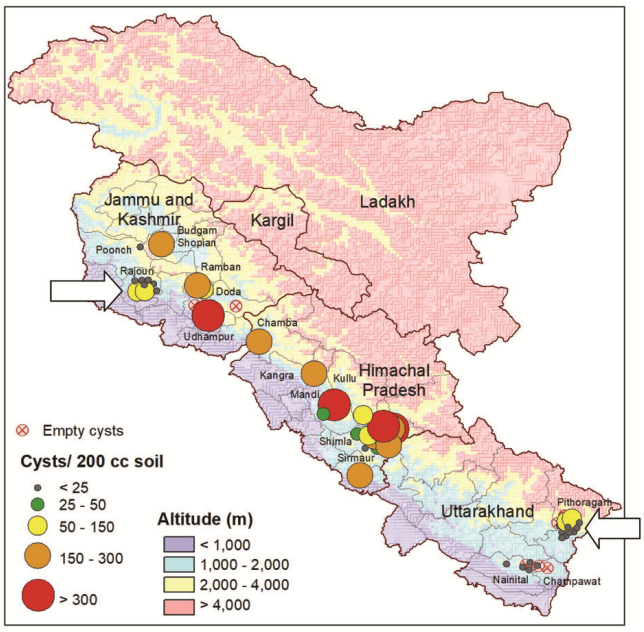

Figure 1. Spatial distribution of potato cyst nematodes in the northern hilly areas of India. Arrows indicate dissemination of nematode from Potato Seed Multiplication Farms (yellow colour) to the adjacent farmers' fields (grey colour).

Table 1. Survey results for the occurrence of *Globodera* spp. in potato in Himachal Pradesh, India

District	Locality	Elevation (m amsl)	Latitude	Longitude	Number of samples	Frequency (%)	No. of cysts/200 cm^3 soil Mean (range)
Shimla	Shilaroo*	2525	N31.12292	E77.25450	40		Not recorded
	Sarapani	2700	N31.18588	E77.41196	3	100.00	28.0 (22–34)
	Kharapani	2750	N31.18077	E77.40146	3	100.00	16.0 (10–22)
	Kheni	2080	N31.11666	E77.38139	3		Not recorded
	Khadrala	2687	N31.12194	E77.35255	8	75.00	26.8 (18–46)
	Khadrala*	2765	N31.16194	E77.39025	12	91.67	377.8 (8–1830)
	Tootupani	2881	N31.15672	E77.37042	5	80.00	277.4 (140–370)
	Umladwar*	2798	N31.13186	E77.34943	4	100.00	77.4 (52–96)
	Hanstari Teer	2772	N31.11544	E77.35431	3	100.00	260.0 (90–430)
	Ponidhar	2715	N31.11067	E77.35741	3	100.00	63.0 (256–270)
	Kharapathar*	2700	N31.06492	E77.37843	7	100.00	347.6 (150–776)
	Ghorna	2237	N31.07276	E77.15237	3		Not recorded
	Deorighat	2694	N31.10136	E77.36971	7	100.00	260.4 (20–824)
Sirmour	Kheradhar*	2032	N30.50035	E77.09235	30	10.00	166.0 (6–284)
Mandi	Phuladhar*	2425	N31.57747	E76.52543	15	80.00	32.0 (4–140)
	Kamraha*	1965	N31.49300	E77.55833	3	100.00	516.6 (410–610)
	Barot	1829	N32.49433	E77.27683	7		Not recorded
Chamba	Ahla*	2232	N31.57747	E76.00257	64	54.69	176.2 (2–560)
Kullu	Khunna*	3200	N31.43350	E77.41710	10	100.00	65.1 (2–166)
Una	Jhalera	389	N32.91933	E76.67433	4		Not recorded
	Oel	437	N31.72800	E76.59283	10		Not recorded
	Ram Nagar	380	N31.55750	E76.42316	5		Not recorded
	Luharli	397	N31.32946	E76.93240	2		Not recorded
	Panjawar	397	N31.32946	E76.93240	4		Not recorded
	Haroli	387	N31.29274	E76.10543	3		Not recorded
	Jhalera	363	N31.28804	E76.13258	3		Not recorded
	Rampur	382	N31.57850	E76.51583	3		Not recorded
	Santoshgarh	374	N31.21966	E76.19650	6		Not recorded
Kangra	Malan	971	N32.71370	E76.25305	25		Not recorded
	Gadiara	925	N32.73200	E76.24131	12		Not recorded
	Rajgunda*	2530	N32.50683	E77.55566	14	100.00	154.6 (130–190)
Lahaul & Spiti	Dalang*	3045	N32.5166	E77.0147	15		Not recorded
	Jispa	3200	N32.5086	E77.0063	3		Not recorded
	Trilokinath	2760	N32.6646	E76.7085	1		Not recorded
	Kishore	4650	N32.6647	E76.7086	5		Not recorded
	Udaipur	2742	N32.7236	E76.6644	5		Not recorded
	Shuiling	3894	N32.4750	E77.0801	4		Not recorded
	Gemur	3200	N32.6115	E77.1463	5		Not recorded
	Tinno	3200	N32.5805	E77.1305	3		Not recorded
	Cheling	4420	N32.5616	E77.0514	2		Not recorded
	Baring	1189	N32.5242	E77.0280	2		Not recorded
	Karpat	1189	N32.8028	E76.7726	10		Not recorded
	Darcha	3360	N32.6779	E77.1950	4		Not recorded
	Mooring	1189	N32.6512	E76.8171	2		Not recorded

*Potato Seed Multiplication Farms (PSMFs) of the Department of Agriculture, Himachal Pradesh.

Figure 1). Samples were collected from all the three blocks of this farm, and the cyst population ranged from 36 to 1565 (mean 411.2 cysts) per 200 cm^3 of soil. All the nine samples from this location were infested with PCNs. Another PSMF located at Kandi Buddhal in Rajouri district of Jammu division also revealed the prevalence of PCNs with 124 cysts per 200 cm^3 of soil. The Ishkunda and Narsingha farms located at Gool in Ramban district recorded a mean population of 60.4 and 232.5 *Globodera* cysts per 200 cm^3 of soil respectively. Several samples collected from farmers' fields in the Jammu division (<2000 m amsl) contained either only empty cysts or no cysts of PCN. In the Kashmir valley, only a limited survey could be conducted. Samples collected from PSMF at Sedew in Shopian district revealed 100% frequency of occurrence with cyst population ranging between 30 and 409 (mean 241 cysts) per 200 cm^3 of soil. The samples collected from PSMF and farmers' fields located in Baramula district from the valley were free from PCNs.

Uttarakhand

Pithoragarh district in Uttarakhand is located at a relatively higher altitude. Two old PSMFs at Balati and

Table 2. Survey results for the occurrence of *Globodera* spp. in potato in Jammu & Kashmir (J&K), India

District	Locality	Elevation (m amsl)	Latitude	Longitude	No. of samples	Frequency (%)	No. of cysts/ 200 cm^3 soil Mean (range)
Ramban	Gool, Ishkunda*	2023	N33.28193	E74.99077	5	100	60.4 (10–172)
	Gool, Narsingha*	1981	N33.28193	E74.98715	4	100	232.5 (70–420)
Ramban	Sanasar	2247	N33.12481	E75.24980	3		Not recorded
Udhampur	Nathatop*	2551	N33.09349	E75.26447	9	100	411.2 (36–1565)
	Marothi, Chenani	1786	N32.940105	E75.417191	1		8 empty
		1720	N32.939650	E75.416096	1		7 empty
	Bupp, Chenani	1760	N32.939260	E75.349963	1		9 empty
		1758	N32.953146	E75.350434	1		5 empty
		1820	N32.943242	E75.352550	1		4 empty
	Lower Madha, Chenani	1856	N33.007001	E75.264405	1		7 empty
		1900	N33.001306	E75.273719	1		6 empty
	Panchayat Meer, Panchari	1810	N33.036312	E75.123047	1		6 empty
		1650	N33.030393	E75.118265	1		4 empty
	Panchari	1728	N33.08304	E75.15304	3		Not recorded
Udhampur	Latti	1885	N32.91463	E75.43001	2		Not recorded
Udhampur	Latti Doona	1931	N32.91626	E75.43071	1		Not recorded
Udhampur	Charat Sewna/Samroli	1600	N32.969873	E75.219919	1		3
Udhampur	Dhar Gaddian Samroli	1000	N33.001109	E75.195797	1		Not recorded
Udhampur	Chatrari Majouri Ram Nagar	1780	N32.816272	E75.485968	1		Not recorded
Udhampur	Jakhed	1750	N32.89004	E75.48718	3		Not recorded
Doda	Vill. Bhalra, Teh, Bhaderwah	1650	N33.022420	E75.690996	1		6 empty
		1650	N33.022066	E75.690765	1		5 empty
Poonch	Makhowalia Loran, Panchayat Diana Kharpa	2320	N33°8447	E74°3563	2	100	8 (6–10)
Kishtwar	Yeerdu	3100	N33.686750	E75.667735	1		Not recorded
Rajouri	Kandi Budhal*	2020	N33.390463	E74.526684	1	100	124
	Rajnagar Buddhal	1970	N33.3720	E74.6382	1	100	10
	Ghondha Buddhal	1650	N33.3512	E74.6228	1	100	115
	Rajnagar Buddhal	1780	N33.3713	E74.6348	1	100	24
	Ghondha Buddhal	1650	N33.3521	E74.6281	1	100	6
	Lower Gulthi	1240	N33.3041	E74.1668	1	100	2
	Lower Gulthi	1500	N.33.2987	E74.1622	1	100	7
	Lower Rajdhani	1570	N33.547526	E74.343353	1	100	5
Shopian	Sedew*	2240	N33.665439	E74.798126	5	100	241 (30–409)
Ganderbal	Haripora	1800	N34.289792	E74.835906	2		Not recorded
Baramula	Tangmarg	2080	N34.068197	E74.436221	3		Not recorded
Baramula	Yari khah-Tangmarg road	2200	N33.575530	E74.939282	1		Not recorded
Baramula	Rafiabad*	1600	N34.253893	E74.390012	7		Not recorded

*PSMFs of the Department of Agriculture, J&K.

Tiksain in Munsiyari tehsil harboured high populations of 68 and 94 cysts per 200 cm^3 of soil respectively (Table 3 and Figure 1). Data from Pithoragarh district showed that many farmers' fields also recorded both full and empty cysts of PCN. In such cases with a few exceptions, most of the locations with full cysts were at more than 2000 m amsl, while those located below this elevation contained only empty cysts. In Champawat district, no incidence of PCNs was recorded and all the sampled locations were below 2000 m amsl, except one at Devidhura Pati (2045 m amsl) that contained four full cysts. Four farmers' field locations in Nainital district (all >2000 m amsl) contained a few full as well as empty cysts.

Identification

Fourteen populations were assigned species on the basis of cone morphology characteristics. All the PCN populations from Shimla and Mandi (HP), and two populations each from Pithoragarh (Uttarakhand) and Nathatop (J&K) were identified as *G. rostochiensis*. Three populations, i.e. one each from Shopian (Kashmir), and Chamba

Table 3. Survey results for the occurrence of *Globodera* spp. in potato in Uttarakhand, India

District/(tehsil)	Village	Elevation (m amsl)	Latitude	Longitude	No. of samples	Frequency of full cyst (%)	No. of cysts/200 cm^3 soil	
							Full	Empty
							Mean (range)	
Pithoragarh	Dor	1468	N30.007257	E80.146352	3	0	0	18
(Munsiyari)	Banik	2166	N30.034429	E80.183950	3	67	2 (0–3)	18
	Ratapani	2455	N30.037434	E80.193402	3	100	22 (20–25)	18
	Nai Basti Bunga	2244	N30.068087	E80.235079	3	100	16 (11–20)	4
	Tiksen Farm*	2119	N30.068041	E80.234976	3	100	94 (85–99)	0
	Balati Farm*	2675	N30.063150	E80.220044	3	100	68 (55–82)	0
	Sarmoli	2091	N30.078902	E80.237441	3	67	4 (0–7)	18
	Jalat	–	–		3	0	0	14
	Darkot	1825	N30.097913	E80.247950	3	0	0	6
	Dhapa	1826	N30.114413	E80.241125	3	0	0	18
	Syangti	–	–		3	0	0	2
	Darkot	1798	N30.097882	E80.247022	3	0	0	8
	Jaitigaon	1713	N30.078839	E80.242926	3	100	8 (3–16)	18
	Dalot	–	–		3	0	Not recorded	
	Dalot	–	–		3	0	Not recorded	
	Mallagorbhatta	–	–		3	33	2 (0–6)	14
	Tallagorbhatta	1957	N30.074364	E80.245586	3	0	Not recorded	
	Baniyagaon	1924	N30.075562	E80.249385	3	67	4 (0–7)	8
	Munsiyari	–	N30.068105	E80.236254	3	100	6 (3–8)	2
	Munsiyari	–	N30.074761	E80.236452	3	33	4 (0–12)	4
	Jaiti	1886	N30.080611	E80.246281	3	0	0	2
	Munsiyari	–	N30.093061	E80.241745	3	0	Not recorded	
	Sarmoli	1844	N30.079662	E80.235624	3	0	Not recorded	
	Quvointhi	1407	N30.081785	E80.267351	3	100	18 (11–29)	6
	Bhadeli	1256	N30.075137	E80.278438	3	0	0	18
	Josa	1279	N30.06255	E80.293422	3	0	0	2
	Suring	1988	N30.091604	E80.241431	3	0	Not recorded	
	Josa	1988	N30.06255	E80.293422	3	0	0	6
	Bata	1147	N30.035217	E80.315036	3	0	Not recorded	
Pithoragarh	Serakhal	1143	N30.012304	E80.321062	3	0	Not recorded	
(Bangapani)	Chhoribagar	982	N29.947553	E80.302130	3	0	Not recorded	
	Bans Bangar	961	N29.931336	E80.301650	3	0	Not recorded	
	Baram	826	N29.850992	E80.354925	3	0	Not recorded	
Champawat	KVK Lohaghat	1718	N29.414685	E80.072054	3	0	Not recorded	
(Lohaghat)	Patan	1895	N29.4215	E80.0879	3	0	Not recorded	
	Suichaube	1732	N29.418349	E80.062274	3	0	Not recorded	
	Chaura dhek	1696	N29.406988	E80.062596	3	0	Not recorded	
	Koli dhek	1716	N29.403828	E80.048776	3	0	Not recorded	
	Tak	1729	N29.401227	E80.043211	3	0	Not recorded	
	Sumalla	1612	N29.399772	E80.040138	3	0	Not recorded	
	Dungari Fartyal	1703	N29.396220	E80.034095	3	0	Not recorded	
	Salna	1715	N29.391151	E80.029360	3	0	Not recorded	
	Tyarson	1745	N29.387445	E80.025234	3	0	Not recorded	
	Sirkot	1847	N29.382373	E80.012127	3	0	Not recorded	
	Khetikhan	1863	N29.380756	E80.006976	3	0	0	4
	Kadwal Gaon	1752	N29.379009	E79.988341	3	0	Not recorded	
	Pokhri	1744	N29.379936	E79.979663	3	0	Not recorded	
	Gunadhar	1691	N29.379452	E79.969787	3	0	Not recorded	
Champawat	Pati	1654	N29.406676	E79.941711	3	0	Not recorded	
(Pati)	Dhanuj	1744	N29.415099	E79.891503	3	0	Not recorded	
	Dhanuj	1807	N29.414195	E79.890398	3	0	Not recorded	
	Valik	1890	N29.413593	E79.880912	3	0	0	2
	Devidhura	2045	N29.414906	E79.864804	3	67	4 (0–9)	10
	Valka	1452	N29.417409	E79.848537	3	0	Not recorded	
Nainital	Vedchula	1994	N29.427531	E79.819525	3	0	Not recorded	
(Dhari)	Vedchula-II	1993	N29.437	E79.437	3	100	5 (3–9)	0
	Vedchula	1943	N29.436773	E79.811766	3	0	Not recorded	
	Sairphatak	2151	N29.458539	E79.764154	3	33	4 (0–12)	0
	Anarpa	2202	N29.458135	E79.719218	3	0	Not recorded	
	Shelalek	2105	N29.430666	E79.717072	3	100	10 (7–14)	0
	Pahadpani	2129	N29.427162	E79.709748	3	0	0	2
	Chaulekh	2097	N29.405804	E79.691567	3	67	8 (0–16)	6
	Managher	2221	N29.396940	E79.680522	3	0	Not recorded	
	Dhanachuli	2167	N29.397293	E79.665423	3	0	Not recorded	

*PSMFs of the Department of Agriculture, Uttarakhand.

Table 4. Identification of selected populations of potato cyst nematodes in North India

Population	Molecular characterization			Cone-top morphology Inference
	Amplicon size			
	G. pallida (798 bp)	*G. rostochiensis* (315 bp)	Inference	
J&K Udhampur Nathatop	+	+	*G. pallida*, *G. rostochiensis*	*G. rostochiensis*
J&K Rajouri, Kandi Budhal	–	+	*G. rostochiensis*	–
J&K Shopian Sedew	+	+	*G. pallida*, *G. rostochiensis*	*G. pallida*, *G. rostochiensis*
UK Pithoragarh Balati			–	*G. rostochiensis*
UK Pithoragarh Tiksen			–	*G. rostochiensis*
HP Shimla Khadrala			–	*G. rostochiensis*
HP Shimla Umladwar			–	*G. rostochiensis*
HP Chamba Alha			–	*G. pallida*, *G. rostochiensis*
HP Sirmour Kheradhar			–	*G. pallida*, *G. rostochiensis*
HP Kullu Chowai			–	*G. pallida*, *G. rostochiensis*
HP Shimla Ponidhar			–	*G. rostochiensis*
HP Mandi Phuladhar			–	*G. rostochiensis*
HP Shimla Kharapathar			–	*G. rostochiensis*
HP Shimla Deorighat			–	*G. rostochiensis*
HP Shimla Tootupani			–	*G. rostochiensis*

J&K, Jammu & Kashmir; UK, Uttarakhand; HP, Himachal Pradesh. +, Present, –, Absent.

and Sirmour (HP) revealed mixed populations of *G. pallida* and *G. rostochiensis* (Table 4).

Only two populations from J&K and one from Uttarakhand were subjected to molecular characterization. Cysts from Nathatop, Rajouri and Shopian (J&K) were tested by species-specific primers for *G. pallida* and *G. rostochiensis*. The Nathatop and Shopian populations showed the presence of both 798 and a 315 bp amplicons, and were positive for both *G. pallida* and *G. rostochiensis*. However, the Rajouri population showed the presence of only a 315 bp amplicon, suggesting that only *G. rostochiensis* cysts were present in samples collected from the region.

Discussion

Considering the present scenario on the distribution of PCNs in the three hilly states/UTs of North India emanating from our surveys, some general inferences can be drawn.

(i) High populations of PCN were recorded at PSMFs; this may be due to continuous cropping of potato in these farms. Circumstantial evidence reveals the nematodes must have been introduced long ago, but due to non-specific above-ground symptoms and lack of awareness, they could not be detected earlier.

(ii) PSMFs may be contributing towards dissemination of PCNs to farmers' fields, which is clearly discernible in Figure 1, but the low populations in PCN-conducive areas could be due to discontinuous cultivation of potato crop.

(iii) Populations of PCN at lower altitudes (<2000 m amsl) may be *G. pallida*, wherever full cysts were encountered. The interception of empty cysts in such locations may be due to unfavourable temperatures. The identity of species, however, needs to be confirmed.

(iv) PCN incidence has been recorded only from *kharif* potato; the autumn and spring season potato crops are grown under low-altitude conditions, not favourable for nematode multiplication.

The distribution pattern of PCNs in the northern hilly States/UTs in India vis-à-vis southern India (the Nilgiris) shows contrasting patterns. While in the Nilgiris, most of the fields are infested harbouring high cyst populations, the same is not valid in the north where such high populations are witnessed mostly in the PSMFs (Figure 1). This leads us to assume that PCNs might have been introduced to the northern hilly states from the south much before the domestic quarantine regulations were enforced against them in 1971. Homologies on the identity of populations from the two geographies through molecular tools can throw light on this. Figure 2 depicts the possible dissemination of PCNs within India.

In view of the economic importance of the nematode and its quarantine importance, the interception of PCNs, particularly in PSMFs, became the main focus of research. AICRP immediately took up the matter with the

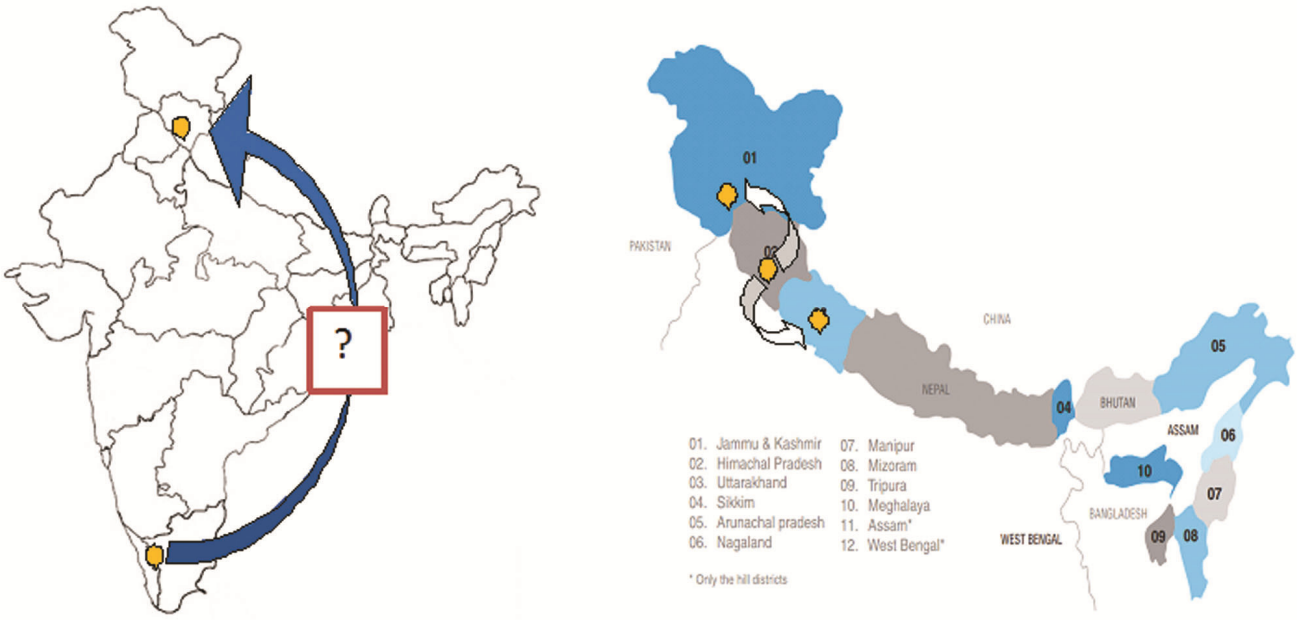

Figure 2. Possible dissemination of potato cyst nematodes within India.

Ministry of Agriculture, Government of India (R. K. Walia, pers. commun.). The Ministry took swift action and besides other measures to contain PCNs, imposed restrictions on the movement of seed potato from these areas to other parts of the country vide Gazette notifications dated 12 October 2018 and 2 November 2018.

1. Stone, A. R., Co-evolution of nematodes and plants. *Symbol. Bot. Upsalinses*, 1979, **22**, 46–61.
2. Evans, K. and Stone, A. R., A review of the distribution and biology of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Proc. Natl. Acad. Sci. USA*, 1977, **23**, 178–189.
3. Joseph, T. A. and Manorma, K., Tamil Nadu, region specific technologies for potato production in India. Chapter 15, AICRP (Potato), ICAR-CPRI, Shimla, 2009.
4. Jones, F. G. W., The potato root eelworm *Heterodera rostochiensis* Woll. in India. *Curr. Sci*., 1961, **30**, 187.
5. Seshadri, A. R. and Sivakumar, C. V., The golden nematode of potatoes (*Heterodera rostochiensis* Woll. 1923) – a threat to potato cultivation in the Nilgiris (Madras State). *Madras Agric. J.*, 1962, **49**(9), 281–288.
6. Thangaraju, D., Distribution of potato cyst nematodes in Kodaikanal hills, Madurai district, Tamil Nadu. *Indian J. Nematol*., 1983, **13**, 222–223.
7. Prasad, K. K. S. and Singh, D. B., Note on the parasitic nematodes associated with potato in Karnataka State, India. *Int. Nematol. Network Newsl*., 1986, **3**, 11–13.
8. Ramana, K. V. and Mohandas, C., Occurrence of potato cyst nematode *Globodera pallida* (Stone 1973) in Kerala. *Indian J. Nematol.*, 1988, **18**, 141.
9. Maqbool, M. A., Occurrence of eight cyst nematodes on some agricultural crops in Pakistan. *J. Sci., Karachi Univ.*, 1980, **8**, 103–108.
10. Ganguly, S., Singh, M. and Ganguly, A. K., Record of potato cyst nematode, *Globodera rostochiensis* and *G. pallid*a in Shimla, Himachal Pradesh, India. *Indian J. Nematol.*, 2010, **40**, 96–102.
11. Anon., Diagnostic protocol for *Globodera rostochiensis* and *G. pallida* PM 7/40(4). *EPPO Bull*., 2017, **47**, 174–197.
12. Subbotin, S., Moens, M. and Waeyenberge, L., Identification of cyst forming nematodes of the genus *Heterodera* (Nematoda: Heteroderidae) based on the ribosomal DNA-RFLP. *Nematology*, 2000, **2**, 153–164.
13. Fullaondo, A., Viribay, M., Salazar, A., Barrena, E., Barrena, I. and Ritter, E., Identification of potato cyst nematode species *Globodera rostochiensis* and *G. pallida* by PCR using specific primer combinations. *Nematology*, 1999, **1**, 157–163.

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