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Research article

Cytoplasmic genome of Indian potato varieties and breeding lines vis a vis prospects in potato breeding

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ABSTRACT

Advances in research resulted in development of a simple, rapid and reliable multiplex PCR protocol for cytoplasm differentiation in potato. Applying this rapid technique, we assessed the cytoplasm diversity in 57 Indian potato varieties, 15 popular exotic varieties and 47 biotic stress resistance breeding parental lines using five DNA based markers. Results revealed that T is the predominant cytoplasm type followed by D in Indian and exotic potato varieties as well as parental lines. The proportion of T and D type cytoplasm was 77.2% and 19.3% and 73.3% and 20.0% in Indian and exotic varieties, respectively. A and W type were found in one variety each, while M and P were missing in Indian varieties. All the popular Indian table potato varieties have tuberosum type cytoplasm with few exceptions of varieties bred for biotic stress resistance namely Kufri Himalini, Kufri Girdhari, carrying demissum cytoplasm. Opposite was true for Indian processing cultivars with the exception of Kufri Chipsona 4, which had T type cytoplasm. Evaluation of biotic stress resistance breeding parental lines showed increasing use of D (34.0%) and W (12.8%) cytoplasm in comparison to previously bred varieties. Although D type cytoplasm is associated with late blight resistance and male sterility, all Indian cultivars with D type cytoplasm are not resistant to late blight, nor they all are male sterile. Male fertile D type cytoplasm and the cytoplasms showing good interaction between cytoplasmic and nuclear gene for agronomic traits should be incorporated in the parental lines. Efforts must also be done to diversify the cytoplasm of cultivated potato with at least semi-cultivated cytoplasm types.

1. Introduction

Multiple copies of chloroplast and mitochondrial DNA along with one copy of nuclear DNA occur in cells of plants. These multiple cytoplasmic genes interact with nuclear genes and affect the expression and function of nuclear genes. The genus *Solanum* is extremely large and contain about 200 tuber-bearing species (Hawkes, 1990). Although, *Solanum tuberosum* is the major cultivated species, resistance or adaptation genes have been introgressed in it from many wild species (Machida-Hirano, 2015). Moreover, the resistance sources identified in one country have been used in other countries to transfer the resistance in the cultivated species. This resulted in transfer of diverse cytoplasm in cultivated potato species across the globe. It is difficult to identify cytoplasmic genomes by tracing back the lineage as pedigree records are sometimes not available for breeding clones.

Potato cytoplasm has been classified into different types and classification has evolved over time with advances in research. Initially, PCR based markers were developed to distinguish chloroplast DNA of cultivated species, *S. tuberosum* subsp. *tuberosum* from other species (Lössl et al., 2000; Hosaka 2002) and to distinguish α-, β-, and γ mitochondrial DNA types (Lössl et al., 2000). Further, a simpler, rapid and more informative classification system was developed using multiplex PCR by Hosaka and Sanetomo (2012) using a set of five cytoplasmic markers which included four chloroplast and one mitochondrial DNA markers. There are six types according to new classification i.e. M (mother), A (Andigena), P (Phureja), W (Wild), D (Demissum), and T (Tuberosum) types. This classification system is validated in cultivated potato and its close wild relatives (Hosaka and Sanetomo 2012, 2014).

The most important trait conferred by cytoplasm is male sterility. Demissum (D) and wild (W) type cytoplasm (some accessions of *S. stoloniferum* having W/gamma type cytoplasm with *Tuberosum* nuclear genes) are strongly male sterile, while T type cytoplasm has poor male fertility. More than 90% of the modern potato varieties have these three cytoplasms. Specific male sterility due to certain cytoplasms has also been reported in potato. Varieties carrying a strong *Potato virus Y* resistance gene (Ry_{sto}) exhibit male sterility caused by mtDNA derived from *S.*

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stoloniferum (Ortiz et al., 1993). *S. demissum* have been the most frequently used wild species in potato breeding to transfer late blight resistance (Plaisted and Hoopes 1989). Similarly, other wild species like *S. acaule* for *Potato virus X* (PVX) resistance, *S. tuberosum* subsp. *andigena* for *Globodera rostochiensis* and *S. vernei* and *S. spegazzinii* for *G. pallida* were used in Europe (Gebhardt et al., 2006).

The cytoplasmic genome is also associated with late blight foliage resistance, yield, and flowering (Sanetomo and Gebhardt 2015). Since the cytoplasm is inherited maternally, it plays an important role in unravelling the history of modern potato breeding. The earliest European potatoes had A type cytoplasm, indicating that they were introduced from the Andes (Sanetomo and Gebhardt 2015). Later A type was replaced by T type cytoplasm following the introduction of late blight to Europe. In 1908, wild potato species *Solanum demissum* was found to have resistance to late blight (Ross 1986). Breeding with *S. demissum* introduced D type cytoplasm to modern potatoes. Later other wild species introduced W type cytoplasm to the modern potato gene pool.

The use of wild species in potato breeding have an intricate history around the world. Because of large species diversity, various cytoplasmic genomes are expected to be present in Indian potato cultivars too. Moreover, accurate identification of the cytoplasm is important in designing efficient mating combinations. Therefore, the present study was done to characterize the Indian potato cultivars, popular exotic varieties and biotic stress breeding parental lines mostly of exotic origin with respect to cytoplasmic diversity for revisiting the potato breeding programmes with respect to cytoplasm diversification and trait introduction.

2. Materials and methods

The material included 57 Indian potato varieties, 15 exotic processing varieties, and 47 parental lines. The 15 exotic varieties are the most popular varieties being used by processing industry. The parental lines include the genotypes used in biotic stress breeding programme, which include exotic varieties and elite breeding lines. Genomic DNA was extracted from 10 to 30 mg of freshly collected leaf samples using DNeasy 50 DNA Plant kit (Qiagen, Germany) as per the supplier's protocol. The DNA quantification was done using nanodrop and DNA concentration was equalized for all the samples for further analysis. Cytoplasmic markers T, S, SAC, A and D for cytoplasm type determination and marker detection procedures were performed as described in Hosaka and Sanetomo (2012) with minor modifications. For primer details kindly refer to Hosaka and Sanetomo (2012). Initially, the PCR reaction was performed individually with each marker in 20 μl total volume, consisting of 2 μl template DNA (approximately 20 ng/μl), 10 ul Emerald PCR master mix (Takara), and 1 μl each of 10X forward primer and 10X reverse primer (10 pM for all the five markers) and 6 ul RNase free water to check the amplification and product size of each marker. PCR thermal conditions were as follows: 4 min at 95 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 54 °C, and 1 min at 72 °C, and terminated with one cycle of 5 min at 72 °C, except for marker D where the annealing temperature was 56 °C. The PCR product (20ul) was loaded on a 1.2% agarose gel with 1xTBE buffer (Figure 1). The gel was stained with syber safe gel stain dye (4ul/100ml). Once annealing temperature for all the markers was standardized, multiplex PCR was performed as described by Hosaka and Sanetomo (2012). We used 2 μl template DNA, 10 ul Emerald PCR master mix (Takara), 2ul of 10X primer mix (T (2uM), S (2uM), SAC (2uM), D (3uM) and A (3uM)) and 6 ul RNase free water. The PCR thermal conditions were kept same as used by Hosaka and Sanetomo (2012) except the annealing temperature, which was decreased to 58 °C from 60 °C. After the PCR reaction, 10ul PCR product was mixed with 10 μl digestion mix including 2 μl 10× FastDigest buffer (Fermentas), 1ul FastDigest *Bam*HI (Fermentas) and remaining RNase free water. Restriction digestions were performed at 37 °C in the thermal cycler for 10 min. After *Bam*HI digestion, 20 μl samples were loaded on a 3% agarose gel in 1× TBE buffer. The bands were visualized, scored and cytoplasm nomenclature was followed as described by Hosaka and Sanetomo (2012).

The data on pollen fertility, late blight resistance and virus resistance was acquired from evaluation of the material at Central Potato Research Institute (CPRI), Shimla, Kufri, Shillong and Ooty over the years (Vinod et al., 2005). The data on pollen fertility was recorded using aceto-carmine staining of pollen grains. The varieties as well as parental lines were screened for late blight resistance at Central Potato Research

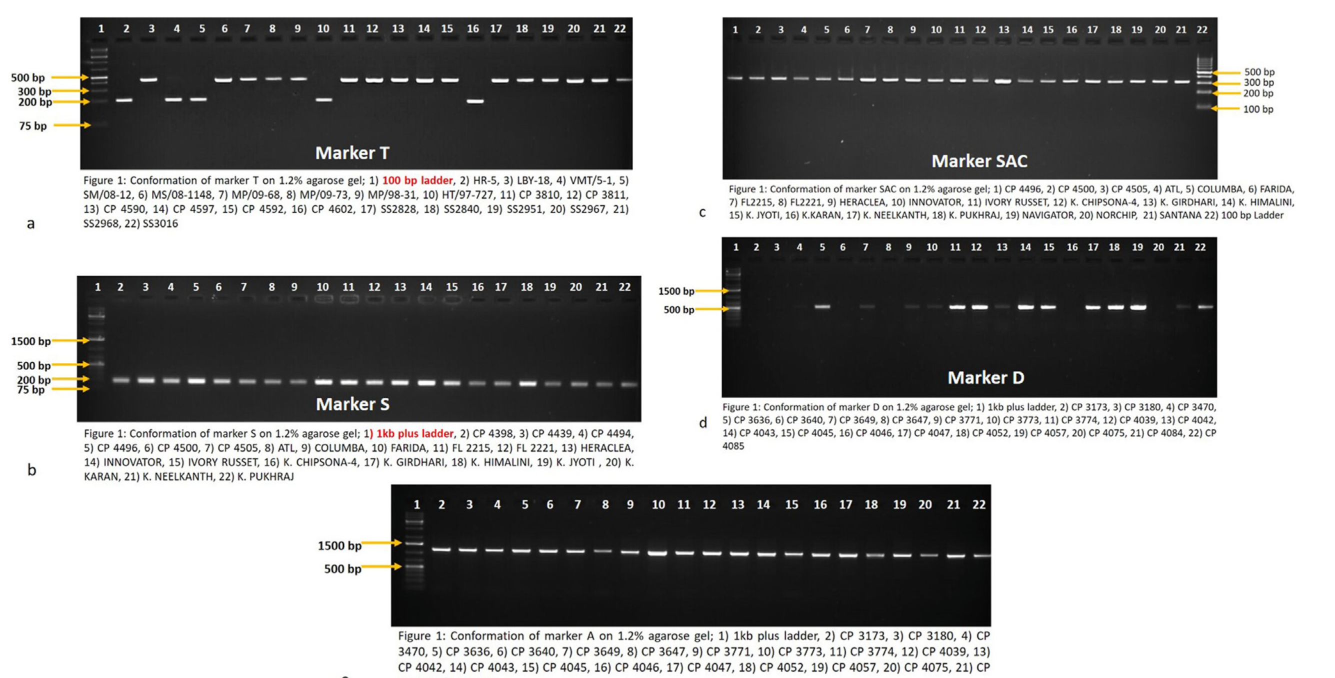

Figure 1. Individual cytoplasmic markers amplification and visualization in potato breeding lines a) T marker, b) S marker, c) SAC marker, d) D marker and e) A marker.

Table 1. Cytoplasmic type of varieties as well as parental lines and relationship with pollen fertility and biotic stress resistance traits.

Variety Name	Salient Characteristics	Year of release	Banding pattern of five cytoplasm markers in multiplex PCR					Cytoplasm	Female Parent	Pollen Fertility	Late blight resistance	Viruses resistance	PCN resistance
Indian table purpose varieties			T	S	SAC	D	A						
Kufri Kuber	Early maturity, white flesh ovoid tubers, North Indian plains and Plateau	1958	3	1	2	0	2	T	*S. curtilobum*	H	MR	PLRV	S
Kufri Red	Medium, maturity, cream flesh round red tubers, North-eastern plains	1958	3	1	2	0	2	T	Darjeeling red round	S	S	S	S
Kufri Safed	Late maturity, cream flesh round purple tubers, North Indian plains	1958	1	1	1	0	1	A	Phulwa	H	S	S	S
Kufri Kundan	Medium maturity, white flesh ovoid tubers, North Indian hills	1958	3	1	2	0	2	T	Ekishirazu	H	S	S	S
Kufri Kumar	Late maturity, white flesh ovoid tubers, North Indian hills	1958	3	1	2	0	2	T	Lumbri	S	S	S	S
Kufri Neela	Late maturity, cream flesh, ovoid tubers, South Indian hills	1963	3	1	2	0	2	T	Katahdin	H	MR	S	R
Kufri Sindhuri	Late maturity, cream flesh, red round tubers, North Indian plains	1967	3	1	2	0	2	T	Kufri Red	L	S	S	S
Kufri Alankar	Medium maturity, cream flesh ovoid tubers, North Indian plains	1968	3	1	2	0	2	T	Kennebec	H	MR	S	S
Kufri Sheetman	Medium maturity, cream flesh round tubers, North western plains	1968	1	1	2	0	2	W	Craigs Defiance	L	S	S	S
Kufri Chandramukhi	Early maturity, white flesh ovoid tubers, North Indian plains and Plateau	1968	3	1	2	0	2	T	Seedling 4485	S	S	S	S
Kufri Khasigaro	Late maturity, cream flesh round tubers, North-eastern hills	1968	1	1	2	1	2	D	Taborky	H	MR	S	S
Kufri Jyoti	Medium maturity, cream flesh ovoid tubers, Hills, plains and plateau	1968	3	1	2	0	2	T	3068d(4)	H	S	S	S
Kufri Jeevan	Late maturity, white flesh ovoid tubers, North Indian hills	1968	3	1	2	0	2	T	M 109-3	H	R	S	S
Kufri Naveen	Late maturity, yellow flesh round tubers, North-Eastern hills	1968	3	1	2	0	2	T	3070d (4)	H	MR	S	S
Kufri Chamatakar	Late maturity, yellow flesh round tubers, North Indian plains	1968	3	1	2	0	2	T	Ekishirazu	S	S	S	S
Kufri Muthu	Medium maturity, cream flesh ovoid tubers, South Indian hills	1971	3	1	2	0	2	T	3046(1)	L	MR	S	S
Kufri Lauvkar	Early maturity, cream flesh round tubers, Plateau region	1972	3	1	2	0	2	T	Serkov	M	S	S	S
Kufri Dewa	Late maturity, cream flesh ovoid tubers, North Indian hills	1973	3	1	2	0	2	T	Craigs Defiance	S	S	S	S
Kufri Badshah	Late maturity, cream flesh ovoid tubers, North Indian hills	1979	3	1	2	0	2	T	Kufri Jyoti	H	R	PVX	S
Kufri Bahar	Medium maturity, white flesh ovoid tubers, North Indian hills	1980	3	1	2	0	2	T	Kufri Red	L	S	S	S
Kufri Lalima	Medium maturity, white flesh round tubers, North Indian hills	1982	3	1	2	0	2	T	Kufri Red	H	R	S	S
Kufri Sherpa		1983	3	1	2	0	2	T	Ultimus	H	MR	S	-

(*continued on next page*)

Table 1 (*continued*)

Variety Name	Salient Characteristics	Year of release	Banding pattern of five cytoplasm markers in multiplex PCR					Cytoplasm	Female Parent	Pollen Fertility	Late blight resistance	Viruses resistance	PCN resistance
Indian table purpose varieties			T	S	SAC	D	A						
	Medium maturity, cream flesh round tubers, North-Bengal hills and Sikkim												
Kufri Swarna	Medium maturity, white flesh ovoid tubers, South Indian hills	1985	3	1	2	0	2	T	Kufri Jyoti	S	S	PVY	G rosto
Kufri Megha	Medium maturity, cream flesh ovoid tubers, North-eastern hills	1989	1	1	2	1	2	D	SLB/K-37	L	MR	PVY	-
Kufri Jawahar	Early maturity, cream flesh round tubers, North-Indian plains and plateau	1996	1	1	2	1	2	D	Kufri Neelamani	H	MR	PVX	S
Kufri Sutlej	Medium maturity, white flesh ovoid tubers, North Indian plains	1996	3	1	2	0	2	T	Kufri Bahar	H	MR	S	S
Kufri Ashoka	Early maturity, white flesh ovoid tubers, North Indian plains	1996	3	1	2	0	2	T	EM/C-1020	H	S	S	S
Kufri Pukhraj	Early maturity, cream flesh ovoid tubers, North Indian plains	1998	3	1	2	0	2	T	Craigs Defiance	H	MR	S	S
Kufri Giriraj	Medium maturity, white flesh ovoid tubers, North-Indian hills	1998	3	1	2	0	2	T	SLB/J-132	L	S	S	S
Kufri Anand	Medium maturity, white flesh oblong tubers, North Indian plains	1999	3	1	2	0	2	T	Kufri Ashoka	H	MR	-	-
Kufri Kanchan	Medium maturity, cream flesh ovoid tubers, North-Bengal hills and Sikkim	1999	3	1	2	0	2	T	SLB/Z-405(a)	H	S	PVY	S
Kufri Shailja	Medium maturity, white flesh ovoid tubers, North Indian hills	2005	3	1	2	0	2	T	Kufri Jyoti	H	S	-	-
Kufri Pushkar	Medium maturity, cream flesh ovoid tubers, North Indian plains	2005	3	1	2	0	2	T	QB/A 9-120	M	MR	-	-
Kufri Arun	Medium maturity, cream flesh ovoid tubers, North Indian plains	2005	3	1	2	0	2	T	Kufri Lalima	H	MR	-	-
Kufri Himalini	Medium maturity, cream flesh ovoid tubers, North-Indian hills	2006	1	1	2	1	2	D	I-1062	H	MR	-	S
Kufri Surya	Early maturity, cream flesh oblong tubers, North Indian plains and Plateau	2006	3	1	2	0	2	T	Kufri Lauvkar	M	S	-	-
Kufri Girdhari	Medium maturity, white flesh ovoid tubers, Indian hills	2008	1	1	2	1	2	D	Kufri Megha	H	R	PVX	S
Kufri Khyati	Early maturity, cream flesh ovoid tubers, North Indian plains	2008	3	1	2	0	2	T	MS/82-638	H	MR	-	S
Kufri Sadabahar	Medium maturity, white flesh ovoid tubers, Uttar Pradesh and adjoining areas	2008	3	1	2	0	2	T	MS/81-145	H	MR	-	
Kufri Gaurav	Medium maturity, white flesh ovoid tubers, North Indian plains	2012	3	1	2	0	2	T	JE 812	H	S	-	R
Kufri Garima	Medium maturity, yellow flesh ovoid tubers, Indo-gangetic plains and plateau	2012	3	1	2	0	2	T	PH/F 1045	H	MR	PVY	S
Kufri Lalit	Medium maturity, yellow flesh round tubers, Eastern plains	2013	3	1	2	0	2	T	85-P-670	H	MR	-	-

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Table 1 (*continued*)

Variety Name	Salient Characteristics	Year of release	Banding pattern of five cytoplasm markers in multiplex PCR					Cytoplasm	Female Parent	Pollen Fertility	Late blight resistance	Viruses resistance	PCN resistance
Indian table purpose varieties			T	S	SAC	D	A						
Kufri Mohan	Medium maturity, white flesh ovoid tubers, Northern and eastern plains	2015	3	1	2	0	2	T	MS/92-1090	H	MR	-	-
Kufri Sukhyati	Early maturity, white flesh ovoid tubers, North Indian plains	2017	3	1	2	0	2	T	MS/82-638	H	MR	-	-
Kufri Kesar	Medium maturity, pale yellow flesh red oval tubers, Eastern plains	2017	3	1	2	0	2	T		-	S	-	-
Kufri Lima	Late maturity, cream flesh ovoid tubers, North Indian plains	2018	3	1	2	0	2	T	C90.266	-	MR	-	-
Kufri Ganga	Medium maturity, cream flesh ovoid tubers, North Indian plains	2018	3	1	2	0	2	T	MS/82-638	-	MR	-	-
Kufri Karan	Medium maturity, cream flesh ovoid tubers, Hills & Plateau region	2018	3	1	2	0	2	T		H	MR	PVY, PVX, PLRV, PALCV	MR
Kufri Neelkanth	Medium maturity, cream flesh ovoid tubers, North Indian plains	2018	3	1	2	0	2	T	MS/89-1095	-	MR	-	-
Kufri Thar 2	Medium maturity, light yellow flesh ovoid tubers, Gangetic plains, plateau region and western dry region	2019	1	1	2	1	2	D		-	-	-	-
Kufri Thar 1	Medium maturity, cream flesh ovoid tubers, East coast plains and middle Gangetic plains	2019	3	1	2	0	2	T		-	-	-	-
Indian processing varieties													
Kufri Chipsona-1	Medium maturity, cream flesh ovoid tubers, North Indian plains	1998	1	1	2	1	2	D	MEX.750826	H	R	S	S
Kufri Chipsona-2	Medium maturity, cream flesh round tubers, North Indian plains	1998	1	1	2	1	2	D	F-6	H	R	S	S
Kufri Chipsona-3	Medium maturity, white flesh ovoid tubers, North Indian plains	2006	1	1	2	1	2	D	MP/91-86	H	MR		S
Kufri Himsona	Late maturity, cream flesh round tubers, Indian hills	2008	1	1	2	1	2	D	MP/92-35	H	MR	-	-
Kufri Frysona	Medium maturity, white flesh long-oblong tubers, North Indian plains	2009	1	1	2	1	2	D	MP/92-30	H	R	-	S
Kufri Chipsona-4	Medium maturity, white flesh round tubers, Karnataka, West-Bengal and Madhya Pradesh	2019	3	1	2	0	2	T	Atlantic		MR	-	-
Exotic varieties													
Atlantic	Early maturity, white flesh round tubers, wide adaptability		3	1	2	0	2	T	Wauseon	H	S	S	G. rosto
Colomba	Medium maturity, yellow flesh round to oval tubers		3	1	2	0	2	T	Blochinger	M	-	-	-
FL2215	Early to medium maturity, white flesh oval to oblong tubers		3	1	2	0	2	T	FL 1840	H	-	-	-
FL2221	Early to medium maturity, white flesh oval tubers		1	1	2	1	2	D	FL 1771	S	-	-	-
Heraclea	Early to medium maturity, yellow flesh round tubers		3	1	2	0	2	T		M	-	-	-

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Table 1 (*continued*)

Variety Name	Salient Characteristics	Year of release	Banding pattern of five cytoplasm markers in multiplex PCR					Cytoplasm	Female Parent	Pollen Fertility	Late blight resistance	Viruses resistance	PCN resistance
Indian table purpose varieties			T	S	SAC	D	A						
Innovator	Early maturity, white cream flesh oblong tubers		3	1	2	0	2	T	Shepody	-	S	-	-
Ivory Russet	Early to medium maturity, white flesh oblong-long tubers		3	1	2	0	2	T	RZ 73-9105	-	S	-	-
Navigator	Medium maturity, yellow flesh oval tubers, big size tubers		3	1	2	0	2	T	Bru 93-136	H	S	-	-
Norchip	Medium maturity, white flesh round to oval tubers, medium to high yield potential		1	1	2	1	2	D	ND 4731-1	H	S	S	
Santana	Medium to late maturity, cream flesh long oval tubers and high dry matter		1	1	2	1	2	D	Spunta	M	S	-	-
Lady Rosetta	Early maturity, light yellow flesh round tubers, wide adaptability		3	1	2	0	2	T	Cardinal	S	S	-	S
Farida	Medium late, light yellow flesh oval tubers		3	1	2	0	2	T	RZ 91-2313	H	S	-	-
FL1533	Early maturity, white flesh ovoid tubers		3	1	2	0	2	T	RD 197-1-7	H	S	-	-
Russet Burbank	Medium to late maturity, white flesh oval tubers, wide adaptability		3	1	2	0	2	T	Burbank mutant	-	S	S	S
Shepody	Early to medium maturity, cream flesh oblong long tubers		1	1	2	0	2	W	Bake-King	-	S	-	S
Parental lines													
CP1717			1	1	2	1	2	D	-	H	S	S	S
CP1909			1	1	2	0	2	W	-	H	MR	S	S
CP1911			3	1	2	0	2	T	-	H	R	S	S
CP1917			3	1	2	0	2	T	-	H	MR	PVY	S
CP1940			3	1	2	0	2	T	-	H	S	-	S
CP1945			1	1	2	0	2	W	-	-	S	S	S
CP1971			3	1	2	0	2	T	-	H	MR	PVY	R
CP1980			3	1	2	0	2	T	-	H	S	S	S
CP2011			3	1	2	0	2	T	-	H	R	S	G rosto
CP2067			1	1	2	0	2	W	-	S	R	S	S
CP2069			3	1	2	0	2	T	-	L	S	S	S
CP2294			1	1	2	1	2	D	-	H	R	PVY, PVX, PLRV	S
CP2350			3	1	2	0	2	T	-	H	MR	PVY, PVS	S
CP2370			3	1	2	0	2	T	-	H	R	PVY, PVX, PVS, PLRV	-
CP2372			3	1	2	0	2	T	-	L	S	S	S
CP2373			3	1	2	0	2	T	-	L	S	S	S
CP2379			3	1	2	0	2	T	-	H	R	PVY	S
CP2416			1	1	2	1	2	D	-	H	R	S	S
CP2418			3	1	2	0	2	T	-	H	MR	S	G rosto
CP2419			3	1	2	0	2	T	-	H	MR	S	S
CP3134			3	1	2	0	2	T	-	L	S	S	S
CP3173			3	1	2	0	2	T	-	H	S	S	S
CP3180			1	1	2	0	2	W	-	H	S	S	S
CP3470			3	1	2	0	2	T	-	H	MR	S	S
CP3636			1	1	2	1	2	D	-	S	R	S	R
CP3640			3	1	2	0	2	T	-	S	R	PVX, PVS, PLRV	S
CP3771			3	1	2	0	2	T	-	H	R	S	S
CP3773			3	1	2	0	2	T	-	H	R	-	S
CP3774			1	1	2	1	2	D	-	H	R	PVY	S

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Table 1 (*continued*)

Variety Name	Salient Characteristics	Year of release	Banding pattern of five cytoplasm markers in multiplex PCR					Cytoplasm	Female Parent	Pollen Fertility	Late blight resistance	Viruses resistance	PCN resistance
Indian table purpose varieties			T	S	SAC	D	A						
CP4039			1	1	2	1	2	D	-	H	R	PVY	S
CP4042			1	1	2	0	2	W	-	S	-	-	G rosto
CP4043			1	1	2	1	2	D	-	H	-	-	S
CP4045			1	1	2	1	2	D	-	M	MR	-	S
CP4046			3	1	2	0	2	T	-	H	MR	PVY	G pal
CP4047			1	1	2	1	2	D	-	H	MR	PVY	S
CP4052			1	1	2	1	2	D	-	H	R	-	S
CP4057			1	1	2	1	2	D	-	M	MR	-	S
CP4084			1	1	2	0	2	W	-	S	-	-	-
CP4085			1	1	2	1	2	D	-	L	S	-	-
CP4087			3	1	2	0	2	T	-	-	-	-	-
CP4175			1	1	2	1	2	D	-	-	MR	PVY	S
CP4311			1	1	2	1	2	D	-	M	R	-	S
CP4398			3	1	2	0	2	T	-	-	-	-	-
CP4494			3	1	2	0	2	T	-	H	-	-	-
CP4496			1	1	2	1	2	D	-	-	-	-	-
CP4500			1	1	2	1	2	D	-	-	-	-	-
CP4505			3	1	2	0	2	T	-	H	-	-	-

Pollen fertility: H-high, M-medium, L-low, S-sterile; Late blight resistance: R-resistant, MR-moderately resistant, S-susceptible; Viruses resistance- S-susceptible, PVY-PVY resistant, PVX-PVX resistant, PVS-PVS resistant, PLRV-PLRV resistant; PCN resistance- R-resistant to both species, G rosto- Resistant to *Globodera rostochiensis*, G pal-resistant to *G. pallida*, S-susceptible; - Not known.

Institute (CPRI), Shimla (through artificial inoculation under controlled conditions), Shillong and Kufri (under natural epiphytotic conditions); viruses resistance at Shimla and PCN resistance at Shimla and Ooty under controlled conditions in pots (Srivastava et al., 2015; Sharma et al., 2014; Bhardwaj et al., 2019; Sudha et al., 2019). The salient characteristics of potato varieties used in the study have been listed in Table 1.

3. Results

A total of 57 Indian potato varieties, 15 exotic processing varieties and 47 biotic stress resistant parental lines were genotyped using multiplex PCR with the five cytoplasmic markers T, S, SAC, A and D for rapid identification of cytoplasm types. The undigested as well as

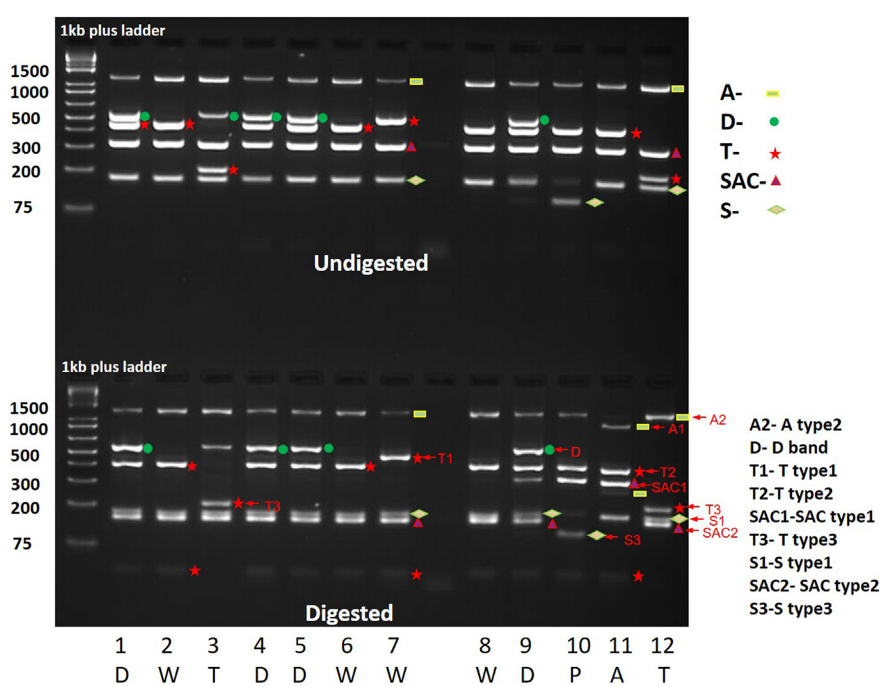

Figure 2. Visualization of undigested and digested multiplex PCR product for cytoplasm type identification. (1-Kufri Girdhari, 2-*S. chacoense*, 3- Kufri Chandramukhi, 4- *S. demissum*, 5- *S. demissum*, 6- *S. canasense*, 7-*S. verneii*, 8- *S. brevicaule*, 9- *S. demissum*, 10- *S. phureja*, 11- *S. tuberosum* subsp. andigena, 12-Lady Rosetta).

Table 2. Frequency of different cytoplasm types in Indian potato varieties and parental lines.

Potato genotypes	Number	Cytoplasm type					
		T	D	A	W	P	M
Indian varieties	57	44 (77.19)	11 (19.30)	1 (1.75)	1 (1.75)	-	-
a) Table purpose varieties	51	43 (84.31)	6 (11.77)	1 (1.96)	1 (1.96)	-	-
b) Processing varieties	6	1 (16.67)	5 (83.33)	-	-	-	-
Exotic processing varieties	15	11 (73.33)	3 (20.0)	-	1 (6.67)	-	-
Parental lines	47	25 (53.19	16 (34.04)	-	6 (12.77)	-	-

Figures in bracket shows the percentage.

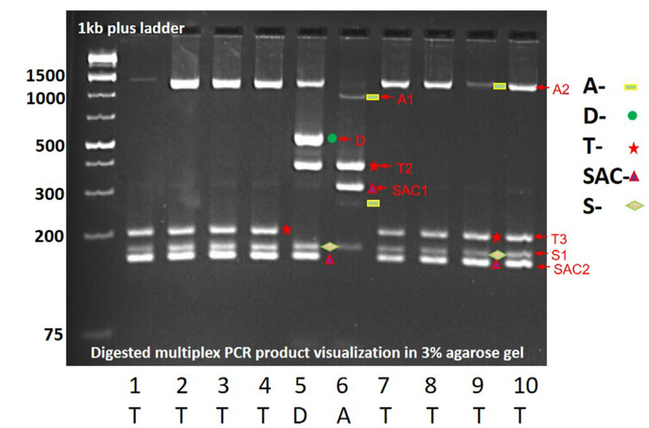

Figure 3. Marker bands of five different cytoplasmic markers in multiplex PCR for cytoplasm type identification in Indian Potato varieties (1-Kufri Pukhraj, 2-Kufri Bahar, 3-Kufri Jyoti, 4-Kufri Pushkar, 5-Kufri Himalini, 6-Kufri Safed, 7-Kufri Khyati, 8-Kufri Surya, 9-Kufri Lauvkar, 10-Kufri Karan).

digested multiplex PCR product were observed for product size of five different markers. In the undigested PCR product, T produced three bands (~450, ~400 and ~200bp), S two bands (~180 and ~140bp), while SAC (~300bp), A (>1000bp) and D (~500bp) produced one band each (Figure 2). BamH1 digestion of PCR products showed digestion of T, SAC and A marker products (Figure 2). The ~450bp (Type1) and ~400 (Type2) bp T-band were cut into 2 bands whereas ~200bp (Type3) band remained intact. The SAC marker ~300bp band was cut into 2 bands in most of the genotypes, where the uncut band of ~300bp was scored as type1 and cut band was scored as type 2. Similarly, the undigested A band was scored as type2 whereas the digested A marker band into three bands was scored as type1. The undigested two bands of S marker were scored as type1 (~180bp) and type3 (~140bp), while D marker band was scored based on its presence. In some varieties and parental lines, low copy light D band was observed, which was not scored as D band (Figure 2).

Four cytoplasm types i.e. T, D, A and W were found in Indian varieties. T type cytoplasm was found in 77.19 per cent (44 varieties), whereas 19.30 per cent (11 varieties) contained D type cytoplasm (Table 2). Among others, Kufri Safed observed A type cytoplasm (Figure 3) while Kufri Sheetman contain W type (Table 1&3). Other two cytoplasm types i.e. M type (mother type, or an ancestral type of Andean cultivated potatoes) and P type (derived from *S. phureja*) were not found in any of the Indian potato varieties. The popular Indian varieties viz., Kufri Pukhraj, Kufri Bahar, Kufri Jyoti, Kufri Pushkar and Kufri Khyati were found to have T type cytoplasm (Figure 3). Only Kufri Himalini which is becoming popular in Southern and Eastern states had D type cytoplasm (Figure 3). Among chipping varieties i.e. Kufri Chipsona 1, 2 and 3 and Kufri Himsona were found to have D type cytoplasm while latest release Kufri Chipsona 4 had T type cytoplasm. The French fry variety Kufri Frysona too had demissum cytoplasm. Heat tolerant varieties, Kufri Lauvkar and Kufri Surya also contained predominant T type cytoplasm. All the newly developed varieties in last 10 years in India observed predominantly T type cytoplasm except a newly identified water use efficient clone Kufri Thar 2, which has D type cytoplasm (Table 1&3).

Inspecting the exotic processing varieties, we found high percentage of T type cytoplasm i.e. 73.3%, followed by D type cytoplasm in 20.0% and W type cytoplasm in 6.7% varieties (Table 2). The result of the exotic varieties was almost similar to the results we obtained for Indian potato varieties, although the number of total varieties was quite less. All popular exotic varieties, namely Atlantic, Lady Rosetta, and Russet Burbank, were found to have predominantly T type cytoplasm, while D type cytoplasm was found in Santana, Norchip and FL2221 (Table 1).

The evaluation of biotic stress resistance breeding parental lines showed the presence of T type cytoplasm in 53.2%, D type in 34.0%, W type in 12.8% genotypes, while A, P and M were absent in the parental lines (Table 2). The most commonly used parents for late blight resistance breeding in recent years, i.e. CP 3774, CP 4052, and CP 4311 had all D cytoplasm types (Table 1&3).

The data on pollen fertility/sterility and biotic stress resistance was used to investigate their relationship with the cytoplasm type (Table 4). There was no clear association of T and D cytoplasm type with pollen fertility but high percentage of W cytoplasm carrying lines were male sterile (23.1%). For late blight resistance, higher percentage of D cytoplasm carriers were resistant (50%) in comparison to susceptible (9.5%) among total resistant and susceptible lines, respectively. The opposite was true for T type cytoplasm lines where the percent susceptible number (78.6%) was higher than resistant lines (45.5%). Similar results were observed for viruses resistance, where higher percentage of D cytoplasm type lines were resistant (38.1%) than T cytoplasm lines. For potato cyst nematode (PCN), higher percentage of T cytoplasm carrying lines were resistant (81.8%) while higher susceptibility was associated with D type cytoplasm (60.9%). The number of W and A cytoplasm lines were too less to compare the results of their association with biotic stress resistance.

4. Discussion

The Indian potato breeding programme started in the middle of 20th century, where all the available collections were evaluated for introduction and as parents in breeding programme. Among biotic stresses, late blight resistance was the major priority in potato breeding because of previous epidemic in Europe. Wild species, introduced from Europe, USA and other countries were also utilized in hybridization for transfer of late blight resistance. Similarly, resistance genes to potato viruses and potato cyst nematodes (PCN) were introduced from wild species into cultivated potato over the time. Besides nuclear genes transfer, the cytoplasm shuffling also occurred. Therefore, we used a new, simpler, rapid and more informative classification system using multiplex PCR (Hosaka and Sanetomo 2012) for cytoplasm identification of Indian potato varieties and breeding lines. This classification system so far been successful for cytoplasmic differentiation in different breeding programs except D marker band, which gets weakly amplified in many genotypes that should not have *S. demissum* cytoplasm (Sanetomo and Hosaka 2013, 2014).

Table 3. Cytoplasmic markers banding pattern for cytoplasm type identification of potato varieties and lines.

Cytoplasm type	Banding pattern of five cytoplasm markers in multiplex PCR					Varieties/lines			
	T	S	SAC	D	A	Indian table purpose varieties	Indian processing varieties	Exotic processing varieties	Parental lines
T	3	1	2	0	2	Kufri Kuber, Kufri Red, Kufri Kundan, Kufri Kumar, Kufri Neela, Kufri Sindhuri, Kufri Alankar, Kufri Chandramukhi, Kufri Jyoti, Kufri Jeevan, Kufri Naveen, Kufri Chamatakar, Kufri Muthu, Kufri Lauvkar, Kufri Dewa, Kufri Badshah, Kufri Bahar, Kufri Lalima, Kufri Sherpa, Kufri Swarna, Kufri Sutlej, Kufri Ashoka, Kufri Pukhraj, Kufri Giriraj, Kufri Anand, Kufri Kanchan, Kufri Shailja, Kufri Pushkar, Kufri Arun, Kufri Surya, Kufri Khyati, Kufri Sadabahar, Kufri Gaurav, Kufri Garima, Kufri Lalit, Kufri Mohan, Kufri Sukhyati, Kufri Kesar, Kufri Lima, Kufri Ganga, Kufri Karan, Kufri Neelkanth, Kufri Thar 1,	Kufri Chipsona-4	Atlantic, Colomba, FL2215, Heraclea, Innovator, Ivory Russet, Navigator, Lady Rosetta, Farida, FL 1533, Russet Burbank	CP1911, CP1917, CP1940, CP1971, CP1980, CP2011, CP2069, CP2350, CP2370, CP2372, CP2373, CP2379, CP2418, CP2419, CP3134, CP3173, CP3470, CP3640, CP3771, CP3773, CP4046, CP4087, CP4398, CP4494, CP4505
D	1	1	2	1	2	Kufri Khasigaro, Kufri Megha, Kufri Jawahar, Kufri Himalini, Kufri Girdhari, Kufri Thar 2	Kufri Chipsona-1, Kufri Chipsona-2, Kufri Chipsona-3, Kufri Himsona, Kufri Frysona	FL2221, Norchip, Santana,	CP1717, CP2294, CP2416, CP3636, CP3774, CP4039, CP4043, CP4045, CP4047, CP4052, CP4057, CP4085, CP4175, CP4311, CP4496, CP4500
A	1	1	1	0	1	Kufri Safed	-	-	-
W	1	1	2	0	2	Kufri Sheetman	-	Shepody	CP1909, CP1945, CP2067, CP3180, CP4042, CP4084

Table 4. Cytoplasm type relationship with pollen fertility and biotic stress resistance traits.

Trait		Cytoplasm type	Number of varieties/lines					% of total*
			Indian table purpose varieties	Indian processing varieties	Exotic processing varieties	Parental lines	Total	
Pollen fertility#	Fertile	T	32	5	6	22	65	69.9
	Fertile	D	6	4	2	12	24	25.8
	Fertile	W	1			2	3	3.2
	Fertile	A	1				1	1.1
	Total Fertile						**93**	
	Sterile	T	6		1	1	8	61.5
	Sterile	D			1	1	2	15.4
	Sterile	W				3	3	23.1
	Sterile	A						
	Total Sterile						**13**	
Late blight resistance#	Resistant	T	3			7	10	45.5
	Resistant	D	1	3		7	11	50.0
	Resistant	W				1	1	4.5
	Resistant	A						
	Total resistant						**22**	
	Moderately resistant	T	21	1		7	29	72.5
	Moderately resistant	D	4	2		4	10	25.0
	Moderately resistant	W				1	1	2.5
	Moderately resistant	A						
	Total moderately resistant						**40**	
	Susceptible	T	18		8	7	33	78.6
	Susceptible	D			2	2	4	9.5
	Susceptible	W	1		1	2	4	9.5
	Susceptible	A	1				1	2.4
	Total susceptible						**42**	
Virus (es) resistance#	Resistant	T	6			7	13	61.9
	Resistant	D	3			5	8	38.1
	Resistant	W						
	Resistant	A						
	Total resistant						**21**	
	Susceptible	T	21		2	12	35	72.9
	Susceptible	D	1	2	1	3	7	14.6
	Susceptible	W	1			4	5	10.4
	Susceptible	A	1				1	2.1
	Total susceptible						**48**	
PCN resistance#	Resistant	T	4		1	4	9	81.8
	Resistant	D				1	1	9.1
	Resistant	W				1	1	9.1
	Resistant	A						
	Total resistant						**11**	
	Susceptible	T	24		2	16	42	60.9
	Susceptible	D	4	4		12	20	29.0
	Susceptible	W	1		1	4	6	8.7
	Susceptible	A	1				1	1.4
	Total susceptible						**69**	

*-Total is sum of varieties/lines of all the four cytoplasm types, in bold font.
-Individual trait wise details of varieties is given in Table 1.

In our study, T type (77.2%) was the predominant cytoplasm, followed by D type (19.3%) in Indian potato varieties. Only two varieties, Kufri Safed and Kufri Sheetman had A type and W type, respectively. Kufri Safed is a selection from local landrace Phulwa, which might be original andigena type. The maternal parent of Kufri Sheetman is Craigs Defiance, which had T type, therefore presence of W type cytoplasm in Kufri Sheetman needs further validation and analysis (Table 1&3). This could be a sampling error also as Kufri Sheetman has been found to have T type cytoplasm in an earlier study (Chimote et al., 2008). Further, we observed T type cytoplasm for old variety Kufri Kuber, although its maternal parent, *S. cutilobum* has been conferred P type cytoplasm (Hosaka 1986). The exotic processing varieties evaluated in the present study followed the same trend with T type as the common cytoplasm, followed by D and W types. The W type cytoplasm results for exotic variety Shepody were spurious, because Innovator which has Shepody as maternal parent observed T type cytoplasm (Table 1&3). Besides, earlier study also conferred T type cytoplasm for Shepody (Hosaka and Sanetomo 2012).

The cytoplasm type prevalence results are quite similar to earlier studies on potato cytoplasm diversity in Europe, Japan and Russia (Sanetomo and Gebhardt 2015; Hosaka and Sanetomo, 2009, 2012; Gavrilenko et al., 2019). In a study of 144 German varieties and 140 elite lines, Lössl et al. (2000) found 47% T type, 40% D type and 10% W type cytoplasm. In another study with a larger collection of European varieties and germplasm by Sanetomo and Gebhardt (2015), T type was the most prevalent cytoplasm followed by D type cytoplasm. The evaluation of Japanese varieties and collections observed the same pattern but the frequency of D (17.4%) type cytoplasm was much lower than the T (73.9%) type cytoplasm (Hosaka and Sanetomo, 2012) while Russian varieties found more usage of D (50.8%) cytoplasm than T (40%) cytoplasm (Gavrilenko et al., 2019). International Potato Center (CIP) is the international supplier of potato germplasm throughout the world and evaluation of CIP breeding germplasm also found T (45%), D (38%) and W (11%) as the predominant cytoplasm types (Mihovilovich et al., 2015). Indian varieties also followed the same trend with T type cytoplasm as the most common cytoplasm followed by D type cytoplasm. The frequency of D type cytoplasm in Indian varieties was much lower than European varieties and was almost in similar proportion to that of Japanese varieties. In an earlier study, 38 Indian varieties showed the predominance of T type cytoplasm in Indian potato varieties which also proved that Indian cultivars are more like tuberosum type than andigena type (Chimote et al., 2008).

Evaluation of biotic stress resistance breeding programme parental lines revealed higher proportion of D (34.0%) and W (12.8%) cytoplasm type genotypes in comparison to Indian and exotic potato cultivar. Although T type was also the predominant cytoplasm in parental lines, the higher proportion of D and W type cytoplasm showed that most of the parents being used in the biotic stress breeding programme carry resistance to various biotic stresses especially, late blight and viruses. This further indicates that D type cytoplasm is being used in higher proportion due to unintended and continuous use of cytoplasmic-based male-sterile maternal lineages derived from *S. demissum* in parental line and variety development (Mihovilovich et al., 2015). These results are in agreement with earlier studies in Europe, America and Japan, which have found extensive use of D and W type cytoplasm (Sanetomo and Gebhardt 2015; Hosaka and Sanetomo, 2012; Mihovilovich et al., 2015).

The T type cytoplasm is obviously the predominant cytoplasm type as observed earlier in global potato germplasm. The reason could be usage of common maternal parents for most of the cultivars as identified as "Rough Purple Chilli" and few other clones from subsp. tuberosum in the case of European cultivars (Plaisted and Hoopes, 1989; Provan et al., 1999; Hosaka and Sanetomo, 2012). It is known that the hybrids between *Solanum tuberosum* subsp. *tuberosum* (T type) and *Solanum tuberosum* subsp. *andigena* (A type) in T×A combination produce higher yields than in A×T or T×T crosses (Maris 1989). Interestingly, higher number of T cytoplasm lines were observed to be resistant to PCN in comparison to D cytoplasm type lines. *S. vernei* and *S. tuberosum* ssp. *andigena* are well known sources of PCN resistance (Bryan et al., 2004) which substantiates our results. The W cytoplasm lines probably could not show PCN resistance association due to limited number of varieties and lines with W cytoplasm type in our study. *Solanum demissum* is the second most abundant cytoplasm in potato varieties and parental lines because of use of late blight resistant hybrids in hybridization carrying resistant genes from *S. demissum*. A small proportion of W cytoplasm type in varieties and parental lines probably indicates the PVY resistance from wild species, *S. stoloniferum* (Lössl et al., 2000; Sanetomo and Geberhardt 2015). There is a weak evidence of Ry_{sto} gene presence in Indian potato varieties (Bhardwaj et al., 2019) which could be correlated to absence of W type cytoplasm in Indian potato varieties. The D and W type cytoplasm are also associated with functional male sterility (Lössl et al., 2000) and therefore can be used as female parents only resulting in the invasion of D and W type cytoplasm in common potato gene pool. Crosses without knowing the cytoplasm type of parental lines could result in increase of male sterile genotypes and limiting the choice of male parents in potato breeding (Provan et al., 1999). This indicates that all the varieties and parental lines carrying D and W type cytoplasm are therefore functionally male sterile and cannot be used as male parents in hybridization programme. We also observed that most D cytoplasm carrying varieties and parental lines are resistant to late blight and viruses. However, higher proportion of D cytoplasm varieties and parental lines were male fertile while W cytoplasm type lines were mostly male sterile in our study (Table 1 & 3). Male fertility of some D type cytoplasm have been noticed earlier also (Hosaka and Sanetomo 2012). Such cytoplasm types could be used to improve the parental lines. Moreover, the cytoplasms showing good interaction between cytoplasmic and nuclear gene for agronomic traits should be incorporated in the parental lines. Cytoplasm genes interact with nuclear genes and affect agronomic and quality traits such as resistance to late blight and tuber bruising, plant maturity, tuber shape, starch content and yield (Sanetomo and Gebhardt 2015). Positive correlations of tuber starch content and foliage resistance to late blight was observed for D and W/γ-type cytoplasm (Sanetomo and Gebhardt 2015).

5. Conclusions

T is the predominant cytoplasm type in Indian potato varieties and breeding lines, followed by D type cytoplasm. Our results agree with the cytoplasm types in potato varieties at global potato. Although the resistance genes from wild species have been transferred into cultivated potato varieties in India, use of W, A, P, M cytoplasm is limited or negligible. In many cases, the D and W cytoplasm have been indirectly used i.e. the exotic cultivars carrying resistant genes have been used as parent for introgression of resistant genes. Increasing use of same *Solanum* species as resistance source have resulted similar cytoplasm types across the countries in cultivated potatoes. The results also suggest that the use of T cytoplasm type has decreased while D and W cytoplasm type is increasing in India as well as at global level. Although diversification is important, efforts must be made to diversify the cytoplasm keeping in view the interactions between cytoplasmic and nuclear genes of two species.

Declarations

Author contribution statement

Salej Sood: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ashwani Kumar; Baljeet Singh: Performed the experiments.

Sundaresha S: Conceived and designed the experiments.

Vinay Bhardwaj: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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