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KUFRI FRYOM: A NEW HIGH YIELDING FRENCH FRY POTATO VARIETY

Vijai Kishor Gupta[1*], SV Singh[1], Vinay Bhardwaj[2], SK Luthra[1], S K Pandey[2], Dinesh Kumar[3], Sanjay Rawal[1], Bandana Kaundal[1], Ashiv Mehta[4], P Manivel[5], Vinod Kumar[2], BP Singh[2], S K Chakrabarti[6] and Manoj Kumar[1]

ABSTRACT: Kufri FryoM is a medium maturing, high yielding main season French fry potato variety suitable for cultivation in North-West and Central Indian plains. It is a clonal selection from a cross between Kufri Chipsona-1 × MP/92-35. It has semi-compact, vigorous, medium-tall plants and produces attractive white cream, oblong tubers with shallow eyes and white flesh. It possesses 20% tuber dry matter content and low reducing sugars (50-90 mg/100 g fresh weight) which results into crispy French fries of very good quality in taste, texture and colour. It produces >80% process grade tuber yield and is capable of yielding 35-40 t/ha under optimum agronomical practices. The new variety Kufri FryoM yields higher than the existing indigenous processing varieties Kufri Frysona and Kufri Chipsona-1. It possesses field resistance to late blight disease and resistance to potato virus Y. The variety has very good keeping quality with over 10 week dormancy period and it can be processed even after 7-8 months of storage with CIPC application at 10-12° C temperature.

KEYWORDS: Kufri FryoM, potato, variety, high yield, French fries, late blight, keeping quality

INTRODUCTION

Frozen French fries are the most popular processed product of potato throughout the world. Frozen prepared or preserved potatoes including French fries amounted to $12.8 billion in global export sales for 2019. Belgium (24.8%), Netherlands (21.9%), USA (14.7%), Canada (11.3%) and France (4.8%) constitute about 75-78% of total export business in the world (Anonymous, 2020). Since 2015, fastest growing exporters of frozen prepared or preserved potatoes are Turkey (526%), India (228%), Poland (61.2%) and Belgium (60%). In India, the domestic demand for processed potato products has also increased considerably during last two decades. Several indigenous and multinational entrepreneurs have established chips, French fries and flakes units in India and new manufacturers are coming up in the field of diversified potato and potato-based products including starch manufacturing. Rising global demand coupled with concerted efforts for development of new indigenous potato varieties for processing and contract farming has transformed India from an importer of French fries to a potential exporter in just over a decade. Among potato based processed products, French fries have higher (>11.6%) annual compound growth rate (ACGR) than other potato based products (Singh *et al*., 2014). Currently, total French fry production in India is around 1, 10,000 tonnes and out of it about 25,000 tonnes are exported to different countries. Existing French fry companies are also doubling their capacities; therefore, in coming time, India is going to be one of the fast-emerging nations

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not only for domestic consumption but also a very competitive exporter for overseas markets (Gupta *et al*., 2020a). To keep pace with rising demand, the development of high yielding new processing potato varieties and their adoption in different agro-ecologies is essential.

Processing of potatoes into French fries requires certain quality attributes and mainly include oblong to long-oval tubers of preferably more than 75 mm size with shallow eyes to yield desirable long sticks of international standard with minimum peeling losses. Tubers need to have 20% or more dry matter with reducing sugars content below 200 mg/100 g fresh tuber weight so as to yield crisp and light coloured French fries (Gupta *et al*., 2020). Presently Kufri Frysona is the only Indian popular variety for making French fries, when grown in northern and central plains. It has medium to late maturity with moderate to high productivity (Singh *et al*., 2010). Although Kufri Frysona is being grown and has been adopted by the processor but there has been a demand of an alternate high yielding variety with a relatively shorter maturity period. Therefore, efforts were directed to meet the above demand and as result a new potato variety Kufri FryoM (MP/4-578) was developed and released in 2020 for North-West and Central Indian plains. Adoption of new potato variety Kufri FryoM by the potato growers will increase their farm productivity and income, thus improving their livelihood besides better sustenance of French fry industry in the country.

MATERIALS AND METHODS

Evaluation and testing of hybrid

Advanced stage hybrid MP/4-578 named as Kufri FryoM originated from hybridization of cross Kufri Chipsona-1 × MP/92-35 attempted in 2004 at Kufri (31° 1'N 77° 3'E; 2501 m above msl) in the mid- hills of Himanchal Pradesh. Seedlings and subsequent clonal generations were raised and evaluated at the Central Potato Research Institute (CPRI), Regional Station, Modipuram, Meerut, UP (29 °4' N and 77° 46' E; 237 above msl) as per procedure described by Luthra *et al*., (2020). The pedigree of Kufri FryoM has been described in **Fig. 1**. The female parent Kufri Chipsona-1 an indigenous processing variety produces white cream ovoid, shallow eyed tubers with cream flesh and possessed resistance to late blight, whereas male parent MP/92-35 an indigenous advanced stage clone produces white-cream ovoid tubers with medium-deep eyes and white-cream flesh.

The clone MP/4-578 was in seedling stage in 2004-05, the five-hill plot in 2005-06, 30 hill plot in 2006-07, multiple-row trial in 2007-08 and in replicated yield trials during 2008-12 at Modipuram. Based on its consistently superior performance over control varieties in yield trials, MP/4-578 was introduced in AICRP in year 2012 for multi-location testing across the country. This advanced stage hybrid had also gone through industrial evaluation and testing during 2012-13. During 2014-18 it was evaluated at 16 locations in three regions i.e. North-Western (4 locations *viz*., Hisar, Jalandhar, Modipuram and Pantnagar), Central (6 locations namely Chhindwara, Deesa, Gwalior, Kanpur, Kota and Raipur) and Eastern plains (6 locations

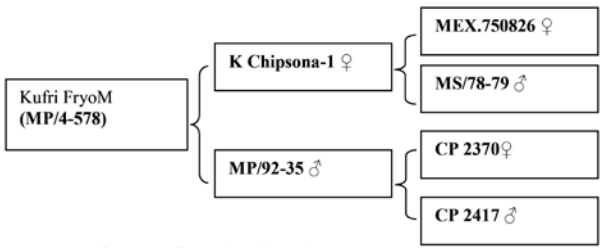

Fig. 1. Pedigree of Kufri FryoM

viz., Bhubaneshwar, Dholi, Faizabad, Jorhat, Kalyani and Patna) in multi-location replicated, on-farm and advanced varietal trails under AICRP.

Estimation of quality parameters

Tuber dry matter: Samples of 5 randomly drawn tubers were used for dry matter estimation. The tubers were chopped into small pieces. The chopped pieces were mixed properly and 50g sample of each variety in 3 replications was kept in oven at 80°C for 72 hours (Gupta *et al*., 2015). The final dry matter content of the sample was estimated when the weight of the sample reached to a constant level.

French fries: For French fry colour score, tubers were peeled, washed and trimmed to remove defects and cut into sticks of 10 × 10 mm thickness. Sticks of more than 75mm length were selected, excess water was removed from the surface of sticks by dryer and these were continuously fried for five minutes or till the bubbling stopped at 180°C. French fry colour score was measured by using the chip colour cards on a scale of 1-10, where 1 was complete white and 10 was black/brown. French fry colour score up to 4.0 is considered acceptable (Ezekiel *et al*., 2003).

Reducing sugars (mg/100g fresh tuber weight): Three tubers were cut from bud to stem end and 10 g of chopped sample was withdrawn after proper mixing. Refluxing was done in 80% iso-propanol (50ml). The extract was filtered using whatman filter paper, evaporated until small quantity remained and volume was made to 100ml. Before estimation of reducing sugars, interfering compounds like soluble proteins were removed by precipitating using saturated potassium oxalate solution and lead acetate solution followed by centrifuging. To the supernatant (0.2 ml) alkaline copper tartarate reagent was added, content was boiled for 10 minutes, cooled and arsenomolybdate reagent (1 ml) was poured. The developed blue colour absorption was measured using spectrophotometer at 620 nm (Nelson, 1944).

Glucose (mg/100g fresh tuber weight): The tubers were washed and peeled, and 200 g sample was drawn and juicerated. Juice was collected in a beaker. Juicerator was washed three times with 100ml portions of buffer diluents (sodium phosphate pH 7.2). Quantitatively combined juice was transferred to volumetric flask and volume was made up to the mark. Samples were covered and combined extract was kept in freeze for one hour prior to analysis. Samples were taken out of freezer and allowed to incubate at room temperature for 20 minutes before analysis. Biochemistry analyser (Make: Yellow Springs Instrument Co. Inc., USA, Model: YSI 2900 series) was calibrated with 2.50 g/l glucose standard solution. Linearity of the membrane was checked by injecting glucose solution (9.00 g/l) after every twenty samples.

Data were analyzed following standard statistical procedures as described by Gomez and Gomez (1984) using the software Windostat 8.5 (Ameerpet, Hyderabad, India). Based on overall performance, the advanced hybrid MP/4-578 was recommended for release in North Indian plains by '36th AICRP on Potato Group Meeting' held during 08-11 September, 2018 at SDAU, Sardar Krushinar, Gujarat. This hybrid was named as potato variety Kufri FryoM, released and notified by the 'Central Sub-Committee on Crop Standards Notification and Release of Varieties for Horticultural Crops, Ministry of Agriculture and Farmer Welfare, Department of Agriculture and Co-operation, Government of India, New Delhi vide Communication No.3-69/2018-SD/lVdated 28th October, 2020'.

Varietal description

Salient morphological attributes of variety Kufri FryoM are described below for identification purpose.

Plant: Medium canopy semi-compact, stem medium thick, predominantly green, wings highly developed and straight.

Foliage: Grey green, leaves intermediate, leaf width medium, leaflets ovate lanceolate, leaflet coalescence absent, anthocyanin in rachis and midrib.

Flower: Flowering medium, inflorescence medium, floral stalk green, floral stalk-pedicle articulation clearly visible and located above the middle, calyx green, corolla red-violet, corolla shape pentagonal, anther yellow, anther cone normal, style longer than stamen column and stigma bi-lobed.

Tubers: Tubers (8-10), oblong, skin white-cream, eyes shallow, eyebrows normal, flesh white, texture mealy.

Sprout: Red-purple, cylindrical, pubescence at sprout base weak.

DNA finger printing: Fingerprint of hybrid, MP/04-578 was generated using 2 SSR markers viz., STU and STIKA using genetic analyzer, ABI 3500. The fingerprints are clearly unique and do not match to any of the existing indigenous varieties (**Fig. 2**).

RESULTS AND DISCUSSION

Yield performance in primary screening: In replicated trials at Modipuram, during

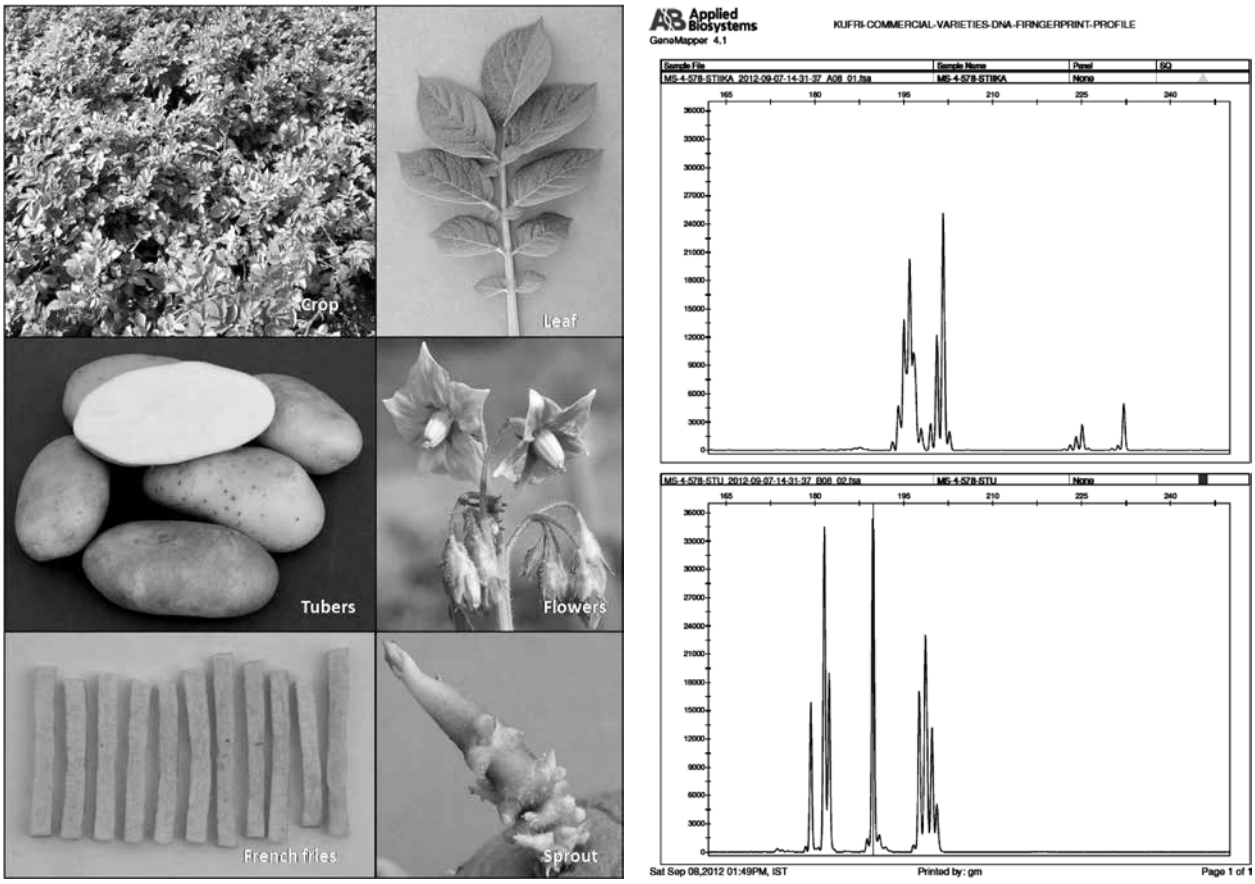

Fig. 2 Morphological features and DNA fingerprint profile of Kufri FryoM

2010-12, MP/4-578 produced higher total tuber yield (38.0 t/ha) as compared to control Kufri Frysona (33.5 t/ha) at 90 days and showed advantage of 14, 14 and 9% higher process, French fry grade and total tuber yields, respectively over control Kufri Frysona (**Table 1**). At Jalandhar, the hybrid had markedly higher process grade tuber yield (30.0 t/ha) than Kufri Frysona (25.3 t/ha) with yield advantage of 18 %, 33% and 11% for process & French fry grade and total tuber yield than Kufri Frysona at 100 days(**Table 2**). Quality parameters were in acceptable range at both the locations.

Yield performance in multi-location AICRP trials

Hybrid MP/4-578 was evaluated for four years at 16 locations (2014-18) across all regions of country, however, hybrid performed well in North-West and Central plains. The results are described below-

First year replicated AICRP trials (2014-15): Hybrid MP/4-578 recorded higher total tuber yield than Kufri Frysona in North-Western (Hisar, Jalandhar, Modipuram, Pantnagar) and Central plains (Deesa, Chhindwara and Raipur). It had significantly higher total tuber yield at Hisar, Deesa and Raipur **(Table 3)**. Mean total tuber yield of MP/4-578 (34.2 t/ha) over different locations of north-western plains and central plains was significantly higher than Kufri Frysona (31.7 t/ha).

Hybrid MP/4-578 also produced higher process grade tuber yield than Kufri Frysona in north-western (Hisar, Jalandhar, Modipuram, Pantnagar) and central plains (Deesa, Chhindwara and Raipur). It had significantly higher total tuber yield at Hisar and Modipuram, Deesa and Raipur **(Table 3)**. Mean process grade tuber yield of hybrid MP/4-578 (25.3 t/ha) over different locations of north-western plains and central plains was significantly higher than Kufri Frysona ((22.2 t/ha). It had acceptable mean tuber dry matter content (19.7%), French fry colour (3.6) and reducing sugars (153.1 mg/100 g fresh weight). **(Table 3 & 4).**

Table 1: Mean yield and quality parameters of MP/4-578 at Modipuram at 90 days (2010-12)

Genotypes	Tuber yield (t/ha)*			PGY (%)	Mean values of processing quality parameters		
	PGY	FFGY	TTY		Tuber dry matter (%)	Glucose (mg/100 g fresh weight)	French fry colour*
MP/04-578	33.9	11.3	38.3	88.3	20.1	32.5	4.8
Kufri Frysona	29.6	10.4	33.5	88.1	22.1	38.0	3.9
% increase over control	14	14	9				

*PGY: Process grade yield; FFGY: French fry grade yield; TTY: Total tuber yield, ** French fry colour score on a scale of 1-10, where up to 4 is acceptable for French fry making

Table 2: Mean yield and quality parameters of MP/04-578 at Jalandhar at 100 days (2010-12)

Genotypes	Tuber yield (t/ha)*			PGY (%)	Processing quality parameters		
	PGY	FFGY	TTY		Tuber dry matter (%)	Reducing sugars (mg/100 g fresh weight)	French fry colour**
MP/04-578	30.0	20.2	37.5	80.1	20.3	75.1	2.1
Kufri Frysona	25.3	15.2	33.8	75.2	21.1	55.5	2.2
% increase over control	18	33	11				

**PGY: Process grade yield; FFGY: French fry grade yield; TTY: Total tuber yield,* **French fry colour score on a scale of 1-10, where up to 4 is acceptable for French fry making

Table 3: Tuber yield and tuber dry matter content of hybrid MP/04-578 in replicated trials in AICRP at 90 days (2014-15)

Region	Location	Total tuber yield (t/ha)		Process grade tuber yield (t/ha)		Tuber dry matter content (%)	
		MP/4-578	Kufri Frysona	MP/4-578	Kufri Frysona	MP/4-578	Kufri Frysona
North-western plains	Hisar	30.1	26.0	24.0	21.5	19.0	18.3
	Jalandhar	35.8	34.7	22.0	21.2	20.5	21.7
	Modipuram	34.4	33.6	27.5	24.5	19.0	21.9
	Pantnagar	30.2	30.0	26.0	25.9	20.2	19.1
Central plains	Deesa	39.7	36.7	32.9	25.6	19.6	21.5
	Chhindwara	43.7	42.5	30.6	29.6	18.7	19.5
	Raipur	25.6	18.6	14.2	7.4	20.8	21.0
	Mean	34.2	31.7	25.3	22.2	19.7	20.4
% increase over controls			7.8		13.8		
	CD 0.05	Variety:0.96	Variety × location: 2.90	Variety: 0.85	Variety × location: 2.67	Variety: 0.29	Variety × locations: 0.93

Table 4: French fry colour score and reducing sugars of hybrid MP/04-578 in replicated trials in AICRP at 90 Days (2014-15)

Location	French fry colour		Reducing sugars (mg/100 g FW)	
	MP/4-578	Kufri Frysona	MP/4-578	Kufri Frysona
Hisar	3.5	4.5	154.7	148.9
Modipuram	4.5	4.4	199.5	169.5
Deesa	2.9	3.0	105.1	111.6
Mean	3.6	4.0	153.1	143.3

**French fry colour score on a scale of 1-10, where up to 4 is acceptable for French fry making

First year on-farm AICRP trial (2015-16): Hybrid MP/4-578 produced a higher total tuber yield than Kufri Frysona at Hisar, Modipuram and Chhindwara **(Table 5).** In north-western plains and central plains, hybrid MP/4-578 had comparable mean total tuber yield (25.2 t/ha) as compared to Kufri Frysona (26.4 t/ha). However, it attained higher process grade tuber yield than Kufri Frysona at Modipuram (north-western plains), and Deesa and Chhindwara (central plains). Mean process grade tuber yield of hybrid MP/4-578

Table 5: Tuber yield and tuber dry matter content of MP/04-578 in AICRP trials at 90 days (2015-16)

Region	Location	Total tuber yield (t/ha)		Process grade tuber yield (t/ha)		Tuber dry matter content (%)	
		MP/4-578	Kufri Frysona	MP/4-578	Kufri Frysona	MP/4-578	Kufri Frysona
North-western plains	Hisar	26.5	25.8	21.4	22.2	18.6	19.3
	Modipuram	32.1	27.5	25.6	20.7	19.1	20.7
Central plains	Deesa	31.2	40.6	18.7	18.1	22.0	22.8
	Chhindwara	29.2	25.8	20.4	18.0	18.8	18.9
	Raipur	18.3	22.7	7.4	10.6	21.8	21.6
	Kota	14.1	15.7	12.0	14.3	24.5	20.0
	Mean	25.2	26.4	18.8	18.4	20.8	20.6
% Yield increase		-	-	-	-	-	-
	CD 0.05		NS		NS		NS

(18.8 t/ha) over different locations of north-western and central plains was higher than Kufri Frysona (18.4 t/ha). Hybrid MP/4-578 (**Table 5 & 6**) recorded acceptable mean tuber dry matter content (20.8%), French fry colour (3.5) and reducing sugars (144.6 mg/100 g fresh weight).

Second year on-farm AICRP trial (2016-17): Hybrid MP/4-578 produced significantly higher total tuber yield **(Table 7)** than Kufri Frysona at Hisar, Modipuram and Pantnagar (north-western plains) and at Kanpur, Chhindwara and Gwalior (central plains). Mean total tuber yield of the hybrid (35.6 t/ha) over different locations of north-western and central plains was significantly higher than control Kufri Frysona (31.6 t/ha). Hybrid MP/4-578 also had significantly higher process grade tuber yield than Kufri Frysona at Hisar, Modipuram and Pantnagar (north-western plains) and at Kanpur and Chhindwara (central plains). Mean process grade tuber yield of the hybrid (30.3 t/ha) over different locations of north-western and central plains was higher than Kufri Frysona (26.1 t/ha). Hybrid MP/4-578 recorded acceptable mean tuber dry matter content (19.4%), French fry colour score (2.9) and reducing sugars (144.5 mg/100 g fresh weight) **(Table 7 & 8)** in multi-location trials.

Advanced varietal AICRP trials (2017-18): In north-western plains, hybrid MP/4-578 had significantly higher (9%) mean total tuber yield (39.4 t/ha) than Kufri Frysona (35.2 t/ha). It recorded significantly higher total tuber yield at Hisar, while statistically at par yield at Modipuram and Pantnagar **(Table 9)**. Hybrid MP/4-578 also produced significantly higher (21%) mean process grade tuber yield than Kufri Frysona. It attained significantly higher process grade

Table 6: Processing quality attributes of MP/04-578 in AICRP trials at 90 days (2015-16)

Location	French fry colour *		Reducing sugars	
	MP/4-578	Kufri Frysona	MP/4-578	Kufri Frysona
Chhindwara	3.5	5.0	174.3	160.8
Gwalior	2.0	2.3	91.2	79.4
Modipuram	5.0	4.8	168.4	145.7
Mean	3.5	4.0	144.6	128.6

*French fry colour score on a scale of 1-10, where up to 4 is acceptable for French fry making

Table 7: Tuber yield and tuber dry matter content of MP/04-578 in AICRP trials at 90 days (2016-17)

Region	Location	Total tuber yield (t/ha)		Process grade tuber yield (t/ha)		Tuber dry matter content (%)	
		MP/4-578	Kufri Frysona	MP/4-578	Kufri Frysona	MP/4-578	Kufri Frysona
North-western plains	Hisar	39.8	34.3	36.2	30.2	20.0	21.0
	Modipuram	39.2	34.5	31.2	23.4	18.0	18.8
	Pantnagar	31.2	26.4	30.7	25.8	17.3	16.4
Central plains	Kanpur	30.7	27.6	27.5	24.6	20.0	21.0
	Chhindwara	34.5	30.8	32.5	29.2	18.7	18.6
	Gwalior	38.2	36.2	23.7	23.1	22.3	20.1
	Mean	35.6	31.6	30.3	26.1	19.4	19.3
% Yield increase			12.5		16.3		
CD 0.05			1.37		1.52		1.52

Table 8: Processing quality attributes of MP/04-578 in AICRP trials at 90 days (2016-17)

Region	Location	French fry colour		Reducing sugars	
		MP/4-578	Kufri Frysona	MP/4-578	Kufri Frysona
North-western plains	Hisar	2.0	4.5	112.4	148.9
	Modipuram	2.4	4.8	120.9	109.7
	Pantnagar	3.3	2.3	-	-
Central plains	Chhindwara	3.4	3.6	-	-
	Gwalior	2.3	2.8	108.5	120.7
	Kanpur	4.0	2.5	236.2	110.4
Mean		2.9	3.4	144.5	122.4

Table 9: Tuber yield of MP/04-578 in AICRP trials at 90 days (2017-18)

Region	Location	Total tuber yield (t/ha)			Process grade tuber yield (t/ha)		
		MP/4-578	Kufri Chipsona-1	Kufri Frysona	MP/4-578	Kufri Chipsona-1	Kufri Frysona
North-western plains	Hisar	38.9	28.7	30.8	35.0	25.5	27.6
	Jalandhar	29.5	32.0	31.3	12.8	13.6	13.6
	Modipuram	49.7	44.8	46.4	26.7	21.4	20.5
	Mean	39.4	35.2	36.2	24.8	20.2	20.6
	% Yield increase		12.0	8.8		23.0	20.8
	CD 0.05	Variety: 2.6; Location × Variety: 4.51			Variety: 2.49; Location × Variety: 4.32		
Central plains	Chhindwara	42.4	36.9	41.2	31.8	27.7	30.9
	Deesa	34.1	34.4	39.2	25.1	14.9	22.0
	Gwalior	29.6	26.1	29.7	24.4	19.9	23.5
	Kanpur	35.8	32.8	25.5	19.2	26.4	18.7
	Kota	23.5	20.8	17.7	21.3	18.8	15.8
	Raipur	20.3	11.4	15.3	19.7	12.0	14.5
	Mean	31.0	27.1	28.1	23.6	20.0	20.9
	%Yield increase		14.3	10.3		18.2	12.9
	CD 0.05	Variety: 1.53; Location × Variety: 3.75			Variety: 1.08; Location × Variety: 2.65		

tuber yield at Hisar and Modipuram and comparable yield at Pantnagar. The hybrid possessed acceptable mean tuber dry matter content (20.8%), French fry colour score (3.1) and reducing sugars (165.2 mg/100 g fresh tuber weight) in this region **(Table 9 & 10)**.

In central plains, hybrid MP/4-578 recorded significantly (10%) higher mean total tuber yield (31.0 t/ha) over control Kufri Frysona (28.0 t/ha). It had significantly higher total tuber yield at Kanpur, Kota and Raipur, while remaining at par at Chhindwara and Gwalior **(Table 9)**. It also produced significantly higher (13%) mean process grade tuber yield (24.0 t/ha) over Kufri Frysona (21.0 t/ha). Significantly higher process grade tuber yield was observed at Deesa, Kota and Raipur, whereas it was at par at Chhindwara, Gwalior and Kanpur. Hybrid MP/4-578 had acceptable mean tuber dry matter content (20.7%), French fry colour score (2.5) and reducing sugars (114.2 mg/ per 100 g fresh weight) in this zone **(Table 9 &11)**.

Table 10: Processing quality attributes of MP/04-578 at 90 days in North- western plains (2017-18)

Genotypes	HIS*	JAL*	MDP*	Mean
	Tuber dry matter content (%)			
MP/4-578	20.08	22.38	20.07	20.84
Kufri Chipsona-1	20.20	24.00	21.07	21.75
Kufri Frysona	19.97	23.38	21.70	21.68
Mean	20.13	23.17	20.74	
CD 0.05	Location=0.45; Genotype =0.52; Location × Genotype= 0.89			
	French fry colour score			
MP/4-578	3.0	2.4	4.0	3.1
Kufri Chipsona-1	1.0	1.6	3.4	2.0
Kufri Frysona	3.2	2.2	4.8	3.4
Mean	2.4	2.1	4.1	
	Reducing sugars (mg/100g Fresh weight)			
MP/4-578	112.0	232.7	151.0	165.2
Kufri Chipsona-1	130.0	98.3	117.0	115.1
Kufri Frysona	134.0	297.3	148.0	193.1
Mean	125.3	209.4	138.7	

*HIS: Hisar, JAL: Jalandhar, MDP: Modipuram

Table 11: Processing quality attributes of MP/04-578 in Central plains (2017-18)

Genotypes	CHN*	DES*	GWL*	KAN*	KTT*	RPR*	Mean
		Tuber dry matter content (%)					
MP/4-578	18.75	19.33	21.93	21.38	22.18	20.68	20.71
Kufri Chipsona-1	18.08	19.40	22.34	20.50	21.80	19.83	20.32
Kufri Frysona	19.15	21.87	23.35	22.23	21.68	19.71	21.33
Mean	18.54	20.83	22.62	20.87	21.96	20.60	
CD 0.05		Location= 0.67; Genotype = 0.55; Location × Genotype= 1.35					
		French fry colour score					
Hybrid/ Variety	CHN*	DES	GWL	KAN			Mean
MP/4-578	1.0	2.4	1.5	5.2			2.5
Kufri Chipsona-1	1.1	1.4	1.4	3.0			1.7
Kufri Frysona	1.0	3.0	2.0	4.0			2.5
Mean	1.0	2.3	1.6	4.1			
		Reducing sugars mg/100g Fresh weight					
MP/4-578	97.7	134.3	84.3	140.3			114.2
Kufri Chipsona-1	70.3	89.9	89.1	120.1			92.4
Kufri Frysona	73.2	108.1	128.5	120.0			107.5
Mean	80.4	110.8	100.6	126.8			

*CHN: Chhindwara, DES: Deesa, GWL: Gwalior, KAN: Kanpur, KTT: Kota and RPR: Raipur

Foliage senescence (%): During 2014-18, hybrid MP/04-578 had higher mean overall foliage senescence (80%) at 90 days at all the locations and crop seasons in comparison to control Kufri Frysona, which had 72% mean foliage senescence (**Table 12**).

Pooled performance of MP/4-578 in AICRP trials:

In north-western plains, hybrid MP/4-578 yielded 10 and 15% higher total and process grade tuber yield (34.5 and 26.5 t/ha) over the control Kufri Frysona (31.4 and 23.0 t/ha), respectively at 90 days **(Table 13)**. The hybrid had acceptable mean tuber dry matter content (20%), French fry colour score (3.7) and reducing sugars (156 mg/100 g fresh weight).

In central plains, Hybrid MP/4-578 yielded 6 and 11% higher total and process grade tuber yield (31.3 and 23.0 t/ha) than Kufri Frysona (26.6 and 20.7 t/ha), respectively at 90 days **(Table 13)**. The hybrid recorded acceptable mean tuber dry matter content (21.0 %), French fry colour score (2.9) and reducing sugars (128 mg/100 g fresh weight)

Overall mean performance in AICRP: Mean values of north-west and central plains revealed that hybrid, MP/4-578 yielded 8 and 13% higher total and process grade tuber yield (32.9 and 24.8 t/ha) as compare to control Kufri Frysona (30.5 and 21.9 t/ha) at 90 days **(Table 13)**. It had acceptable mean tuber dry matter content (20.1%), French fry colour score (3.3) and reducing sugars (142 mg/100 g fresh weight). This hybrid possessed very good storability under ambient storage owing to a longer dormancy period (>6 weeks), lesser rotting, physiological weight loss and total weight loss (11.5%) at 75 days after storage (**Table 13**).

STORAGE BEHAVIOUR

Hybrid MP/04-578 exhibited better keeping quality as compared to control Kufri Frysona during storage at room temperature (**Table 14**). The hybrid was also found suitable for French fry making even after 8 months of long-term storage at 10-12°C with CIPC fogging (**Table 15**).

Industrial Testing

Hybrid MP/4-578 was developed primarily for its use in processing by the French fry industries; therefore, it was evaluated and tested at McCain Foods, Mehsana (Gujarat), Fresh-O-Veg, Indore (Madhya Pradesh) and at Pepsico India Pvt Ltd, Burdwan (West Bengal) during 2012-13. Mean performance (**Table 16**) in processing industry evaluation indicated 20, 23 and 12% superiority of MP/4-578 in process grade, French fry grade and total yield, respectively (40.5, 32.2, 45.4 t/ha) over the control variety K. Frysona (33.6, 26.1, 40.5 t/ha). MP/4-578 has shown ability to produce 90% process grade tubers as compared to

Table 12: Foliage senescence (%) of MP/04-578 at 90 days (2014-15 to 2017-18)

Location	MP/4-578	Kufri Frysona	MP/4-578	Kufri Frysona	MP/4-578	Kufri Frysona
	2014-15		2016-17		2017-18	
Modipuram	60.0	52.6	66.0	50.0	67.0	53.3
Chhindwara	91.3	90.3	83.8	81.5	84.5	83.5
Deesa	100.0	40.0	-	-	72.6	67.6
Kota	-	-	76.0	77.0	81.0	82.0
Raipur	92.0	93.0	90.0	90.0	-	-
Mean	85.8	69.0	79.0	74.6	76.3	71.6

Table 13: Pooled performance of MP/04-578 in AICRP trials at 90 days (pooled over locations*)

Year	MP/4-578					Kufri Frysona				
	North-western plains (HIS, JAL, MOD, PNT)*									
	TTY	PGY	DM%	FFC	RS	TTY	PGY	DM	FFC	RS
2014-15	32.6	24.9	19.7	4.0	177.1	31.1	23.3	20.3	4.5	159.2
2015-16	29.3	23.5	18.9	5.0	168.4	26.7	21.5	20.0	4.8	145.7
2016-17	36.7	32.7	18.4	2.5	114.8	31.7	26.5	18.7	3.4	129.3
2017-18	39.4	24.8	20.8	3.1	165.2	36.2	20.6	21.7	3.4	193.1
Mean	34.5	26.5	19.5	3.7	156.4	31.4	23.0	20.2	4.0	156.8
% yield increase						10				
	Central plains (CHN, DES, GWL, KAN, RPR, KTT)*									
2014-15	36.3	25.9	19.7	2.9	105.0	32.6	20.9	20.7	3.0	112.0
2015-16	23.2	14.6	21.8	2.8	132.8	26.2	15.3	20.8	3.7	120.1
2016-17	34.5	27.9	20.3	3.3	160.2	31.5	25.6	19.9	3.7	142.1
2017-18	31.0	23.6	20.7	2.5	114.2	28.1	20.9	21.3	2.5	107.5
Mean	31.3	23.0	20.6	2.9	128.1	29.6	20.7	20.7	3.2	120.4
% yield increase						6	11			
	Pooled over north-west and central plains									
2014-15	34.5	25.4	19.7	3.5	141.1	31.9	22.1	20.5	3.8	135.6
2015-16	26.3	19.1	20.4	3.9	150.6	26.5	18.4	20.4	4.3	132.9
2016-17	35.6	30.3	19.4	2.9	137.5	31.6	26.1	19.3	3.6	135.7
2017-18	35.2	24.2	20.8	2.8	139.7	32.2	20.8	21.5	3.0	150.3
Mean	32.9	24.8	20.1	3.3	142.3	30.5	21.9	20.5	3.6	138.6
% yield increase						8	13			

*HIS-Hisar, JAL-Jalandhar, MOD-Modipuram, PNT-Pantnagar, CHN-Chhindwara, DES-Deesa, GWL-Gwalior, KAN-Kanpur, KTT-Kota, RPR-Raipur
TTY: Total tuber yield/ha, PGY: Process grade tuber yield/ha, DM: Tuber dry mater content (%), FFC: French fry colour score, RS: Reducing sugars mg/100g fresh weight of potato

Table 14: Storage behaviour of MP/04-578 under ambient conditions at Modipuram (2010-11 to 2017-18)

Genotypes	Dormancy	% sprouting		Weight loss in sprouting (%)	Rottage (%)	Physiological weight loss (%)	Total weight loss (%)
		45 days	75 days				
MP/04-578	> 6 weeks	50.6	62.0	0.1	0.2	10.8	11.0
Kufri Frysona	> 6 weeks	34.9	50.8	0.1	0.6	13.4	14.1

Table 15: Performance of MP/04-578 under CIPC long term storage at McCain Foods, Mahesana, Gujarat (2012-13)

Sample Date	Genotypes	USDA Fry Color					Sugar End	Fry Index	Avg. Index	Sugar End
		0	1	2	3	4				
15-06-13	MP/04-578	12	7	1	0	0	0	1.5	-	-
15-06-13	Kufri Frysona	16	2	1	1	0	0	1.4	-	-
22-08-13	MP/04-578	17	3	0	0	0	0	1.2	-	-
22-08-13	Kufri Frysona	19	1	0	0	0	0	1.1	-	-
14-11-13	MP/04-578	19	0	1	0	0	1	1.1	-	-
14-11-13	Kufri Frysona	12	7	1	0	0	0	1.5	1.3	0.5

Table 16: Performance of MP/04-578 in trials with processing industry

Genotypes	Yield (t/ha)*			% PGY	Fry colour (Visual)	Fry colour (USDA)	DM (%)*
	PGY	FFGY	TTY				
McCain Foods, Mehsana, Gujarat (100 days)							
MP/4-578	52.1	38.0	57.6	90.5	1.5	0	20.60
K Frysona	42.4	30.8	50.6	83.9	1.5	0	21.30
CD 0.05	2.5	2.3	2.7				
% Yield increase	23	23	14	8			
Fresh-O-Veg, Indore, Madhya Pradesh (100 days)							
MP/4-578	30.1		32.0	93.9	1.5		22.0
K Frysona	24.3		27.0	90.2	1.8		22.2
CD 0.05	3.0		2.8				
% Yield increase	24		19				
Pepsico India Pvt Ltd, Burdwan, West Bengal (95 days)							
MP/4-578	39.2	26.3	46.5	84.4	2.0		21.1
K Frysona	34.1	21.4	44.0	77.6	2.5		22.8
CD 0.05	2.7	1.9	2.8				
% Yield increase	15	23	6	9			
Mean performance with processing industry (2012-13)							
MP/4-578	40.5	32.2	45.4	89.6	1.7	0.0	21.2
K Frysona	33.6	26.1	40.5	83.9	1.9	0.0	22.1
% Yield increase	20.4	23.2	11.9				
Additional traits at Pepsico India Pvt Ltd, Burdwan, West Bengal (95 days)							
Genotypes	Gross solid (%)	GCD*	DB*	UC*	ID*	ED*	TPoD*
MP/4-578	21.07	0	0	1	1.5	3	5.5
K Frysona	22.77	0	0	0	6.5	5	11.5

*PGY: Process grade yield, FFGY: French fry grade yield, TTY: Total tuber yield, DM: Tuber dry matter content, GCD: Gross Color Defects; DB: Dried Bruise; UC: undesirable Color; ID: Internal Defects; ED: External Defects; TPoD: Total Potato Defects.

Kufri Frysona (84%). The hybrid possessed >21% tuber dry matter, 1.5 French fry colour score and other quality traits comparable to control Kufri Frysona. French fries made from French fry grade tubers of hybrid MP/4-578 were excellent and met the standards of all required quality traits.

Disease resistance

Late blight: Hybrid, MP/04-578 was evaluated for two years under natural epiphytotic conditions at Kufri for late blight resistance. The plant foliage was found to be field resistant (AUDPC 158) to all races of late blight (*Phytophthora infestans*) compared to best control Kufri Chipsona-1 (AUDPC 213) and other indigenous and exotic processing controls

Potato Virus Y: Potato Virus Y (PVY, Potyvirus) is the most damaging potato virus that causes severe yield reduction up to 80% alone or in combination of other viruses. Breeding PVY resistant cultivars through introgression of resistance (Ry) gene is the best strategy to overcome this problem. The Ry_{adg} gene derived from *Solanum tuberosum*

ssp *andigena* provides extreme resistance to PVY. The hybrid possesses, Ry_{adg} gene (**Fig 3**) which imparts durable resistance against Potato Virus Y (PVY).

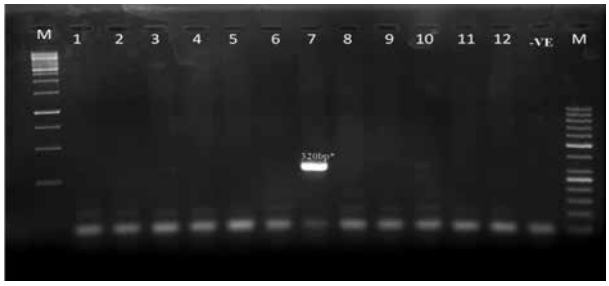

Fig. 3: Presence of R_{yadg} gene (320bp) in hybrid, MP/04-578 (Lane 7)

Agronomic management

Optimum tuber yield of hybrid MP/4-578 could be obtained by adopting a standard agronomical schedule for medium maturing varieties. In north-western and central plains, it could be planted as main crop during 15 October to 5 November when maximum day time temperature falls around 32°C by adopting recommended seed rate of 3.5-4.0 t/ha consisting seed size tubers (40-60 g). This hybrid requires more space, so plant spaced at 20-25 cm in 66 cm rows had optimum tuber size distribution for production of maximum proportion of French fry and process grade tubers. As far as nutrient management is concerned, generally 135 N-40 P_2O_5-100 K_2O kg/ha should be band placed at the time of planting and remaining half dose of 135 kg N/ha should be applied 20-25 days after planting at earthing-up. At Modipuram, nitrogen, phosphorous and potassium levels of 270, 40 and 100 kg/ha, respectively were found optimum for the production of maximum proportion of French fry grade tubers. Nutrient management in other agro-ecologies may differ and thus needs to be fine-tuned. Otherwise, regional recommendations may be followed for optimum productivity of this hybrid. Inter-culture and earthing-up operation is recommended to provide aeration in root zone, improving nitrogen use and controlling second flush of weeds. This is done after 22-25 days of planting when the crop is 8-12 cm in height. Cartap hydrochloride 4G @ 20 kg/ha should be applied during earthing-up for management of cutworms, white grubs, beetles and leaf-eating caterpillars. It will also take care of sucking pest like leafhopper and aphids. In case of seed crop, yellow sticky traps (15 × 30 cm^2 size) may be placed just above the canopy height @ 60 traps/ha at equidistance from each other for mass trapping of whiteflies/aphids. Seed treatment with imidacloprid (200SL) @ 0.04% (4 ml/10 l water) for 10 minutes before planting should be adopted. First spray of imidacloprid (200SL) @ 0.03% (3 ml/10 l water) should be done at 85% of crop emergence. Second spray of thiamethoxam (25 WG) @ 0.05% (5 gm/10 l water) should be carried out 10-15 days after the first spray. Integrated management schedule should be adopted for late blight. Prophylactic spray just at the time of canopy closure should be given with mancozeb or propineb or chlorothalonil @ 0.2% (2 g/l of water). If disease appears then need based application of cymoxanil + mancozeb or dimethomorph + mancozeb or fenamidone + mancozeb @ 0.3% (3 g/l of water) is followed for effective management of late blight. Irrigation is stopped 12-15 days before haulm killing. Crop is harvested 12-15 days after haulm cutting. It is left in the field for 2-3 hours for air drying. Tubers are kept in heaps (1.5m height) for 10-15 days in shade for skin curing. For seed, treatment is done after harvest of seed crop and before cold storage. Seed tubers are washed in clean water and treated with 3% boric acid for 25-30 minutes. Afterwards, tubers are dried in shaded place before storage. Solution once prepared can be used for 20 times dipping. This is effective in management of black scurf and common scab.

Adaptability

Hybrid MP/4-578 named as cv. Kufri FryoM has been recommended for Punjab, Haryana and western Uttar Pradesh (north-western plains), and Madhya Pradesh, Chhattisgarh, Gujarat and Rajasthan (central plains). This variety can provide raw material for processing industry a longer period and can be an apt choice for making French Fries due to its higher yield in shorter growing period, field resistance to late blight, acceptable quality traits, attractive oblong tubers, long tuber dormancy and better shelf life under CIPC storage.

Usage

The new potato variety Kufri FryoM is suitable for the production of raw material for processing into crispy French fries. Variety Kufri FryoM is likely to be preferred by the processors for its white oblong tubers, white flesh, shallow eyes, pleasant flavour, mealy texture and expected lower peeling losses.

CONCLUSION

Kufri FryoM (MP/4-578) has performed well in multi-location trials conducted under AICRP on potato in north and central Indian plains. Therefore, it can be grown successfully for increasing productivity and diversified utilization of potatoes for making French fries in these areas. Kufri FryoM can provide one more alternative genotype to fast-growing processing industry and the potato farmers with white-cream skin oblong tubers, shallow eyes, white flesh, excellent keeping and culinary quality with field resistance to late blight and tolerance to potato virus Y in addition to higher process grade and total tuber yields in comparatively shorter duration.

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KUFRI SANGAM: A HIGH YIELDING DUAL PURPOSE POTATO VARIETY FOR TABLE AND PROCESSING

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ABSTRACT: Kufri Sangam is a medium maturing, main season high yielding dual purpose potato variety suitable for cultivation in northern plains (for table use) and central plains (for processing and table use). It is a clonal selection from a cross between Kufri Himsona × Kufri Pukhraj. It has compact, vigorous and medium-tall plants. It produces attractive white-cream, ovoid tubers with shallow eyes and cream flesh. It possesses 18-20% tuber dry matter, low reducing sugars (<150mg/100 g fresh weight), mealy texture, very good taste. It produces >90 marketable and >80% processing grade tuber yield and is capable of yielding 35-40 t/ha under optimum agronomical practices. Kufri Sangam yields higher than the presently grown popular table variety Kufri Bahar and processing varieties viz., Kufri Chipsona-3 and Kufri Frysona. Kufri Sangam is moderately resistant to late blight and has excellent keeping quality under country storage conditions with over 10 weeks dormancy period.

KEYWORDS: Potato, Kufri Sangam, dual purpose variety, high yield, late blight resistance, keeping quality, northern and central plains

INTRODUCTION

Potato is the third most important food crop in the world after rice and wheat. There has been phenomenal growth in production and productivity of potato in India. Besides best management practices, the key for sustaining higher production and productivity lies in superior genotypes and diversified utilization. During last two decades focus has been to develop varieties with higher yield, resistant to diseases along with superior quality and storability traits. To meet the demand of burgeoning population, India would be requiring about 125 million tones of potato in the year 2050 (Singh *et al* 2014). The demand for processed potato products from last two decades in the country has increased many fold and several Indian and multinational entrepreneurs have established chips, French fries and flakes making units and several companies are coming-up in the field of diversified potato and potato based products including starch manufacturing (Gupta *et al* 2020a). Potatoes' having multiple traits to suit fresh market consumption as well as for processing market are the most sought after objectives of now a day's breeding programme. To get the right combination of characteristics in one variety takes a large investment in time and resources. The sincere effort directed in this regards enabled identification of Kufri Sangam having high yield, quality, storability and disease resistance to late blight than to existing varieties like Kufri Bahar (table variety) and Kufri Chipsona-3 and Kufri Frysona (processing varieties).

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MATERIALS AND METHODS

Advanced stage hybrid MP/6-39 christened as Kufri Sangam originated from hybridization of cross between Kufri Himsona × Kufri Pukhraj (Fig. 1). The hybridization was done in 2006 at Kufri (32°N 77°E; 2501 m above MSL) in the hills. The pedigree of MP/6-39 is described in figure-1. The female parent Kufri Himsona is an indigenous processing variety produces white-cream ovoid, shallow eyed tubers with cream flesh and possessed resistance to late blight; whereas male parent Kufri Pukhraj an indigenous popular table potato variety produces yellow, ovoid tubers with shallow eyes and light yellow flesh.

The seedling and subsequent clonal stages were raised and evaluated (Gupta *et al*., 2020) at ICAR-Central Potato Research Institute, Campus, Modipuram, Meerut (29° N and 77.7° E; 300 masl). The clone MP/6-39 was in seedling stage in 2006-07, the five-hill plot in 2007-08, 30 hill plot in 2008-09, multiple-row trial in 2009-10 and in replicated yield trials during 2010-2011 to 2014-2015 at Modipuram, Jalandhar, Amirgarh, Indore and Burdhwan. Based on consistently superior performance over control varieties in replicated yield trials, the hybrid was introduced in All India Coordinated Research Project on Potato (AICRP on Potato) in 2015 for multi-location testing across the country and also evaluation and testing of hybrid at industrial conditions during 2015-16. In multi-location initial varietal trial (IVT)/advanced varietal trial (AVT) during 2015-2016 to 2018-2019, the hybrid was evaluated at 17 locations in four regions, however, MP/6-39 performed well in North (4 locations-Hisar, Jalandhar, Modipuram and Pantnagar) and Central plains (6 locations-Chhindwara, Deesa, Gwalior, Kanpur, Kota, Raipur) of the country.

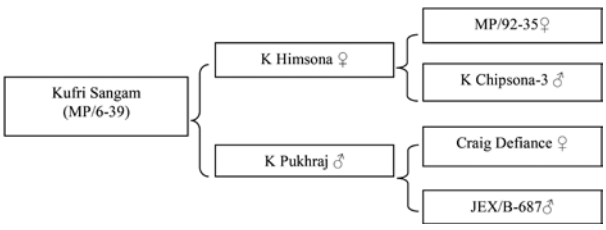

Fig. 1. Pedigree of Kufri Sangam

Estimation of quality parameters

Tuber dry matter: Samples of 5 randomly drawn tubers were used for dry matter estimation. The tubers were chopped into small pieces. The chopped pieces were mixed properly and 50g sample of each variety in 3 replications was kept in oven at 80° C for 72 hours (Gupta *et al*., 2015).The final dry content of the sample was estimated when the weight of the sample reached to a constant level.

French fries: The tubers were peeled, washed and trimmed to remove defects and cut into sticks of 10 × 10 mm thickness. Sticks of more than 75mm length were selected, excess water was removed from the surface of sticks by dryer and these were continuously fried for five minutes or till the bubbling stopped at 180°C. French fry colour score was measured by using the chip colour cards on a scale of 1-10, where 1 was complete white and 10 was black/brown. French fry colour score up to 4.0 is considered acceptable (Ezekiel *et al*., 2003).

Reducing sugars (mg/100g fresh tuber weight): Three tubers were cut from bud to stem end and 10 g of chopped sample was withdrawn by after proper mixing. Refluxing was done in 80% iso-propanol (50ml). The extract was filtered using whatman filter paper, evaporated until small quantity remained and volume was made to 100ml. Before estimation of reducing sugars, interfering compounds like soluble proteins were removed by precipitating using saturated

potassium oxalate solution and lead acetate solution followed by centrifuging. To the supernatant (0.2 ml) alkaline copper tartarate reagent was added, content was boiled for 10 minutes, cooled and arsenomolybdate reagent (1 ml) was poured. The developed blue colour absorption was measured using spectrophotometer at 620 nm (Nelson, 1944).

Glucose (mg/100g fresh tuber weight): The tubers were washed and peeled, and 200 g sample was drawn and juicerated and juice was collected in a beaker. Juicerator was washed three times with 100ml portions of buffer diluents (sodium phosphate pH 7.2). Quantitatively combined juice was transferred to volumetric flask and volume was made up to the mark. Samples were covered and combined extract was kept in freeze for one hour prior to analysis. Samples were taken out of freezer and allowed to incubate at room temperature for 20 minutes before analysis. Biochemistry analyser (Make: Yellow Springs Instrument Co. Inc., USA, Model: YSI 2900 series) was calibrated with 2.50 g/l glucose standard solution. Linearity of the membrane was checked by injecting the glucose solution (9.00 g/l) and after every twenty samples.

The data were analyzed following standard statistical procedures as described by Gomez and Gomez (1984) using the software Windostat 8.5 (Ameerpet, Hyderabad, India). Based on its performance, the advanced hybrid MP/6-39 was recommended for release in north and central Indian plains by 38th AICRP Potato group meeting held during 03-4 September, 2019 at JNKV, Jabalpur, Madhya Pradesh. The hybrid MP/6-39 has been christened as potato variety Kufri Sangam and it was released and notified by the Central Sub-Committee on Crop Standards Notification and Release of Varieties for Horticultural Crops, Ministry of Agriculture, Department of Agriculture and Co-operation**, Government of India, New Delhi vide No.3-69/2018-SD'lVdated 28th October, 2020.**

Varietal description

For identification purpose, the salient morphological attributes (**Fig 2**) of variety Kufri Sangam are described below-

Plant: Medium, plant canopy compact, stem medium thick, predominantly green, wings highly developed and straight.

Foliage: Grey green, leaves large, leaf width medium, leaflets ovate lanceolate, leaflet

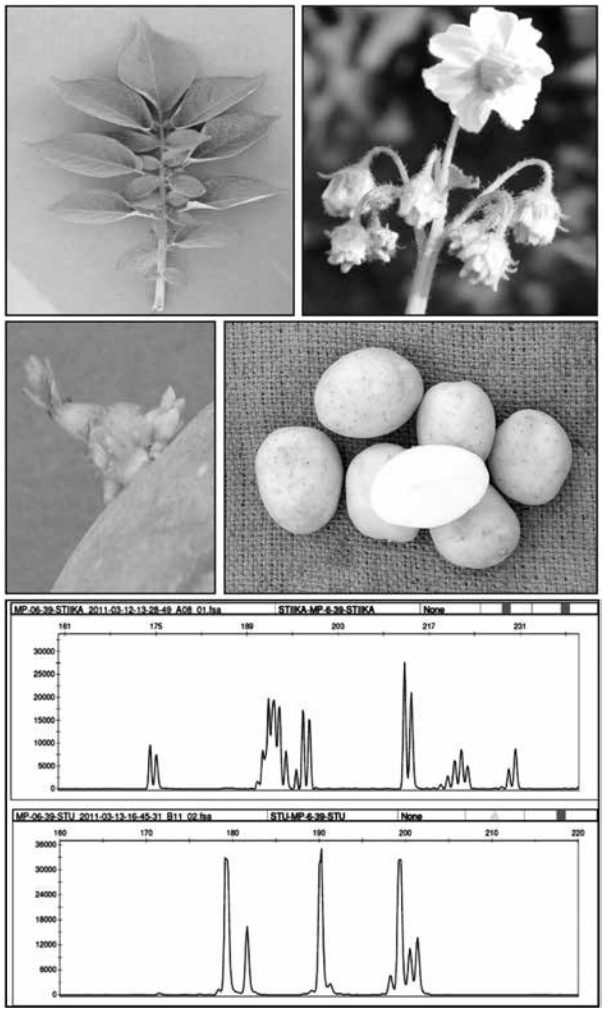

Fig.2 Morphological features and DNA fingerprint profile of Kufri Sangam

coalescence absent, anthocyanin in rachis and midrib not present.

Flower: Flowering medium, inflorescence small, floral stalk green, floral stalk-pedicle articulation clearly visible and located above the middle, calyx green, corolla white, corolla shape pentagonal, anther orange, anther cone normally developed, stylar length longer than stamen column and stigma bi-lobed.

Tubers: Tubers (10-12), ovoid, skin white-cream, eyes shallow, eyebrows normal, flesh cream, texture mealy.

Sprout: Sprout pink, shape spherical, pubescence at sprout base is weak.

RESULTS AND DISCUSSION

Yield performance in yield trials: Advanced stage hybrid MP/6-39 consistently out-yielded the controls Kufri Chipsona-3 at 90 days in replicated yield trials during 2012-2013 to 2014-2015 at Modipuram, (Uttar Pradesh) and Jalandhar (Punjab) and in industrial testing with French fry industry McCain Foods, Mahesana **(**Amirgarh, Gujarat), chips industry Pepsico facility **(**Burdwan, West Bengal) and chip grade potato supplier M/s Fresh-O-Veg (Indore Indore, Madhya Pradesh). MP/6-39 produced higher processing grade (37 t/ha; 28%) and total tuber yield (43 t/ha; 22%) than the best control Kufri Chipsona-3 (29 and 35 t/ha) at 90 days **(Table 1)**. MP/6-39 also showed superiority for processing tuber (39%) and total tuber yield (33%) than the French fry variety Kufri Frysona (27 and 32 t/ha) at 90 days (Gupta *et al*., 2016 and Gupta *et al.,* 2019). It has acceptable chip colour (2.8) and 19% tuber dry matter content **(Table 2)**

Yield performance in AICRP trials: The hybrid MP/6-39 was evaluated for four years in replicated yield (2015-2016) and advanced varietal trials (2016-2017, 2017-2018 and 2018-2019). The results on performance of MP/6-39 are presented below

Initial varietal trial (2015-16): MP/6-39 produced a significantly higher or at par total tuber yield **(Table 3)** than the best control Kufri Chipsona-3 at Hisar,

Table 1. Yield performance of advanced stage hybrid MS/6-39 during 2012-2013 to 2014-2015 in yield trials

Genotypes	Processing grade yield (t/ha) at 90 days					
	UP*	PNB	GUJ	WB	MP	Mean
MP/6-39	33.4	34.7	43.8	46.3	26.7	37.0
Kufri Chipsona-3	25.1	28.7	36.5	33.1	20.8	28.9
Kufri Frysona	23.7	26.6	29.9	31.2	22.4	26.7
Atlantic	26.2	21.6	35.7	33.5	16.8	26.8
% increase over Kufri Chipsona-3						28.1
Genotypes	Total tuber yield (t/ha) at 90 days					
	UP	PNB	GUJ	WB	MP	Mean
MP/6-39	40.7	39.7	46.7	55.5	31.4	42.8
Kufri Chipsona-3	31.4	36.3	43.1	39.7	24.5	35.0
Kufri Frysona	29.3	33.7	34.1	37.1	26.2	32.1
Atlantic	29.7	25.4	38.1	36.5	19.0	29.7
% increase over Kufri Chipsona-3						22.3

*Modipuram, UP (2012-13 to 2014-15), Jalandhar, Punjab (2013-14 to 2014-15), Burdhwan, WB (2012-2013), Amirgarh, Gujarat and Indore, Madhya Pradesh (2012-13 and 2014-15)

Table 2. Dry matter content and chip colour of advanced processing hybrid during 2012-13 to 2014-2015

Genotype	Tuber dry matter (%)					
	UP*	PNB	GUJ	WB	MP	Mean
MP/6-39	18.6	18.7	18.8	18.2	21.2	19.1
Kufri Chipsona-3	21.6	22.5	22.3	21.0	21.8	21.8
Kufri Frysona	21.2	20.9	22.0	22.0	22.4	21.7
Atlantic	20.3	22.2	19.8	21.1	22.3	21.1
Genotype	French fry colour					
	UP	PNB	GUJ	WB	MP	Mean
MP/6-39	4.7	1.7	1.3	3.3	3.2	2.8
Kufri Chipsona-3	3.8	1.6	1.8	3.3	3.0	2.7
Kufri Frysona	3.2	2.7	1.3	3.0	2.4	2.5
Atlantic	3.4	1.7	1.8	2.8	2.3	2.4

Table 3. Performance of MP/06-39 at 90 days in initial varietal trials (2015-2016)

Region	Locations	Total yield (t/ha)			Processing grade tuber yield (t/ha)		
		MP/6-39	Atlantic	K Chip-3	MP/6-39	Atlantic	K Chip-3
Northern Plains	Hisar	43.1	19.5	25.5	33.0	17.5	21.1
	Modipuram	38.5	24.9	32.2	29.3	21.0	24.3
Central Plains	Chhindwara	36.2	27.0	30.6	25.3	18.9	21.3
	Deesa	34.9	23.1	34.0	29.1	18.0	29.1
	Gwalior	28.8	26.5	29.4	12.0	15.1	10.1
	Kota	13.7	10.9	12.7	11.2	9.7	11.3
	Mean	32.5	22.0	27.4	23.3	16.7	19.5
% Yield increase			48.0	18.7		39.6	19.4
$CD_{0.05}$	-	Variety:1.17 Variety × locations: 3.31			Variety: 0.96 Variety × locations: 2.7		

Modipuram (Northern plains), Chhindwara, Deesa, Gwalior and Kota (Central plains) The mean total tuber yield of MP/6-39 (33 t/ha) over different locations of northern plains and central plains were significantly higher (19%) than the best control Kufri Chipsona-3 (27 t/ha). MP/6-39 also produced a significantly higher or at par processing grade tuber yield than the best control Kufri Chipsona-3 at Hisar and Modipuram (Northern plains), Chhindwara, Deesa and Kota (Central plains). The mean processing grade tuber yield of MP/6-39 (23 t/ha) over different locations of northern plains and central plains were significantly higher (19%) than best control Kufri Chipsona-3 (20 t/ha). MP/6-39 has acceptable tuber dry matter content (20%) and 66 % mean foliage maturity **(Table 4, 5).**

Advanced varietal trial-1 (2016-17): MP/6-39 produced a significantly higher or at par total tuber yield **(Table 5)** than the best control Kufri Chipsona-3 at Hisar, Modipuram, Pantnagar (northern plains), Deesa, Gwalior and Kanpur (Central plains). The mean total tuber yield of MP/6-39 (36 t/ha) over different locations of northern plain and Central plain were significantly higher (14%) than the best control Kufri Chipsona-3 (32 t/ha). MP/6-39 also produced a significantly higher or

Table 4. Tuber dry matter (%) and foliage maturity of MP/6-39 at 90 Days (2015-2016)

Region	Locations	Tuber dry matter (%)			Foliage maturity (%)		
		MP/6-39	Atlantic	K Chip-3	MP/6-39	Atlantic	K Chip-3
Northern Plains	Hisar	16.4	19.9	19.9	-	-	-
	Modipuram	18.6	18.8	18.6	70.0	80.0	66.7
Central Plains	Chhindwara	18.6	18.8	18.7	91.8	89.0	87.5
	Deesa	17.8	20.7	20.8	26.7	46.7	40.0
	Gwalior	24.2	18.8	26.7	-	-	-
	Kota	22.2	23.2	21.0	74.0	78.3	76.7
	Mean	19.6	20.0	21.0	65.6	73.5	67.7
$CD_{0.05}$	-	Variety: NS Variety × locations: 1.56			Variety: 3.16 Variety × locations: 7.07		

Table 5. Total tuber yield and processing grade tuber yield at 90 Days in advanced varietal trials-1 (2016-2017)

Region	Locations	Total tuber yield (t/ha)				Processing grade tuber yield (t/ha)			
		MP/6-39	Atlantic	Kufri Chip-3	Kufri Frysona	MP/6-39	Atlantic	Kufri Chip-3	Kufri Frysona
Northern Plains	Hisar	39.7	29.5	37.6	34.3	36.2	27.3	32.8	30.2
	Modipuram	40.0	30.3	38.4	34.5	28.9	26.4	30.8	23.4
	Pantnagar	30.4	28.4	26.2	26.4	29.8	27.9	25.7	25.8
Central Plains	Deesa	29.9	19.9	25.7	22.2	24.4	14.0	13.6	13.6
	Gwalior	37.0	34.5	37.8	36.2	21.5	22.0	26.4	23.1
	Kanpur	38.7	27.9	23.5	27.6	35.5	24.9	22.6	24.6
	Mean	36.0	28.4	31.5	30.2	29.4	23.8	25.3	23.5
Yield increase (%)			26.5	14.0	19.0		23.7	16.1	25.3
		$CD_{0.05}$: 2.64		CV: 9.23		$CD_{0.05}$: 2.86		CV: 12.18	

at par processing grade tuber yield than the best controls Kufri Chipsona-3 at Hisar, Modipuram, Pantnagar (northern plains), Deesa and Kanpur (Central plains)**.** The mean processing grade tuber yield of MP/6-39 (29 t/ha) over different locations of north-western plain and central plain were significantly higher (16%) than Kufri Chipsona-3 (25 t/ha). It is pertinent to mention that MP/6-39 produced higher total (19%) and processing grade (25%) tuber yield over French fry variety Kufri Frysona (30 and 24 t/ha) **(Table 5)**. MP/6-39 has acceptable tuber dry matter content (20%), foliage maturity (66%), French fry colour (3.7) and reducing sugars (138 mg/100g fresh weight of potato) **(Table 6 and 7).**

Advanced varietal trial-2 (2017-2018): MP/6-39 produced higher total tuber yield **(Table 8)** than Kufri Bahar at Modipuram, Pantnagar (northern plains) and Chhindwara, Gwalior Kanpur and Kota (central Plains). The mean total tuber yield of MP/6-39 (41 t/ha) over different locations of northern and central plains was higher (8%) than Kufri Bahar (38 t/ha). MP/6-39 also produced higher marketable tuber yield than Kufri bahar at Modipuram, Pantnagar (northern plains), Chhindwara, Deesa, Gwalior and Kota (Central plains). The mean marketable tuber yield of MP/6-39 (37 t/ha) over different locations of North-Western plains and Central plains was higher (8%) than Kufri Bahar (34 t/ha).The tuber dry matter (20%) of MP/6-

Table 6. Tuber dry matter (%) and foliage maturity (%) at 90 Days (2016-2017)

Region	Locations	MP/6-39	Atlantic	K Chip-3	K Frysona	MP/6-39	Atlantic	K Chip-3	K Frysona
		Tuber dry matter %				Foliage maturity %			
Northern Plains	Modipuram	18.0	19.1	18.2	18.8	60.0	70.0	50.0	50.0
	Pantnagar	16.9	16.7	16.5	16.4	94.7	95.0	95.0	95.7
Central Plains	Deesa	20.8	19.9	21.8	24.3	80.0	80.0	80.0	80.0
	Gwalior	22.3	23.0	23.3	20.1	-	-	-	-
	Kanpur	-	-	-	-	46.4	63.0	64.1	66.5
	Mean	19.5	19.7	20.0	19.9	70.3	77.0	72.3	73.1
		$CD_{0.05}$: NS		CV: 5.96					

Table 7. Quality attributes at 90 Days (2016-2017)

Locations	French fry colour			Reducing Sugar mg/100 g of fresh weight of the potato		
	MP/6-39	Atlantic	K Chip-3	MP/6-39	Atlantic	K Chip-3
Modipuram	4.2	3.0	4.2	118.9	66.1	124.6
Pantnagar	3.3	2.7	2.7			
Gwalior	3.6	2.7	3.3	157.2	84.8	85.4
Mean	3.7	2.8	3.4	138.1	75.5	105.0

Table 8. Performance of MP/6-39 and popular variety Kufri Bahar at 90 Days in advanced varietal trial-2 (2017-2018)

		MP/6-39				Kufri Bahar			
Region	Location	Foliage Senescence (%)	Total yield (t/ha)	Marketable yield (t/ha)	Tuber dry matter (%)	Foliage Senescence (%)	Total yield (t/ha)	Marketable yield (t/ha)	Tuber dry matter (%)
Northern Plains	Modipuram	65.0	50.8	46.8	17.0	70.0	45.4	40.8	15.9
	Pantnagar	86.0	29.3	27.6		86.7	28.0	26.7	
Central Plains	Chhindwara	84.5	42.4	34.8	19.1	85.3	37.6	30.8	18.7
	Deesa	66.2	41.6	40.6	20.2	78.6	42.6	40.6	18.8
	Gwalior		37.9	37.9	22.7		33.5	33.5	21.0
	Kanpur	16.3	46.3	37.9	19.5	37.8	43.8	38.5	19.5
	Kota	85.2	35.8	33.9	23.1	86.6	31.8	29.3	20.4
Mean		67.2	40.6	37.1	20.3	74.1	37.5	34.3	19.1
Yield increase (%)							8.2	8.0	

39 was higher as compared to Kufri Bahar (19%).

Advanced varietal trial-3 (2018-19): MP/6-39 produced a significantly higher or at par total tuber yield **(Table 9)** than the best control Kufri Chipsona-3 at Hisar, Jalandhar, Modipuram (North-western plain), Deesa, Gwalior, Kanpur, Kota and Raipur (Central plains). The mean total tuber yield of MP/6-39 (39 t/ha) over different locations of north-western plain and central plain were significantly higher (12%) than the best control Kufri Chipsona-3 (35 t/ha).

MP/6-39 also produced a significantly higher or at par processing grade tuber yield **(Table 9)** than the best controls Kufri Chipsona-3 at Hisar, Jalandhar, Modipuram

Table 9. Total tuber yield and processing grade tuber yield at 90 Days (2018-2019)

Region	Locations	Total tuber yield (t/ha)				Processing grade tuber yield (t/ha)			
		MP/6-39	Atlantic	K Chip-3	K Frysona	MP/6-39	Atlantic	K Chip-3	K Frysona
Northern Plains	Hisar	31.3	18.9	30.9	22.4	30.3	18.0	29.2	21.4
	Jalandhar	36.5	35.7	30.3	26.3	31.6	32.7	25.3	22.1
	Modipuram	49.8	31.6	44.3	40.6	41.0	28.5	36.3	34.0
Central Plains	Deesa	43.6	31.2	38.3	39.9	39.1	29.5	33.3	37.9
	Gwalior	48.3	40.1	42.3	44.0	40.8	35.7	35.1	37.2
	Kanpur	41.1	32.7	36.4	36.0	35.5	28.1	30.2	27.7
	Kota	31.6	15.1	26.1	16.5	30.5	14.0	25.1	15.3
	Raipur	27.6	28.8	29.0	22.9	22.0	17.4	16.7	11.3
Mean		38.7	29.3	34.7	31.1	33.9	25.5	28.9	25.9
Yield increase (%)			32.3	11.6	24.6		32.3	11.6	24.6
$CD_{0.05}$			Genotype: 0.83 Location × Genotype: 3.1				Genotype: 0.8 Location × Genotype: 3.0		

(north-western plain), Deesa, Gwalior, Kanpur, Kota and Raipur (Central plains). The mean processing grade tuber yield of MP/6-39 (34 t/ha) over different locations of north-western plain and central plain were significantly higher (12%) than Kufri Chipsona-3 (29 t/ha). MP/6-39 has acceptable tuber dry matter content (21%), French fry colour (2.8), reducing sugars (98 mg/100g fresh tuber weight) and 69 % mean foliage maturity **(Table 10 and 11).**

Pooled performance in AICRP during different years and locations

Northern plains: MP/6-39 (39 t/ha and 32 t/ha) yielded 19 and 17% higher total and processing grade tuber yield than the best control Kufri Chipsona-3 (33 t/ha and 28 t/ha), respectively at 90 days **(Table 12).** MP/6-39 has tuber dry matter content (18%), French fry colour (3.2) and reducing sugars (106 mg/100 g fresh tuber weight).

Table 10. Tuber dry matter (%) and foliage maturity (%) at 90 Days (2018-19)

Region	Locations	Tuber dry matter				Foliage maturity (%)			
		MP/6-39	Atlantic	K Chip-3	K Frysona	MP/6-39	Atlantic	K Chip-3	K Frysona
Northern Plains	Hisar	17.2	15.8	19.9	18.8	-	-	-	-
	Jalandhar	19.5	23.9	19.6	20.8	-	-	-	-
	Modipuram	18.6	21.8	20.7	21.0	55.0	60.0	50.0	50.0
Central Plains	Deesa	22.1	23.6	23.9	21.4	71.2	89.6	82.8	77.2
	Gwalior	23.4	22.7	24.1	21.6	-	-	-	-
	Kanpur	19.8	20.1	20.5	21.1	-	-	-	-
	Kota	23.8	21.3	20.6	22.2	80.3	87.7	81.8	84.6
	Raipur	23.8	22.4	22.3	18.1				
	Mean	21.0	21.5	21.5	20.6	68.8	79.1	71.5	70.6
	$CD_{0.05}$	Genotype: 0.25 Location × Genotype: 0.90							

Table 11. Quality attributes (Chip/ French fry colour) at 90 Days (2018-19)

Region	Locations	French fry colour				Reducing Sugar mg/100 g of fresh weight of the potato			
		MP/6-39	Atlantic	K Chip-3	K Frysona	MP/6-39	Atlantic	K Chip-3	K Frysona
Northern Plains	Hisar	2.5	3.0	3.0		84.3	87.1	75.5	
	Jalandhar	1.5	2.3	2.3	2.4	102.3	323.7	231.1	355.7
	Modipuram	4.0	3.0	3.6	4.5	66.7	85.0	90.9	88.2
Central Plains	Deesa	3.0	1.0	2.0	3.0	94.5	127.3	67.9	78.5
	Gwalior	3.0	2.5	3.0		140.5	121.0	89.1	
	Mean	2.8	2.36	2.78	3.3	97.7	148.8	110.9	174.1

Table 12. Performance of MP/6-39 in AICRP trials at 90 days (pooled over location)

Northern Plains												
Stage	MP/6-39			ATL			K. Chip-3			K Frysona		
Trial	TTY	PGY	DM%	TTY	PGY	DM%	TTY	PGY	DM%	TTY	PGY	DM%
IVT*	40.8	31.2	17.5	22.2	19.3	19.4	28.9	22.7	19.3			
AVT1**	36.7	31.6	17.5	29.4	27.2	17.9	34.1	29.8	17.4	31.7	26.5	17.6
AVT3**	39.2	34.3	18.4	28.7	26.4	20.5	35.2	30.3	20.1	29.8	25.8	20.2
Mean	38.9	32.4	17.8	26.8	24.3	19.3	32.7	27.6	18.9	30.8	26.2	18.9
Yield increase (%)				45.3	33.3		19.0	17.3		26.5	23.8	
Central Plains												
IVT*	28.4	19.4	20.7	21.9	15.4	20.4	26.7	18.0	21.8			
AVT1**	35.2	27.1	21.6	23.9	19.5	19.9	29.0	20.9	22.6	28.7	20.4	22.2
AVT3**	38.4	33.6	22.6	29.6	24.9	22.0	34.4	28.1	22.3	31.9	25.9	20.9
Mean	34.0	26.7	21.6	25.1	19.9	20.8	30.0	22.3	22.2	30.3	23.2	21.5
Yield increase (%)				35.4	33.9		13.3	19.8		12.4	15.3	
G Mean	36.5	29.5	19.7	26.0	22.1	20.0	31.4	25.0	20.6	30.5	24.7	20.2
Yield increase (%)				40.5	33.6		16.2	18.3		19.4	19.7	

Northern plains: IVT (Hisar and Modipuram), AVT1 (Hisar, Modipuram and Pantnagar), AVT3 (Hisar, Jalandhar and Modipuram); Central plains: IVT (Chhimdwara, Deesa, Gwalior and Kota), AVT1 (Deesa, Gwalior and Kanpur), AVT3 (Deesa, Gwalior, Kanpur, Kota and Raipur)

Central plains: MP/6-39 (34 t/ha and 27 t/ha) yielded 12 and 15% higher total and processing tuber yields than the best control Kufri Frysona (30 t/ha and 23 t/ha), respectively at 90 days crop duration **(Table 12).** MP/6-39 has acceptable tuber dry matter content (22 %), French fry colour (3.3) and reducing sugars (137 mg/100 g fresh tuber weight)

***Mean performance MP/4-578 in AICRP trials (Pooled over northern and central plains)*:** MP/6-39 (37 t/ha and 30 t/ha) yielded 16 and 18% higher total and processing grade tuber yields respectively, than the best controls Kufri Chipsona-3 (31 t/ha and 25 t/ha) at 90 days of crop duration **(Table 12).** MP/6-39 has acceptable tuber dry matter content (20%), French fry colour (3.3) and reducing sugars (122 mg/100 g fresh tuber weight) **(Table 13)**.

Keeping Quality

Kufri Sangam has better keeping quality as compared to Kufri Chipsona-3 and Kufri Bahar during storage at room temperature

Table 13. Quality attributes MP/6-39 in AICRP trials at 90 days (pooled over location)

Northern Plains								
Trials	MP/6-39		ATL		Chip-3		K Frysona	
	CC/FFC	RS	CC/FFC	RS	CC/FFC	RS	CC/FFC	RS
AVT1	3.8	118.9	2.9	66.1	3.5	124.6		
AVT3	2.7	93.3	2.8	205.4	3.0	153.3	3.5	355.7
Mean	3.2	106.1	2.8	135.8	3.2	139.0	3.5	355.7
Central Plains								
Trials	FFC	RS	FFC	RS	FFC	RS	FFC	RS
AVT1	3.6	157.2	2.7	84.8	3.3	85.4		
AVT3	3.0	117.5	1.8	124.2	2.5	78.5	3.0	78.5
Mean	3.3	137.4	2.2	104.5	2.9	82.0	3.0	78.5
G Mean	3.25	121.75	2.5	120.15	3.05	110.5	3.25	217.1

TTY: Total tuber yield, PGY: Processing grade tuber yield, DM; Dry mater (%), French fry colour: on 1-10 scale (1 very light colour & 10 very dark).RS: Reducing sugars: mg/100g FTW

Table 14. Storage behavior of MP/6-39 under ambient conditions at Modipuram*

Genotypes	Dormancy period (weeks)	Sprouting (%)		Weight loss (%)			
		45 days	75 days	Sprouts	Rottage	Physiological	Total
MP/6-39	>10 week	0.0	8.2	0.0	3.4	7.8	11.2
Kufri Chipsona-3	>6 weeks	70.0	96.6	0.5	2.0	11.7	14.3
Kufri Bahar	>6 weeks	69.9	86.7	0.5	0.8	11.8	13.1

*Four years means (2015-2016 to 2018-2019)

(Table 14). Kufri Sangam has long dormancy (>10 Week), less physiological weight loss (8%) and total weight loss (11%) as compared to popular variety Kufri Bahar (12 and 13%) and Kufri Chipsona-3 (12 and 14%) after 75 days storage under ambient conditions.

Industrial Testing of hybrid

To see suitability of MP/6-39 to provide the raw material for French fries industry, industrial testing was done at McCain Foods, Mahesana (2015-16). MP/6-39 produced significantly high total and processing grade tuber yield than Kufri Frysona and Kennebec. The results **(Table 15)** of MP/6-39 with 49 and 46 t/ha total and processing grade tuber yield showed 32% and 31% yield superiority over the French fry variety Kufri Frysonsa (38 and 36t/ha). The advanced hybrid MP/6-39 with 49 and 46 t/ha total and processing grade tuber yield showed 23% showed yield superiority over exotic French fry variety

Table. 15 Industrial testing of MP/6-39 with McCain Foods, Mahesana (2015-16)

Genotypes	TTY/ha	PGY/ha >40mm	% PGY	Tuber form index (> 40 mm)	Tuber count per 10 kg	Tuber dry matter %
MP/6-39	49.4	46.4	93.9	0.93	98	20.6
K Frysona	37.5	35.5	94.5	0.93	92	20.2
Kennebec	40.1	37.7	93.9	1.03	75	20.2
$CD_{0.05}$	4.5	6.0	6.6	-	11.0	

Kennebec (40 and 38 t/ha). The variety produced nearly 94% processing grade tuber yield with acceptable (21%) dry matter and comparable processing traits as compared to indigenous and exotic genotypes. The industry remarked MP/6-39 to be with white tuber with good size and good for French fry.

Disease resistance

Kufri Sangam has moderate level of resistance against late blight as it has recorded lower area under disease progress curve (AUDPC) values (290) as compared to check variety Kufri Bahar (750) under artificial epiphytotic conditions during 2013-15 at Modipuram. Similarly on detach leaf method Kufri Sangam showed lesser lesion area (2.95 cm^2) as compared to Kufri Bahar (7.2 $cm^{2)}$.

Agronomic management

The optimum tuber yield of Kufri Sangam can be obtained by adopting a standard agronomical schedule for medium maturing varieties. *Planting time:* northern plains (15-25 October) and central plains (5-15 November). *Seed rate:* 35-40 q/ha. *Seed size*: 40-60 g. *Spacing:* Plant spaced at 20-25 cm in 66 cm rows provides optimum tuber size distribution for production of desirable tuber size for the production of ware or processing potatoes. *Fertilizer:* For seed crop, generally 90, 80, 100 kg/ha nitrogen, phosphorous and potassium at the time of planting and remaining 90 kg/ha nitrogen at the time of earthing-up is recommended. At Modipuram, optimum nitrogen (240 kg/ha), phosphorous (40 kg/ha) and potassium (100 kg/ha) dose was worked out for the production of ware or processing potatoes. Nutrient management in other agro-ecologies may differ and thus needs to be fine-tuned or regional recommendation may be followed for optimum productivity of this variety. *Irrigations:* In conventional flood irrigation, first irrigation is given 12-15 days after planting at 2-5% crop emergence. Second irrigation is given 20-25 days just after earthing up. Subsequent irrigations are given as and when required at 10-12 days interval. Crop should not face moisture stress at stolon formation and during tuberization. Stop irrigation about 10-12 days before haulm cutting when 25-30% plants have shown senescence. *Plant Protection Measures:* For management of cutworms, white grubs, beetles and leaf-eating caterpillars, apply cartap hydrochloride 4G @20 kg/ha during earthing-up. It will also take care of sucking pest like leafhopper and aphids. For seed crop, place yellow sticky traps (15 × 30 cm^2 size) just above the canopy height @ 60 traps/ha at equidistance from each other for mass trapping of whiteflies/aphids. Seed treatment with imidacloprid (200SL) @ 0.04% (4 ml/10 lit) for 10 minutes before planting. First spray with imidacloprid (200SL) @ 0.03% (3 ml/10 lit of water) at 85% crop emergence. Second spray with thiamethoxam (25 WG) @ 0.05% (5 gm/10 lit of water) after 10-15 days of the first spray.

Adaptability

Kufri Sangam has been recommended for Punjab, Haryana, Uttar Pradesh, Uttrakhand (northern plains) and Madhya Pradesh, Chhattisgarh, Gujarat, Rajasthan (Central plains). Due to its higher yield, moderate resistance to late blight, acceptable quality traits and attractive ovoid tubers, long dormancy, better shelf life under ambient store conditions, the variety can provide option to long distance transportation of tubers for table or processing use.

Usage

The new potato variety Kufri Sangam is suitable for ware (northern plains) as well as

for production of potatoes for processing into French fries (central plains). Kufri Sangam is likely to be preferred by processors for its white- ovoid tubers with cream flesh and shallow eyes resulting in lower peeling losses. It possesses a pleasant flavour, mealy texture.

CONCLUSION

Kufri Sangam has performed well in multi-location trials conducted under AICRP on potato in northern and central Indian plains and therefore can be grown successfully for increasing productivity and utilization of potatoes for table as well as for processing (making French fries) in these areas. Kufri Sangam can provide one more very good alternative to consumer for fresh consumption and also to processing industry from single variety with white-cream skin ovoid tubers, shallow eyes, cream flesh, excellent keeping and culinary quality and moderate resistance to late blight in addition to high marketable/processing and total tuber yields.

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PROTEIN QUALITY OF SOUTH AFRICAN POTATOES TO INFORM DIETARY CHOICES

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ABSTRACT: Protein content was determined using both the nitrogen to protein conversion factor of 6.25 and the summation of amino acids for four of the potato cultivars (*Solanum tuberosum* L.) most commonly consumed in South Africa. Protein content for the cultivars varied between 1.65 g/100 g and 2.14 g/100 g fresh-weight when using protein conversion and 1.64 g/100 g and 2.14 g/100 g when using the sum of amino acids. Significant differences were found between the amino acid contents of the different cultivars. Leucine had the lowest chemical score of essential amino acids. Protein quality was evaluated using various methods. Even though the protein content of the cultivars is low, it is of a high quality and can contribute to overall dietary protein consumption.

KEYWORDS: Amino acids, Nutrition, Crude Protein (Crude), Protein digestibility.

INTRODUCTION

The metabolic effect of specific individual dietary amino acids is important, demanding accurate information about the amino acid profile of foods. According to the Food and Agricultural Organisation (FAO), when evaluating dietary protein: "dietary amino acids should be treated as individual nutrients and wherever possible data for digestible or bioavailable amino acids should be given in food tables on an individual amino acid basis" (FAO 2011).

Protein is considered an important macronutrient in the diet as it provides both essential amino acids and is a source of energy. There has been much discussion regarding protein and amino acid requirements for both adults and children in recent years (Ghosh et al. 2012). Internationally, the 2007 Food and Agriculture Organization/ World Health Organization (FAO/WHO) report entitled 'Protein and amino acid requirements in human nutrition', concluded that protein quality is of greater importance than actual quantity, emphasising the presence of individual amino acids in a food, instead of simply focussing on total protein (WHO, 2007). Determining the amounts of dietary essential amino acids, digestibility of protein and bioavailability of amino acids, facilitates the quantification of quality parameters of protein source foods (Gilani et al. 2005).

Protein quality has been defined as the ability of a protein to achieve metabolic actions by providing specific patterns of amino acids and it is as important as protein content (Pretorius et al. 2019; MIllward et al. 2008). The FAO report suggested that on average the recommendations for essential amino acids, particularly for lysine, should be doubled, and the recommended requirement for adults should be increased from 12 mg/ kg body mass to 30 mg/kg (WHO 2007). Data on the amino acid composition of foods is thus essential to improve global protein intake and health. The subject of the contribution of high-quality foods to the diet is questioned at length in the recently released EAT-Lancet report. In this report it is estimated that there are 820 million people suffering from low quality diets which leads

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to a deterioration in human health. The report further promotes dietary changes to include foods that provide nutrients of high quality that can significantly decrease the number of individuals consuming low quality diets (EAT-Lancet 2019).

Alternative sources of protein can make a significant contribution to protein intake of vulnerable individuals especially in countries where high quality proteins from animal source foods is scarce. It has been noted that protein malnutrition is a causative factor of 49% of more than 10 million annual deaths of children under five (Wang et al. 2017). It is estimated that almost one third of children in developing countries are stunted, caused by long term insufficient intake of protein and energy. This emphasises the need for alternative sources of high-quality protein to meet the growing need (Global Nutrition Report, 2018). Therefore, research on various supplementary crops is encouraged to determine the true and total nutritional value of food protein (EAT-Lancet 2019).

The South African Food-Based Dietary Guidelines (FBDG) encourage consumers to plan their meals around starchy foods and state that these foods should form the basis of most meals. The carbohydrates in starchy foods contribute significantly to the energy intake in the diet but can also be a source of other macronutrients and micronutrients that can contribute to human and dietary health. Potatoes are the most important non-cereal food crop with global production figure reaching 370 million tonnes (FAO 2020). These tubers are consumed worldwide and regarded as a staple food in many countries, including South Africa. The versatility of this commodity has led to an expansion in the demand for potato products which has inevitably led to an increased contribution to dietary diversity through the production and consumption of various potato products (King & Slavin 2013). In South Africa, potato consumption has increased by 26.8% associated with a 14.6% population growth showing that the total demand for potatoes increased by more than population growth, i.e., increased per capita consumption (BFAP 2019). This increase in consumption clearly shows what an important role potatoes play in the national diet. Due to some of the preparation methods, potatoes are often maligned in nutrition domains as being unhealthy and accused of contributing to the obesity epidemic. However, this is not a true reflection of the commodity, as a potato in its original form, cooked without the addition of fats and oils, can form part of a balanced diet (Bártová et al. 2015). Furthermore, it was recently reported that the biological value of potato protein is high (Camire et al. 2009).

Objective

The purpose of the study was to determine the protein content and the amino acid profile of four different potato cultivars commonly consumed in South Africa. The protein as derived from the nitrogen content of the samples and multiplied with the Jones factor was compared to the sum of total amino acids. In addition, the protein quality scores were calculated.

MATERIALS AND METHODS

Sampling

Duplicate nutrient analyses were done on composite samples of four potato cultivars obtained from various production regions across South Africa. Four cultivars that contribute to the largest market share (as shown in brackets) in South Africa were chosen for this study. Sampling was conducted from October – December 2018. Mondial (55%) and Sifra (23%) were sourced from three different production regions in South Africa, i.e., Free State, Limpopo and

Sandveld. The other cultivars, Valor (6%) and BP1 (2%), were sourced from Limpopo as this was the region that was harvesting at the time of the study. All of the potatoes were cultivated according to the common agricultural practices of each specific region under irrigation. Once harvested, 3 kg of tubers of each cultivar were packed in brown paper bags and transported to the laboratory. Upon arrival at the laboratory the tubers were stored in a cool dark room for six days to mimic market conditions. Prior to analyses, tubers were washed with water to remove all excess dirt and allowed to air-dry. Three whole tubers were randomly selected from each cultivar sample and analysed as a composite sample.

Determination of protein

Total nitrogen was determined using the Kjeldahl method. The nitrogen content was then used to calculate crude protein using the Jones conversion factor of 6.25. In 1981 a study to determine the nitrogen to protein conversion factor for potatoes was conducted using 34 different cultivars, which gave a factor of 6.24 which is similar to the empirical factor mostly used for foodstuffs. It was therefore decided that there is no reason to implement a potato specific conversion factor (Van Gelder 1981; Greenfield & Southgate 2003).

Determination of amino acids

The amino acid profile was determined by the ARC Irene Analytical Laboratory using high-performance liquid chromatography (HPLC) with florescence detection. The determination was carried out during three separate hydrolyses. The first hydrolysis analysed arginine, hydroxyproline, serine, aspartic acid, glutamic acid, threonine, glycine, alanine, tyrosine, proline, methionine, valine, phenylalanine, isoleucine, leucine, histidine and lysine. The ground freeze dried potato was weighed and hydrolysed with 6 N hydrochloric acid. An internal standard was added to the hydrolysate and filtered. A portion of the hydrolysate was dried under nitrogen-flow. The hydrolysate was derivatized with 9-fluorenylmethy chloroformate (FMOC-Cl) reagent and the amino acid content was determined by HPLC with an eluent of a tertiary gradient of pH, methanol and acetonitrile (Einarsson et al. 1983).

The second hydrolysis determined cysteine and followed an identical approach as described above with the exception that prior to hydrolysis, cysteine was oxidised to cystic acid with a peroxide formic acid solution (Gehrke et al. 1985). The third hydrolysis determined tryptophan. Ground freeze dried potato was hydrolysed enzymatically using protease. The hydrolysis was filtered through 0.45 µg filter and tryptophan was determined by means of HPLC equipped with an AMinoTAg column and florescence detection (De Vries et al. 1980).

Evaluation of the protein quality of foods for human consumption

In addition to the concentration of amino acids in foods, it is important to consider the digestibility of essential and non-essential amino acids in foods. Protein quality is typically measured using biological assays or chemical analysis as discussed below.

Analyses

Table 1 shows the summary of adult essential amino acid requirements which will be used for further comparison, discussion and calculation in this article. The values presented in Table 1 are the best currently available estimates for essential amino acid requirements (FAO/WHO/UNU 2007).

Table 1 Summary of the adult essential amino acid requirements (FAO/WHO/UNU 2007)

Amino Acid[a]	mg/kg body weight/day	mg/g maintenance protein
Histidine	10	15
Isoleucine	20	30
Leucine	39	59
Lysine	30	45
Sulphur Amino Acids	15	22
Methionine	10	16
Cysteine	4	6
Aromatic Amino Acids (Phenylalanine and Tyrosine)	25	38
Threonine	15	23
Tryptophan	4	6
Valine	26	39
Total essential amino acids	184	277

[a]Mean protein requirement of 0.66 g protein/kg per day

Chemical score of essential amino acids

Once the quantity of amino acids in the different cultivars was determined, the chemical score (CS) of the essential amino acids (CSEAA) was calculated in relation to the amino acid pattern of the reference requirements (Table 1) proposed by the FAO (FAO/WHO/UNU 2007; FAO 2013), using the following equation (Eq 1) as first described by Mitchell and Block (1946).

$$\text{CSEAA} = \left[\frac{(\text{gEAA in test protein})}{(\text{gEAA in reference protein})}\right] \quad \text{(Eq 1)}$$

Essential amino acid index

The essential amino acid index (EAAI) measures the presence of amino acids that the human body cannot synthesise and gives a stronger indication of potential nutritive value. The essential amino acids index (IEAA) was calculated using the following equation (Eq 2) described by various researchers (Oser 1959; Huang et al. 2018; Rolinec et al. 2018; Abdualrahman et al. 2019).

$$\text{EAAI} = 100 \times \sqrt[n]{\frac{a}{ap} \times \frac{b}{bp} \times \frac{c}{cp} \times \ldots\ldots\ldots \frac{i}{ip}} \quad \text{(Eq 2)}$$

where a, b, c, . . ., i = content of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, threonine and valine in each sample; ap, bp, cp, . . ., ip = content of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, threonine and valine in the reference protein (FAO, 2013); n = number of amino acids used (counting pairs such as methionine and cysteine, and phenylalanine and tryptophan, as one).

Protein digestibility corrected amino acid score (PDCAAS)

Protein digestibility-corrected amino acid score (PDCAAS) is a method of evaluating the quality of a protein based on both the amino acid requirements of humans and their ability to digest the protein. The protein digestibility can be determined analytically, or can be approximated by using existing tables on protein digestibility of foods. PDCAAS was calculated using the equation proposed by the FAO technical working group on protein quality and human requirements (Eq 4) (FAO/WHO 1991; FAO/WHO/UNU 2007). PDCAAS values > 1 were truncated to 1 (FAO 2013).

$$\text{PDCAAS} = \left[\frac{(\text{g of limiting amino acid in test protein})}{(\text{g of same amino acid in reference protein})}\right] \times \text{digestibility} \quad \text{(Eq 4)}$$

Digestible indispensable amino acid score (DIAAS)

The FAO developed the digestible indispensable amino acid score (DIAAS) in 2013 as the new recommended method to determine protein value (FAO 2013; FAO 2014). This method takes into account both the individual amino acid concentration and its digestibility at the end of the small intestine (Ileal), determined best with human models, and if not, with pig or rat models,

respectively (Rutherfurd et al. 2015). It is calculated by dividing the digestible dietary indispensable amino acid (mg) in one gram of the dietary protein by the same dietary indispensable amino acid (mg) in one gram of the reference protein (Eq 5) (FAO, 2013). For the calculation of DIAAS, the 2013 FAO Report recommends using human true ileal amino acid digestibility coefficients, or pig true ileal amino acid digestibility coefficients, or rat true ileal amino acid digestibility coefficients, in that order of preference (FAO, 2013). An ileal digestibility value of 90 was used for the calculation (Branco-Pardal, et al., 1995). DIAAS values were not truncated.

$$\text{DIAAS} = \left[\frac{\text{(mg of digestable dietary indispensable amino acid in 1 g test protein)}}{\text{(mg of the same dietary indispensable amino acid in 1 g reference protein)}} \right] \times 100 \qquad \text{(Eq 5)}$$

Statistics

Data received from the laboratory was arranged in tabular format and statistically analysed using the GenStat for Windows (2008) statistical computer programme (Payne et al. 2012). A one-way analysis of variance (ANOVA) test was applied with Fisher's protected *t*-test least significant difference at the 5% level of significance among cultivar means.

RESULTS AND DISCUSSION

This study measured and compared the amino acid profile of four different potato cultivars. These four cultivars form 86% of the South African potato market and are subsequently the most commonly consumed tubers. In Table 2 the protein and amino acid content of these four cultivars are reported.

As shown in Table 2, protein values using Nitrogen conversion did not differ significantly (P=0.055) between the various cultivars, ranging from the lowest value for Valor (1.65 g/100 g) to the highest value for BP1 (2.14 g/100 g). These values are similar to those found in the United States Department of Agriculture Food Composition Tables for the protein content of potatoes (2.03 g/100 g) (USDA 2019), as well as for traditional potatoes cultivated in the Canary Islands (1.94 g/100 g) (Galdon et al. 2010).

The protein content of foodstuffs is determined by using a nitrogen to protein conversion factor. In the case of potatoes, the standard Jones factor of 6.25 is used. Critical reviews of the accuracy of the Jones factor for protein determination have delivered varying results depending on the food analysed. Some researchers state that the Jones factor is merely nitrogen expressed using a different unit and does not provide accurate results for true protein content (Mariotti et al. 2008). A study of red meat found an underestimation of protein content when the Jones factor of 6.25 was used in the determination (Hall & Schönfeldt, 2013). In this study it was found that the Jones factor of 6.25 delivered accurate results when determining the protein content of potatoes. Variance between the calculated protein content and the sum of amino acids differed between 0% and 1% allowing the researchers to accept that the nitrogen to protein conversion factor of 6.25 can be used.

It is known that the nutritional, and more specifically the amino acid, contents of potatoes do vary and that the three greatest influencers, in order of effect are cultivar (34.3%), nitrogen fertilisation (17.9%) and the conditions that occur during the growth phase (2.1%), i.e., year and site interactions (Bártová et al.,2015; Grubben,et al. 2019). These findings were borne out when comparing the results from a previous study on the nutritional content of South African potatoes conducted in 2013 where tubers were planted under dry land conditions and had an overall lower protein content than tubers planted under irrigation as seen in the current study.

Table 2 Amino acid content (mg) of four different potato cultivars

Analysis	P-value	Mondial	Sifra	Valor	BP1
Protein (N × 6.25) (g/100 g)	0.055	1.770	1.940	1.650	2.140
Sum of amino acids		1.750	1.930	1.640	2.140
Essential amino acids (EAA)		0.675	0.740	0.675	0.860
Arginine	≤0.05	0.135^{a}	0.135^{a}	0.105^{b}	0.12^{ab}
Histidine	0.603	0.055	0.050	0.040	0.040
Isoleucine	≤0.05	0.05^{c}	0.06^{b}	0.065^{b}	0.08^{a}
Leucine	≤0.05	0.075^{c}	0.095^{b}	0.09^{bc}	0.115^{a}
Lysine	0.052	0.115	0.120	0.110	0.145
Methionine	*	0.030	0.030	0.030	0.040
Phenylalanine	≤0.05	0.06^{b}	0.065^{b}	0.07^{b}	0.095^{a}
Threonine	≤0.05	0.055^{c}	0.07^{b}	0.06^{c}	0.08^{a}
Tryptophan	*	0.02	0.02	0.02	0.03
Valine	≤0.05	0.080^{b}	0.095^{b}	0.085^{b}	0.115^{a}
Non-essential amino acids (NEAA)		1.075	1.185	1.965	1.275
Alanine	≤0.05	0.055^{c}	0.08^{a}	0.06^{c}	0.07^{b}
Aspartic acid	≤0.05	0.345^{b}	0.315^{b}	0.355^{b}	0.475^{a}
Cysteine	0.258	0.025	0.025	0.020	0.040
Glutamic acid	≤0.05	0.42^{b}	0.495^{a}	0.27^{c}	0.375^{b}
Glycine	*	0.05	0.05	0.05	0.06
Proline	0.4	0.055	0.075	0.08	0.08
Serine	≤0.05	0.055^{c}	0.07^{b}	0.06^{c}	0.08^{a}
Tyrosine	≤0.05	0.06^{b}	0.065^{b}	0.06^{b}	0.085^{a}
Sulphur Amino Acids Methionine + Cysteine	0.08	0.055	0.055	0.050	0.080
Aromatic Amino Acids Phenylalanine +Tyrosine	≤0.05	0.12^{b}	0.13^{b}	0.13^{b}	0.18^{a}
EAA/NEEA Ratio		0.628	0.624	0.699	0.675
PDCAAS		1	1	1	1
DIAAS		101	99	108	114
Essential amino acid index (EAAI)		1.37	1.37	1.49	1.52
Essential amino acid index %		137	137	149	152

*Values for these amino acids did not show variance and therefore no statistical analysis was possible
#Means with different superscripts in a row differ significantly

Tubers from the 2013 study found overall lower protein values: Mondial 1.45 g/100 g, Sifra 1.00 g/100 g, Valor 1.35 g/100 g and BP1 1.96 g/100 g (van Niekerk et al. 2016).

In the current study, of the nine essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine), five amino acid values differed significantly between the four cultivars (Table 2). The values for methionine and tryptophan were too similar for statistical analysis. Amino acid values for histidine and lysine did not differ significantly between the cultivars.

There are numerous methods to assess the nutritional value of proteins. Oser's

method (one of the earlier methods) was used to rate the quality of protein based on the contribution of all the essential amino acids (Rao et al. 1959). The essential amino acid index (EAAI) ranged from 1.367 for Mondial to 1.518 for BP1. All four cultivars had an EAAI value above 0.90 and can therefore be classified as a good quality protein according to Oser's method.

In Table 3 the chemical score of essential amino acids (CSEAA) with the lowest value indicates the first limiting amino acid. For potatoes leucine was found to be the first limiting amino acid as it had a score of less than 1 for all four cultivars. These results are in agreement with the results of a study conducted in Denmark (Jorgens & Lauridsen, 2004). Leucine is one of the three branched chain amino acids (BCAA) which play an essential role in protein synthesis and is critical in metabolic processes (Layman, 2003). Consuming plant foods, such as beans, soya, lentils or split peas, which are high in leucine, together with potatoes, can contribute to the intake of complete protein (Pretorius et al. 2019). Lysine is an essential amino acid and the contribution of lysine from plant sources broadens the intake of essential amino acids within a changing modern diet. From this study it became evident that potatoes are a plant based source of lysine (Table 3).

Table 3 Chemical score of essential amino acids (CSEAA)

Analysis	Mondial	Sifra	Valor	BP1
Histidine	2.10	1.73	1.63	1.25
Isoleucine	0.95	1.04	1.32	1.25
Leucine	0.73	0.84	0.93	0.91
Lysine	1.46	1.39	1.49	1.51
Sulphur Amino Acids Methionine and Cysteine	1.43	1.30	1.39	1.70
Aromatic Amino Acids Phenylalanine and Tyrosine	1.80	1.78	2.09	2.22
Threonine	1.37	1.58	1.59	1.63
Tryptophan	1.90	1.73	2.03	2.34
Valine	1.17	1.27	1.33	1.38

The protein digestibility– corrected amino acid score (PDCAAS), was adopted in 1993 as the preferred method for measurement of the protein quality in human nutrition. However, the truncation of PDCAAS to 1.0 means that important information on highly nutritious proteins is discarded. This truncation is done to 1 if the protein value exceeds 1 using the PDCAAS calculation (Millward, 2012; FAO, 2013; Joye, 2019). This is the case in the current study where all the PDCAAS values were truncated to 1 and therefore no conclusions could be drawn. A more recent protein quality score, as proposed by the FAO in 2013, is the digestible indispensable amino acid score (DIAAS), that compares the content of all digestible essential amino acids in a protein to the level of these digestible amino acids in a reference protein. The adoption of this new method to measure the quality and digestibility (bioavailability) of dietary proteins reflects the advances made in analytical methods in order to provide more accurate data (British Nutrition Foundation, 2013). The DIAAS values for the cultivars ranged from 99 for Sifra to 114 for BP1. In comparison, soya, which is commonly regarded as a high-quality protein plant-based food, has a DIAAS value of 90 (Pretorius et al. 2019).

In Table 4, the protein, sum of amino acids, PDCAAS, DIAAS, EAAI and ratio of essential to non-essential amino acids of potatoes is compared to high protein plant-based foods. The values for potatoes are the averaged values of the four cultivars analysed in this study. In this comparison potatoes are the food with the lowest protein content on a fresh-weight basis. The PDCAAS values for potatoes, beans, lentils and soy were truncated to one. Chickpeas and split peas had PDCAAS values of 0.96 and 0.92,

Table 4 Protein quality measures for potatoes and high protein plant-based foods

	Potatoes	Dry Haricot Beans*	Tinned Chick-peas*	Dry Lentils*	Dry Split peas*	Dry Soy*
Protein (N × 6.25) (g/100 g)	1.88	20.87	19.87	24.83	21.77	36.45
Sum of Amino Acids	1.87	19.93	18.69	22.56	21.06	35.78
PDCAAS	1	1	0.96	1	0.92	0.6
DIAAS	106	81	94	132	106	90
Essential amino acids index EAAI	1.44	1.98	1.66	1.65	1.66	1.53
EAA/NEAA ratio	0.66	0.84	0.61	0.62	0.66	0.64

*(Pretorius et al. 2019)

respectively. DIAAS values ranged between 81 for beans and 132 for lentils. Potatoes had a DIAAS value of 106 which is an average score compared to the other foods in Table 4. Potatoes had the lowest score for EAAI at 1.44 with beans scoring highest at 1.98. All the foods had an EEAI score above 0.90, which indicates that they are all classified as a good quality protein. Potatoes, chickpeas, lentils, split peas and soy had similar EAA/NEEA ratios, and beans had the highest score of 0.84.

CONCLUSION

On average raw potatoes contained 1.6 – 2.1 g/100 g protein. In general crude protein content as calculated using the Jones factor of 6.25 corresponded well with the sum of amino acids. When using the essential amino acid index and DIAAS all four potato cultivars that were analysed can be classified as containing protein of good quality. Leucine was found to be the first limiting amino acid in potatoes, which is an amino acid that is commonly limited in plant-based products. If PDCAAS was used to describe the protein quality, all values were truncated to 1, which may mean that valuable information was lost due to truncation. DIAAS values ranged from 99 for Sifra to 114 for BP1. This is numerically higher than the DIAAS values for soya of 90.

Even though potatoes are not typically considered a source of protein, due to their low protein content, the unique amino acid composition allows potatoes to be regarded as a plant-based food that contains high quality protein. Investigations to identify cultivars that contain more protein are recommended. The development and cultivation of such cultivars should be studied.

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POTATO PEEL: A WONDERFUL SOURCE OF POLYPHENOLS DUE TO HIGHER BIOACCESSIBILITY

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ABSTRACT: Type and concentration of various antioxidants present in potato peel is well reported. However, bioaccessibility of potato peels' antioxidants, particularly polyphenols has not been reported yet. Therefore, present study aimed at evaluation of bioaccessibility of polyphenols and carbohydrates from peel of Kufri Chipsona-1 (processing variety); Kufri Jyoti and Kufri Bahar (table varieties). *In-vitro* bioaccessibility of total polyphenols, chlorogenic acid, caffeic acid and rutin revealed that bioaccessibility of total and individual phenols is maximum in the gastric phase. On average, the release of chlorogenic acid, caffeic acid and rutin was 3, 18 and 1.1 folds higher in intestinal phase compared to their content in undigested peel, respectively. Starch decreased from oral to intestinal phase with concomitant increase in sugars. Though potato peel is known to contain high concentration of polyphenols, the bioaccessibility of chlorogenic acid and caffeic acid is many folds higher than the ranges reported through biochemical procedures in undigested peels. Potato peel has a huge potential to be used as a fortificant in development of various food products not only due to presence of high concentration of polyphenols but also because of their high bioaccessibility that can help to improve the health of consumers.

KEYWORDS: Potato peel, polyphenols, carbohydrates, *in vitro* bioaccessibility.

INTRODUCTION

Potato is one of the major cash crops in many provinces of India. Besides being a main source of carbohydrates, it is also a good source of vitamin C, polyphenols and minerals. Potato is used majorly as vegetable in cooked form either alone or in combination with the other vegetables. Conventionally, potato peel is removed before processing the potatoes into different products. Thus potato processing industries generates a lot of peel waste with no value. Depending on the peeling method, losses in form of potato peel waste can vary from 15 to 40% in processing industry (Arapoglou *et al.,* 2010). However, potato peels contain lot of nutritional and phytochemical components such as dietary fibre, antioxidants and polyphenols etc. Almost 50% of the potato polyphenols are located in potato peel and adjoining tissues and concentration of polyphenols decreases towards tuber pith (Al-Weshahy and Rao, 2009). Potato peel can be used as natural antioxidants in food system due to high polyphenol content, which was reported 10 times higher than their levels in potato flesh. Potato peel phenols show higher radical scavenging activity as compared to potato tuber (Nara *et al.,* 2006). Phenolic compounds present in human diet prevents degenerative diseases, render positive effects on certain types of cancer, cardiovascular diseases, various inflammatory disorders and thus, value added use of this by-product is

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of great interest to potato industry (Shahidi, 2000; Birt, 2006). Interestingly, chlorogenic acid is significantly high in potato peels as compared to potato flesh. Chlorogenic acid constitutes 90% of total phenolic compound in potato peel and is known to function as an antioxidant and insulin sensitizer and exhibit anti-inflammatory properties (Chaplick *et al.*, 2010). Caffeic acid also shows immunomodulatory and anti-inflammatory activity (Park *et al.*, 2004).

Potato peel is also good source of dietary fibre consisting of insoluble carbohydrates such as cellulose, hemicellulose, lignin, pectin and gums (Camire *et al.*, 1997). Oxidative deterioration of fats and oils causes rancid odours and flavors, decrease the nutritional quality and leads to formation of secondary toxic compounds which has adverse effects to human health. Therefore, addition of antioxidants in processing is an important step to preserve flavour and to prevent nutrient loss and thus, potato peel can be an alternate substitute for synthetic antioxidants and can be used as natural antioxidant without any harm to human health.

Bioaccessibility can be defined as amount of compound released from the food matrics, solubilised in the small intestine and available for subsequent absorption (Tagliazucchi *et al.*, 2010). Studies regarding bioaccessibility of phytochemical compounds require *in-vivo* experiments with humans. However, *in-vitro* studies have proven to be useful in determining the behaviour changes and stability of phytochemicals under gastrointestinal (GI) conditions as it is cost effective, have no ethical issues, almost mimic the ideal human conditions by simulated GI models (Thakur *et al.*, 2020). Though various reports are present indicating potato peel as a good source of phytonutrients, but to the best of our knowledge, there are no studies reported, regarding bioaccessibility/ changes during *in-vitro* GI digestion of phytochemicals and other nutritional compound present in potato peel. Various reports have suggested utilization of potato peel as a fortificant due to high antioxidant concentration, however, information is lacking on the behaviour of potato peels' antioxidants and carbohydrates; and extent of their release during digestion. Thus, effect of *in-vitro* gastrointestinal digestion on potato peels' antioxidants (total polyphenols, chlorogenic acid, caffeic acid, rutin) and carbohydrates (starch, reducing sugars, sucrose) was evaluated to establish nutritional value of potato peel. In the present study, Indian potato cultivars viz. Kufri Jyoti, Kufri Bahar and Kufri Chipsona 1 were used. Among these, Kufri Jyoti and Kufri Bahar are quite popular and consumed at large scale in India. These two are the table purpose varieties and each of these has occupied almost 30% of the cultivated area under potato. Kufri Chipsona 1 being processing cultivar is used by both organized and unorganized sector for preparation of chips and other value added products. Because of more use of these varieties, there will be more wastage of peels.

MATERIALS AND METHODS

Reagents

HPLC grade methanol and HPLC grade acetic acid, Folin-Ciocalteau reagent, Bovine Serum Albumin Fraction V and glucose was purchased from Merck. Chlorogenic acid, caffeic acid, rutin, pepsin (675U/mg) from porcine, α-amylase (13U/mg) from porcine, bile salt, pancreatin was purchased from Sigma Aldrich. All the other reagents and chemicals of analytical grade were purchased from Himedia.

Sample collection and preparation

Freshly harvested tubers of potato variety Kufri Jyoti (used as both table and processing variety), Kufri Bahar (table variety) and Kufri Chipsona 1 (processing variety) were

procured from Central Potato Research Institute, Modipuram, Meerut, UP, India (29° 05'19'' N, 77° 41'50'' E, 237 m asl) during 2019. Tubers were washed with tap water to remove macroscopic impurities, surface dried and peeled manually. Fresh peel of all varieties was used for *in-vitro* digestion and biochemical estimations {total polyphenols, reducing sugars, sucrose, total starch and individual phenols (chlorogenic acid, caffeic acid and rutin)} in both digested (oral, gastric intestinal) and undigested samples. Results are reported on fresh weight basis.

Sample extraction and analysis

Extractions from undigested samples for reducing sugars, sucrose and total starch were carried out as mentioned by Raigond *et al.* (2014). For estimation of polyphenols, the method described by Thimmaiah (2000) was adopted.

Extraction of Individual phenols: Extraction of potato peel for individual phenols was carried out as per method described by Albishi *et al*. (2013) with some modifications. Fresh peel (1g) was ground in 5ml of methanol/ water/ glacial acetic acid (80:19.5:0.5 v/v/v) solution. Sample was sonicated for 15min and centrifuged at 9000 rpm for 10 min at 4°C. Supernatant was collected and residue was again extracted with 2ml of methanol/ water/ glacial acetic acid (80: 19.5:0.5 v/v/v) solution and centrifuged. Both supernatants were pooled together. Supernatants were freeze dried in lyophilizer (-56°C, 4.0 mbar, Christ, Germany). Residue was re-suspended in 1ml of water: methanol (90:10v/v) solution, vortexed and filtered through 0.2µm PVDF filter.

In-vitro digestion of polyphenols, individual phenols, reducing sugar and sucrose

The *in-vitro* GI digestion process was followed as per the method described by Miranda *et al*. (2013) with slight modifications. Potato peel (2g) was used for *in-vitro* digestion and digestions were performed in triplicate.

Oral Phase

Oral digestion was performed by adding 5ml of α-amylase from porcine pancreas (450U/g of potato peel) dissolved in 0.9% sodium chloride (pH 6.9 with 1N sodium hydroxide) in 2g of potato peel. Sample was ground in presence of α-amylase to mimic oral conditions. After 2 min of incubation at 37°C samples were centrifuged for 10min at 10,000 rpm and aliquot (approximately 1ml) was collected for further analysis.

Gastric Phase

Gastric phase was started by adding 9ml of pepsin (from porcine gastric mucosa) solution (pepsin 6500U/g of potato peel prepared in 0.9% sodium chloride solution) to the oral solution. Then the pH was decreased to 2 with 6M hydrochloric acid and mixture was incubated in incubator shaker at 37°C for 1 hr with continuous agitation. Sample was centrifuged for 10 minutes at 10,000 rpm. Aliquot (approximately 1ml) was collected for further analysis and remaining sample was used for intestinal phase.

Intestinal Phase

The pH of solution was brought to 5.5 using 1M sodium bicarbonate and intestinal digestion was simulated by addition of bile extract (11mg/g of potato peel) and pancreatin (from porcine pancreas) (1.8mg/g of potato peel) for 2 hour at 37°C. Final digestion extract was centrifuged at 10,000 rpm for 10 min and aliquot was used for analysis.

In-vitro starch digestion

In-vitro starch digestion was carried out in the same manner as discussed above. In case of starch digestion, reaction was terminated

at the end of each phase i.e oral, gastric and intestinal phase. After centrifugation step, the residues were washed twice with 10ml of 80% ethanol to remove excess sugars and supernatant was drained carefully every time. Residue left after washing was extracted with 11.5ml of 52% perchloric acid and centrifuged at 10,000 rpm for 10 min. Supernatant was collected and volume was adjusted to 50ml with distilled water.

Analysis of oral, gastric and intestinal aliquots

Total polyphenols

Total polyphenols were estimated as per the method described by Malik and Singh (1980). To 50µl of aliquot collected from three phases, 950 µl distilled water was added. To this, 0.5ml of phenol reagent (Folin-Ciocalteu) was added, vortexed and incubated for 3 minutes at room temperature. Further, 2ml of 20% sodium carbonate was mixed, incubated for 1 hr (room temp) and absorbance was recorded at 650nm (Spectrophotometer, Model-4001/A, ThermoSpectronic, USA). Standard curve was prepared using 1mg/ml chlorogenic acid as stock solution.

Individual phenols

Aliquots (oral/ gastric/ intestinal) were filtered through PVDF membrane (0.2µm) before further analysis. Quantification of individual phenols was carried out as per the method of Albishi *et al*. (2013) using HPLC. A reverse phase column (125 × 4mm Purospher RP-18e, Merck, Germany) was used on the LaChrom HPLC system (Merck-Hitachi Darmstadt, Germany). Individual phenols were estimated at wavelengh of 324nm and injection volume of 20µl was used for analysis. Gradient was used where Mobile phase A contained 1% formic acid in water and mobile phase B consisted of methanol. Gradient composition of two mobile phases was as follows: 0 min-95% A; 2.5 min-84% A; 5 min-73% A; 7.5 min-62% A; 10 min-50% A; 12.5 min-40% A; 15 min-30% A, 17.5 min-25% A; 20 min-20% A; 22.5min-10% A and 25 min-95% A. Stock solution was prepared by using standard chlorogenic acid, rutin and caffeic acid at 1mg/ ml methanol concentration.

Reducing Sugars

To 50µl of aliquot (oral/ gastric/ intestinal) 950µl of distilled water was added followed by addition of 1ml of Nelson reagent. Samples were boiled for 20 min (60-80°C) and then placed in cold water. After the samples were cooled, 1ml arsenomolybedate solution was added, vortexed and kept for 5 min at room temperature. Distilled water (7ml) was added to the above sample and absorbance was recorded at 620nm (Spectrophotometer, Model-4001/A, ThermoSpectronic, USA). Standard curve was prepared using 1mg/ml dextrose as stock solution.

Sucrose

To 100µl of aliquot (oral/ gastric/ intestinal) 900µl of distilled water was added followed by addition of 100µl of 30% KOH. The above solution was boiled for 10 min (60-80°C) and placed in cold water. To the above solution, 3ml of 0.15% anthrone reagent (prepared in 76% sulphuric acid) was added. Samples were incubated for 15 min at 40°C and absorbance was recorded at 620 nm (Spectrophotometer, Model-4001/A, ThermoSpectronic, USA). Standard curve was prepared using 1mg/ml sucrose as stock solution.

Total Starch

To 50µl of aliquot (oral/ gastric/ intestinal) 950µl of distilled water was added followed by addition of 2 ml of 0.2% anthrone reagent (prepared in 95% sulphuric acid). Samples were boiled for 8 min (60-80°C) and cooled to room temperature. Absorbance was recorded at 620nm (Spectrophotometer, Model-4001/A,

ThermoSpectronic, USA). Standard curve was prepared using 1mg/ml glucose as stock solution.

Statistical Analysis

A completely randomised design was used for each biochemical parameter having three replications. The data were analysed using MSTAT 4.0C software and the procedure of Gomez and Gomez (1984). Differences between means were compared using Tukey's test ($P < 0.05$). Different letters have been used to indicate significant differences between the varieties and phases ($P < 0.05$).

RESULTS AND DISCUSSION

Bioaccessibility of polyphenols

Total polyphenols

Significantly higher concentration of total polyphenols was obtained in undigested peel of K. Jyoti (95 mg/100g) followed by K. Chipsona 1 (70 mg/100g) and K. Bahar (52 mg/100g) on fresh weight basis (Fig. 1). After *in-vitro* GI digestion significant decrease was observed in total polyphenolic content in oral, gastric and intestinal phases in peel of all varieties, however, polyphenols were highly bioaccessible in gastric phase followed by intestinal and oral phase (Fig. 1). The bioaccessible phenolic content in oral phase was 37 mg/100g, gastric phase was 43 mg/100g and intestinal phase was 38 mg/100g (average of three varieties).

Higher range of total phenols in peel of K. Chipsona 1 (148 mg/100g fresh weight (FW)) and K. Jyoti (148 mg/100g) was reported by Bandana *et al.* (2016). However, almost similar range was obtained for potato peel extract (70.82 mg of catechin equivalent/100 g) by Kanatt *et al*. (2005). Freeze dried potato peel extract was found to contain total polyphenols ranging from 1.5 to 3.3 mg of gallic acid equivalent/gram (Al-Weshahy and Rao, 2009). In another study, Albishi *et al.* 2013 reported that total phenolic content in freeze dried potato peel ranged from 4.54 to 13.85 (mg GAE/g) and in flesh varied from 1.36 to 2.09 mg GAE/g, respectively. Our results of *in-vitro* GI digestion are in accordance with those reported by Miranda *et al.* (2013), who reported more release of polyphenols from boiled potatoes in

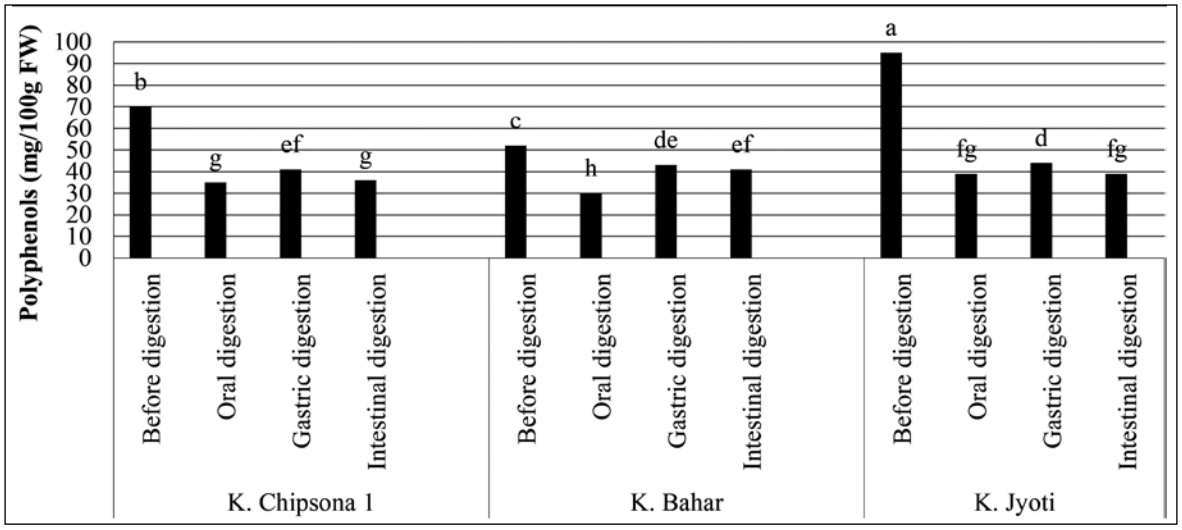

Fig. 1: Changes in total polyphenols (mg/100g FW) during in-vitro gastrointestinal digestion of potato peel. Different letters indicate significant differences between varieties and phases (p< 0.05).

gastric phase. Simulated *in-vitro* digestion of orange peel treated with pulse electric field and high voltage electrical discharge decreased the concentration of total phenols compared to non-digested sample. Decrease was non-significant in gastric phase, whereas significant decrease in phenol concentration was observed in intestinal phase and non-dialysed sample as compared to undigested sample (Buniowska *et al.*, 2015). After *in-vitro* GI digestion of pomegranate peel flour, the total polyphenols decreased in oral, gastric and intestinal phase as compared to the test sample before digestion. The recovery percent of total polyphenols in pomegranate peel flour was maximum in oral phase (69%) followed by gastric phase (65%) and intestinal phase (43%) indicating that maximum degradation occurred during intestinal phase. Bioaccessibility of phenolic and flavonoid compounds in pomegranate peel flour at the end of intestinal digestion was found to be 35.90 and 64.02% respectively (Gullon *et al.*, 2015). Argyri *et al.* (2006) reported that solubility and availability of phenolic compounds are greatly influenced by pH conditions and interaction with dietary constituents, such as iron, fibre or proteins. Results suggested that several changes in phenolic compounds such as modification in chemical structure, increased or reduced solubility or interaction with other compounds might have happened during the GI digestion of potato peel which influenced the phenol bioaccessibility in different phases.

Chlorogenic acid, caffeic acid and rutin

Chlorogenic acid was found to be the most dominant individual phenol in potato peel. Peel contained significantly higher concentration of chlorogenic acid (87 µg/g FW) followed by rutin (24 µg/g FW) and caffeic acid (6 µg/g FW) (average of varieties) (Fig. 2, Table 1). *In-vitro* digestion significantly increased the release of chlorogenic acid, caffeic acid and rutin with maximum bioaccessibility observed in gastric phase (Table 1). Irrespective of the varieties, chlorogenic acid and rutin showed maximum bioaccessibility and trend was gastric phase> oral phase > intestinal phase. However, in case of caffeic acid trend in K. Chipsona 1 and K. Jyoti was gastric phase> intestinal phase > oral phase. Overall average significant increase in chlorogenic acid was 3 folds in intestinal phase (262 µg/g FW) after *in-vitro* digestion. Caffeic acid on an average increased to 18 folds (102 µg/g FW) in

Table 1: *In-vitro* gastro intestinal digestion of potato peel individual phenols (µg/g FW)

Individual Phenols	Treatment	Before Digestion	Oral Phase	Gastric Phase	Intestinal Phase
Chlorogenic acid	K. Chipsona 1	85 $^{(j)}$	333 $^{(d)}$	484 $^{(a)}$	211$^{(h)}$
	K. Bahar	76 $^{(j)}$	321 $^{(e)}$	362 $^{(c)}$	264 $^{(g)}$
	K. Jyoti	101$^{(i)}$	327 $^{(de)}$	383 $^{(b)}$	310 $^{(f)}$
Caffeic acid	K. Chipsona 1	3.08 $^{(i)}$	82 $^{(f)}$	162 $^{(a)}$	141$^{(b)}$
	K. Bahar	0.43 $^{(i)}$	78 $^{(f)}$	66 $^{(g)}$	55 $^{(h)}$
	K. Jyoti	2.20 $^{(i)}$	103 $^{(e)}$	125 $^{(c)}$	110 $^{(d)}$
Rutin	K. Chipsona 1	45.0 $^{(h)}$	202 $^{(d)}$	290 $^{(a)}$	24 $^{(j)}$
	K. Bahar	5.9$^{(k)}$	172 $^{(f)}$	252 $^{(b)}$	33 $^{(i)}$
	K. Jyoti	21.0$^{(j)}$	143 $^{(g)}$	180 $^{(e)}$	25 $^{(j)}$

Different letters indicate significant differences between varieties and phases ($P < 0.05$). Statistical analysis of chlorogenic acid, caffeic acid and rutin was carried out separately.

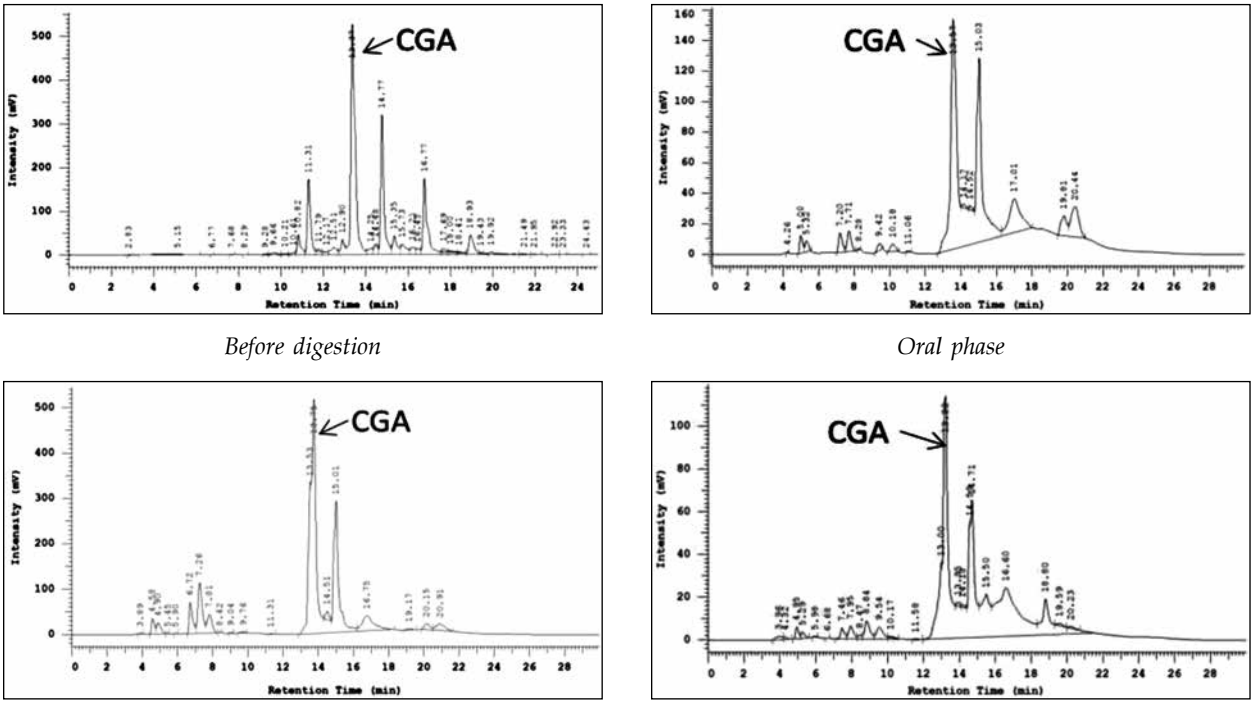

Fig. 2: HPLC Chromatograms of polyphenolics obtained from raw potato peel and during various phases of digestion of raw peel (CGA-Chlorogenic acid)

intestinal phase. Rutin increased significantly to 1.1 folds (27 µg/g FW) in intestinal phase. Miranda *et al*. (2013) reported 5 folds increase in chlorogenic acid in variety Vitelotte and 2.3 folds increase in variety Nicola from boiled potatoes to gastric filtrate. Caffeic acid was reported more in intestinal phase in variety Nicola and in case of variety Vitelotte the content was more in gastric phase followed by intestinal and oral phase. Peel from two of our varieties i.e K. Chipsona-1 and K. Jyoti showed similar trend for caffeic acid as reported by Miranda *et al*. (2013).

Sukrasno *et al*. (2014) reported that fresh potato peel contained approximately 1.0 mg/g (0.1 %) of chlorogenic acid, whereas Kanatt *et al*. (2005) reported chlorogenic acid to be 25mg/100g. Wu *et al*. (2012) quantified phenolic compounds from potato peel and flesh for two consecutive years and reported that chlorogenic acid content was 3.87 and 1.26 mg/g dry powder in potato peel and 1.0 and 0.60 mg/g dry powder in potato flesh during both years. Caffeic acid content was 2.23 and 0.72 mg/g dry powder in potato peel during both years, however, caffeic acid was not detected in potato flesh indicating that potato peel contained higher amount of phenolic compounds than flesh. Chlorogenic acid and caffeic acid content in freeze dried potato peel ranged from 16 to 307 mg /100g and 23 to 66 mg /100g as reported by Albishi *et al*. (2013). In a study conducted by Rowayshed *et al*. (2015) chlorogenic acid was found to be 21 mg/100g DW and caffeic acid was 12 mg/100g DW in potato peel extract.

Our results of chlorogenic acid, caffeic acid and rutin behaviour during *in-vitro* GI are similar to those reported by Miranda *et al.* (2013). However, in a study carried out by Colantuono *et al.* (2016) *in-vitro*

bioaccessibility of phenols from pomegranate peel (PPe) and pomegranate peel cookies (PPeC) showed different trend than the present study. During simulated *in-vitro* digestion of PPe, maximum bioaccessible amount of total polyphenols was found in oral phase (3278 mg/100g) followed by gastric phase (2502 mg/100g) and intestinal phase (1483 mg/100g). Pomegranate peel cookies have maximum amount of polyphenols in oral phase (223 mg/100g) followed by intestinal phase (154mg/100g) and gastric phase (62 mg/100g). The concentration of the polyphenols at each digestion step was associated with the total antioxidant capacity of the potentially bioaccessible compounds. The difference in phenol release trend could be due to difference in the food matrix.

The study indicated that the concentration of polyphenols being estimated from tubers (peel and flesh) is far low than the concentration of polyphenols that is released during digestion and is actually available/ bioaccessible for body. Digestion of proteins during gastric step may be responsible for more release of polyphenols that are available for body to be absorbed. This study has shown that the intake of polyphenols from potato peel might be quite high compared to the quantified concentrations reported from potato peel.

Bioaccessibility of carbohydrates

Total starch

Total starch in potato peel ranged from 12 to 15% (FW) before digestion and decreased significantly during *in-vitro* digestion (Fig. 3). Concentration of total starch was the maximum before digestion, and decreased from oral to intestinal phase. Average decrease in the total starch during *in-vitro* GI digestion was 92%, 94% and 96% in oral, gastric and intestinal phase, respectively. Bandana *et al.* (2016) reported 12.6 and 13% (FW) total starch in raw peel of K. Chipsona 1 and K. Jyoti, respectively. In another study, Arapoglou *et al.* (2009) reported total starch content to be 7.8% in raw potato peel. The decrease in starch content in gastric and intestinal phase is due to presence of digestive enzymes that lead to starch breakdown and release of sugars. Our results of starch hydrolysis are in accordance with those reported by Singh *et al*. (2020) and Tian *et al.* (2018), who reported more starch hydrolysis in intestinal phase compared to gastric phase.

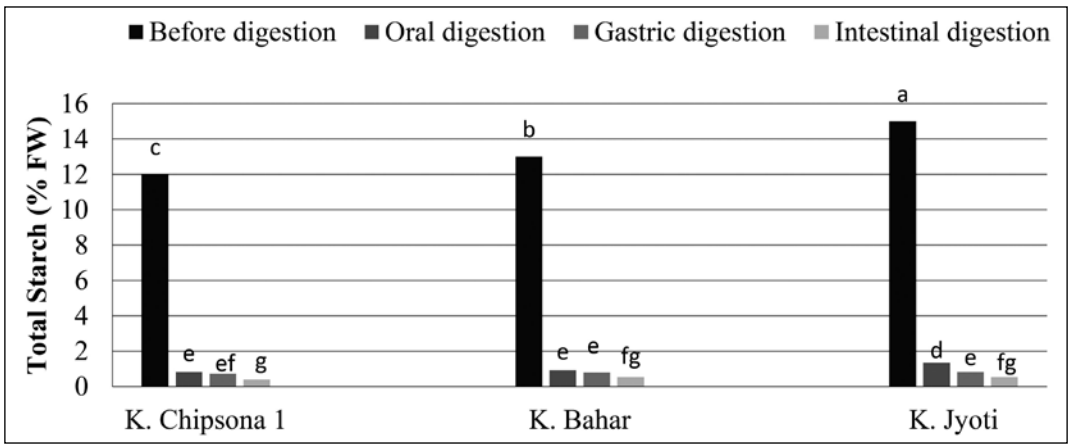

Fig. 3: Total starch during in-vitro gastrointestinal digestion of potato peel. Different letters indicate significant differences between varieties and phases (p< 0.05).

Sugars

Reducing sugars ranged from 199 to 223 mg/100g FW in peel of three varieties (Fig. 4). Significant increase was observed in reducing sugar after *in-vitro* digestion and maximum amount of reducing sugars was obtained in intestinal phase compared to gastric and oral phase. Varieties K. Chipsona 1 and K. Jyoti showed similar trend for reducing sugars as intestinal phase> gastric phase> oral phase, however, in K. Jyoti, the order of release of reducing sugar was oral phase> intestinal phase> gastric phase. Overall 7 folds (intestinal phase) increase in reducing sugars was noticed in potato peel during *in-vitro* digestion compared to undigested samples.

Average 114 mg/100g FW sucrose was reported in undigested potato peel (Fig. 4). Sucrose was found to be significantly increased after *in-vitro* GI digestion. Maximum amount of sucrose was bioaccessible in intestinal phase followed by gastric and oral phase. Similar trend was noticed in all the three varieties and average increase during digestion was 4.9 folds (555mg/100g) in intestinal phase.

Bandana *et al.* (2016) reported 35 and 26 mg/100 FW of reducing sugars in peel of K. Chipsona 1 and K. Jyoti and similar range of sucrose in peel of K. Chipsona 1 (98 mg/100FW) and K. Jyoti (73 mg/100g FW). The study indicated that the starch content decreased from oral to intestinal phase with concomitant increase in sugars. Starch hydrolysis starts from oral phase itself due to presence of α amylase in salivary solution. Further hydrolysis in gastric phase occurred due to acidic conditions of gastric phase. In intestinal phase, presence of pancreatin further leads to breakdown of starch. During each phase enzymatic/acidic hydrolysis of starch resulted in release of sugars. Singh *et al.* (2020) reported similar trend for reducing sugars as reported in the present study.

CONCLUSION

Results showed that potato peels contain significantly higher levels of antioxidants such as total polyphenols, chlorogenic acid, caffeic acid and rutin. The present study indicated that these antioxidants are highly bioaccessible from potato peel. Due to high

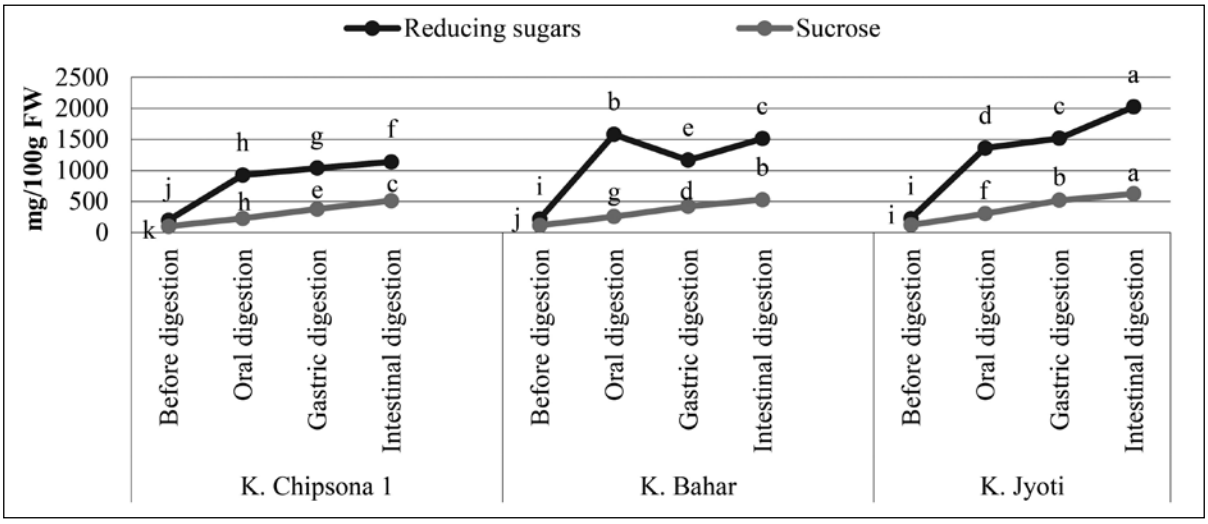

Fig. 4: Reducing sugar and sucrose during in-vitro gastrointestinal digestion in potato peel. Different letters indicate significant differences between varieties and phases (p< 0.05).

bioaccessibility of antioxidants from potato peel, it can be used as fortificant in many food preparations. Incorporation of potato peels to other foods may not only enhance nutritional quality due to high polyphenolics bioaccessibility but may also help to enhance iron bioavailability due to high concentration of ascorbic acid from potato peel. Because of high chlorogenic acid bioaccessibility in potato peels, they can be utilized for development of health foods for diabetics and health conscious consumers by mixing potato peels with other ingredients. Thus, potato processing wastes may be used in food formulations and their extracts could potentially be employed as an effective source of natural antioxidants in food systems.

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MANAGEMENT OF LATE BLIGHT OF POTATO THROUGH FUNGICIDE SCHEDULING

Shailbala Sharma

ABSTRACT: Late blight of potato caused by oomycetes pathogen *Phytophthora infestans*, is a disease of major concern all over the world responsible for 20-75 % yield loss. For more than a decade, management of late blight has become increasingly demanding because of emergence of aggressive and more resistant new strains of the pathogen. In present studies, potential management of late blight has been studied with a view to develop an effective and safe spray schedule for management of late blight of potato. Results revealed that minimum disease intensity and disease incidence were reported from prophylactic spray with fungicide Chlorothalonil 75 % WP @ 0.25 % followed by Dimethomorph 9 % + Mancozeb 60 % WP @ 0.3 % and one more spray with Chlorothalonil 75 % WP @ 0.25 % treated plots. Minimum tuber yield was reported from control plots while maximum tuber yield (42.60 t/ha in K. Khyati and 34.50 t/ha in K. Bahar) were also recorded from the same treatment i.e. treatment Chlorothalonil 75 % WP @ 0.25 % followed by Dimethomorph 9 % + Mancozeb 60 % WP @ 0.3 % and one more spray with Chlorothalonil 75 % WP @ 0.25 %. This treatment proved as the best fungicide scheduling treatment for management of late blight of potato by providing the maximum returns and highest B:C ratio.

KEYWORDS: Potato, late blight, *Phytophthora infestans*, fungicide scheduling, Chlorothalonil

INTRODUCTION

Potato (*Solanum tuberosum L.*) mainly vegetative propagated by seed tubers, is affected by many diseases. Among various fungal diseases, late blight of potato is considered as one of the most dreaded disease, is responsible for considerable economic losses. This disease is weather dependent and mostly appears on regular basis in epiphytotic proportion in all over the country. It is responsible to cause considerable reduction in quality and tuber yield. If fungicides are not applied at right time, it will not provide adequate control. Even there is chances of development of new races which showed resistant reaction to systemic fungicides. Moreover, there is high risk of development of fungal races resistant to systemic fungicides. So, combination of systemic and contact fungicides may be use as one of the options to manage this problem. Mixture of different fungicides have different mode of action which may delay the build-up of resistance. The present studies were conducted with the aim to develop an effective and safe spray schedule for management of late blight of potato.

MATERIALS AND METHODS

A field experiment was conducted for consecutive two years during *rabi* season at Vegetable Research Centre (VRC), Pantnagar. Two potato cultivars namely Kufri Bahar and K. Khyati were planted in the last week of October with purpose to expose the potato crop to extremely congenial atmospheric situation for late blight disease development. The experiment was laid out in randomized block design with plot size 3 × 2 m^2 plot size. Total four treatments with four replications were given to the crop. The treatments comprised of sprays of fungicides either alone or in combinations.

These treatments are fungicide Chlorothalonil 75 % WP @ 0.25 % followed by 2 more sprays at weekly interval (T1),

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Prophylactic spray (just at the time of canopy closure) with Chlorothalonil 75 % WP @ 0.25 % followed by Dimethomorph 9 % + Mancozeb 60 % WP @ 0.3 % and one more spray with Chlorothalonil 75 % WP @ 0.25 % (T2), prophylactic spray (just at the time of canopy closure) with Mancozeb 75 % WP @ 0.2 % followed by Dimethomorph 9 % + Mancozeb 60 % WP @ 0.3 % and one more spray with Mancozeb 75 % WP @ 0.2 % (T3) and Control (T4). All the fungicides were sprayed at the time of appearance of late blight. Total three fungicides i.e. Chlorothalonil 75 % WP (0.875 -1.250 % ai per hac), Dimethomorph 9 % + Mancozeb 60 % WP (ai 90 g/kg and 600 g/kg) and Mancozeb 75 % WP (ai 1.125-1.5 kg/hac) either alone or in combinations were used.

The first appearance of disease was noticed at second week of December. Disease incidence was recorded at 10 days interval. Tuber yield (kg/plot) was recorded at the time of harvesting and then converted into t/ha. B:C ratio were calculated at the market rate of fungicides i.e. Mancozeb (Rs 500/kg), Chlorothalonil (Rs 1400/kg) and Dimethomorph +Mancozeb (Rs 1300/kg). Sale price of produce were Rs 5/kg.

RESULTS AND DISCUSSION

Effect of fungicides on disease incidence

Pooled data of two cropping seasons revealed **(Table 1)** that, maximum disease incidence (100 %) was recorded in control plots of potato cultivar K. Bahar and all the test treatments (T1, T2, T3) were significantly superior to control. Treatment Chlorothalonil 75 % WP @ 0.25 % three sprays at weekly interval (T1) recorded 80 % disease incidence while treatment prophylactic spray (just at the time of canopy closure) with Chlorothalonil 75 % WP @ 0.25 % followed by Dimethomorph 9 % + Mancozeb 60 % WP @ 0.3 % and one more spray with Chlorothalonil 75 % WP @ 0.25 % (T2) reported 55 % and treatment prophylactic spray (just at the time of canopy closure) with Mancozeb 75 % WP @ 0.2 % followed by Dimethomorph 9 % + Mancozeb 60 % WP @ 0.3 % and one more spray with Mancozeb 75 % WP @ 0.2 % (T3) recorded 88 % disease incidence in case of potato cultivar K. Bahar. The percent reduction in disease over control were 20 %, 45 % and 12 % in treatment T1, T2 and T3 respectively over control.

Table 1: Effect of fungicidal scheduling on disease incidence of late blight recorded at 10 days interval (Pooled data of two years)

Cultivars	Treatments	Disease incidence (%) at 10 days interval			% Reduction in disease over control
		D1	D2	D3	
Kufri Bahar	T1	21.0	46.0	80	20.0
	T2	8.0	20.0	55	45.0
	T3	32.0	52.0	88	12.0
	T4	55.0	74.0	100	-
Kufri Khyati	T1	10.0	20.0	54.0	40.0
	T2	5.0	12.0	38.0	57.78
	T3	12.0	25.0	60.0	33.33
	T4	15.45	45.50	90.00	-
CD (cultivar)		12.56	13.81	12.86	
CD (treatment)		15.31	18.56	16.57	

In case of potato cultivar K. Khyati, the percent reduction in disease over control were 40 %, 57.78 % and 33.33 % in treatment T1, T2 and T3. Treatment T1 recorded 54 % final disease incidence while treatment T2 and T3 gave 38 % and 60 % final disease incidence. Control plots showed 90 % disease incidence. In Potato cultivar K. Khyati reported less disease incidence as compare to potato cultivar K. Bahar.

Effect of fungicides on tuber yield

Potato cultivar K. Bahar recorded maximum potato yield (34.50 t/ha.) with the

sprays of Chlorothalonil 75 % WP @ 0.25 % followed by Dimethomorph + Mancozeb @ 0.3 % and one more spray with Chlorothalonil 75 % WP @ 0.25 % (T2) followed by treatment T1 which recorded 30.90 t/ha tuber yield. Treatment T3 gave 29.55 t/ha tuber yield. Minimum tuber yield (23.30 t/ha) was reported from control plot. All the treatments were significantly superior to control plots. 32.62 %, 48.07 % and 26.82 % increase in yield over control were recorded from treatment T1, T2 and T3 respectively.

In case of potato cultivar K.Khyati, treatment T2 recorded the highest tuber yield (42.60 t/ha) while control recorded the minimum tuber yield (26.80 t/ha.). Treatment T1 gave 38.20 t/ha while treatment T3 recorded 37.50 t/ha tuber yield. Both cultivars followed the same disease pattern. There were 42.54 %, 58.96 % and 39.93 % increase in tuber yield over control in case of treatment T1, T2 and T3 respectively.

Economics of fungicide spray schedule

An economics of the treatments were calculated at the market price of fungicides as well as potato produce. The cost of cultivation includes labour charges, cost of fertilizer, irrigation, field preparation, land rent, vehicle charges, fungicide charges, sale price of produce etc which was Rs 103000.00 per ha. The economics of fungicide spray schedule **(Table 2)** indicated that treatment T2 gave the highest benefit cost ratio i.e. 1.59 in case of potato cv. K.Bahar with maximum returns (Rs 64050.00 per ha) followed by treatment T1 (1.43 B:C ratio and Rs 46250 per ha net return). The control plots showed the lowest B:C ratio (1.13) with minimum returns (Rs 13500.00 per ha) in case of potato cv. K.Bahar.

In case of potato cultivar K.Khyati, control plots recorded 1.30 B:C ratio with minimum returns (Rs 31000.00 per ha). Treatment T2 showed the maximum gross returns (Rs 213000.00 per ha) as well as net returns (Rs 104550.00 per ha). Treatment T1 showed 1.76 and treatment T3 gave 1.77 B:C ratio respectively. The cultivar K.Khyati showed the more returns and B:C ratio as compare to K.Bahar.

In one study regarding fungicidal scheduling, Chakraborty and Banerjee, (2016) revealed that prophylactic spray with fungicide Mancozeb @ 0.2 % followed by combi products of Fenamidone + Mancozeb @ 0.3% as second spray at the time of appearance of disease followed by third

Table 2: Effect of fungicide scheduling on tuber yield and benefit cost ratio

Cultivars	Treatments	Tuber yield (t/ha)	Percent increase in yield over control	Cost of cultivation (Rs/ha)	Gross returns (Rs/ha)	Net returns (Rs/ha)	B:C ratio
K. Bahar	T1	30.90	32.62	108250.00	154500.00	46250.00	1.43
	T2	34.50	48.07	108450.00	172500.00	64050.00	1.59
	T3	29.55	26.82	105950.00	147750.00	41800.00	1.39
	T4	23.30	-	103000.00	116500.00	13500.00	1.13
K. Khyati	T1	38.20	42.54	108250.00	191000.00	82750.00	1.76
	T2	42.60	58.96	108450.00	213000.00	104550.0	1.96
	T3	37.50	39.93	105950.00	187500.00	81550.00	1.77
	T4	26.80	-	103000.00	134000.00	31000.00	1.30
CD (cultivar)		2.20					
CD (treatment)		3.12					

spray with fungicide Mancozeb @ 0.2 % gave the best results against late blight of potato. Gurjar, *et al*., (2019) showed that first prophylactic spray with fungicide Mancozeb @ 0.2 %, second spray with combi product of Dimethomorph + Mancozeb @ 0.3 % and third spray with fungicide Mancozeb @ 0.2 % was found significantly superior for management of disease as disease severity reaches upto 39.44 % at 35 days after appearance of disease. Tiwari, *et al*., (2020) also worked on same problem and revealed that several chemicals are developed to manage various plant diseases by preventing or destroying pathogens causing the disease and their knowledge is important in reducing losses in production.

Cohen and Rubin (2020) revealed that chemical oxathiapiprolin benthiavalicarb or mixture of this chemical can be use as treatment to manage the oomycetes fungus and it provides the systemic protection against disease caused by foliar oomycetes pathogen. Mhatre, *et al*., (2020) reported that combi formulations like M-Cymoxanil + Mancozeb and Chlorothalonil-Ametoctradin + Dimethomorph was highly effective and could considered as the best treatments for southern hills of India against late blight of potato. Tiwari, *et al*., (2021) reported that incorporation of fungicide spray schedule with other practices is the most effective, environment friendly and low-price approach.

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POTATO APICAL LEAF CURL DISEASE SCALE FOR SCREENING OF ADVANCED POTATO HYBRIDS USING NOVEL AGRO-INOCULATION TECHNIQUE

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ABSTRACT: Potato apical leaf curl disease (PALCD) caused by ToLCNDV has emerged as a serious threat to potato cultivation in India. Host resistance to the virus is the potent strategy to curb the menace of this disease. Identification of potato germplasm free from ToLCNDV is a major challenge for healthy seed production and also for an effective resistance breeding programme. Novel and simple agro-inoculation of ToLCNDV was done to select resistant genotypes of potatoes. A set of 9 advanced hybrids and 4 control varieties were evaluated for resistance to ToLCNDV under controlled conditions at ICAR-Central Potato Research Institute, Shimla. Clear symptoms were visible in susceptible genotypes within 30 days after the first inoculation. Based on symptoms of the disease, a scale (0-5) was developed for ToLCNDV resistance screening. Phenotyping results showed the resistant reaction of SM/00-42, SM/00-120 and VMT5-1 to ToLCNDV. However, virus load quantification of inoculated plant samples revealed low virus load in LBY 18, VMT5-1 and SM/00-42. All the four control varieties showed moderately susceptible to susceptible reaction with Kufri Pukhraj showing the highest virus load among all genotypes. The hybrid, SM/00-42 was found to promising due to its resistant reaction and very low virus load. This can be effectively utilized in ToLCNDV resistance breeding or direct deployment after agronomic evaluation. Moreover, the scale developed in the present study could be used in preliminary screening for the disease.

KEYWORDS: Potato hybrids, Screening, ToLCNDV- [potato], Potato apical leaf curl disease scale, Virus load.

INTRODUCTION

Potato has been supporting food security globally for over 400 years since its spread in new continents. It is a vital industrial crop for many nations including India however, there are many hurdles and challenges in potato cultivation. The crop is attacked by many diseases, which are widespread and affect crop growth and production. Viruses are of particular importance in the Indian context due to the cultivation of potatoes in warmer areas. The begomoviruses are the most destructive and are particularly important in this climate change scenario. Particularly, the recombinants of tomato leaf curl viruses are being reported to affect new hosts and cause severe economic losses (Ashwathappa *et al*., 2020; Hanamasagar *et al*., 2021; Venkataravanappa *et al*., 2021). Garg *et al*. (1996) reported a new virus disease in potatoes named potato apical leaf curl disease and the virus was earlier named as Potato Apical Leaf Curl Virus (Garg *et al*., 2001). This whitefly transmitted begomovirus of the geminiviridae family has unique twin particle morphology and bipartite genomes, i.e. DNA-A and DNA-B. Usharani *et al*. (2004) cloned the genomic components of this virus and based on the nucleotide sequence, identified it to be a strain of Tomato leaf curl New Delhi virus (ToLCNDV) in tomato and designated it as Tomato leaf curl New Delhi virus- potato (ToLCNDV - Pot). Symptoms appear as curling of apical leaves with distinct mosaic due to primary infection (Usharani *et al*., 2004) and in secondary infection, the entire plant shows severe leaf

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curling and stunting symptoms (Sohrab et al., 2013). Initially, the sporadic incidence of the disease was reported by Lakra (2003) at Hisar, Haryana, while the severe infection was later observed in Western UP and other areas of North India (Chandel *et al*., 2010; Saha *et al*., 2014). At present, it is one of the important viral diseases of potatoes as severe infection (40-100%) results in heavy yield losses up to 50% in susceptible varieties (Jeevalatha *et al*., 2017a; Kumar *et al*., 2021). Keeping the above in view, it is important to screen the potatoes for ToLCNDV incidence for healthy seed production and an effective resistance breeding program. In the present study, 9 advanced hybrids and 4 varieties as control were evaluated for resistance to ToLCNDV.

MATERIALS AND METHODS

Nine advanced multiple resistant hybrids and four varieties viz., LBY-18, SM/00-120, SM/08-12, VMT 5-1, SM/08-11, SM/00-42, SM/03-23, SM/09-161, SM/05-75, Kufri Jyoti, Kufri Pukhraj, Kufri Surya and Kufri Himalini were used in the present study. The advanced hybrids used in the present study have been developed from the hybridization of elite parental lines involving one of the parents with high resistance to late blight. The hybrids have many desirable tuber traits apart from good tuber yield & other agronomic traits.

Infection through agro-inoculation

The Agro-inoculation method was used to create disease in the potato hybrids by following previously standardized methods (Jeevalatha *et al*., 2017b). The procedure for inoculation involves gentle pricking of seed potato sprouts with fine 5ml syringe needles, followed by incubation in a mixture of agrobacterium cells carrying infectious clones of ToLCNDV isolate MOD-21 (DNA A and DNA B components in 1:1 ratio) for 4-5 hrs in a rotary shaker maintained at 28°C. The treated tubers were planted in medium-size plastic pots at ICAR-Central Potato Research Institute, Shimla in the year 2017. The 2-3 leaf stage plants were again twice inoculated with the same agrobacterium mixture through stem injection and leaf infiltration methods after 15 and 20 days of planting/first inoculation. The pots were kept in the glasshouse, where the temperature was congenial (>25°C) for the virus to multiply. For each genotype five plants were raised, which included three inoculated plants and two un-inoculated controls. The plants were observed for the appearance of ToLCNDV symptoms in the hybrids after 30 days of the first inoculation. The observations in each genotype were recorded in triplicate at 15 days intervals i.e. 30, 45 and 60 days after planting/first inoculation.

Viral load quantification

Leaf samples were collected randomly from the top and middle part of the plants at 30 days after planting/first inoculation and were immediately frozen in liquid nitrogen and stored at -80°C. Total genomic DNA was extracted from the leaf samples using the GenElute™ Plant Genomic DNA Miniprep kit (Sigma-Aldrich, Missouri, USA) following the manufacturer's instructions. The total DNA was quantified and diluted to 20 ng/µl before using in real-time PCR quantification. Real-time PCR assay was carried out to quantify the viral load using coat protein gene-specific primers (LCV-CPFP- 5′-ACCGTCGTCCTACAGGATCTC-3′ and LCV-CPRP-5′-GCTCGGTTCATTGTCAAACATGT-3′) and an increase in viral load was compared between control and inoculated plants as well as susceptible and resistant potato hybrids by plotting C_T values. Absolute quantification of the viral load was performed using serially diluted (10^1 to 10^9 copies) plasmid carrying coat protein gene of ToLCNDV. Leaf samples of three biological replicates were pooled and used in the real-time PCR assay. Three technical replicates were maintained for each sample.

ToLCNDV scale

The disease symptoms on plants were assessed categorically to propose a scale for ToLCNDV resistance screening.

RESULTS AND DISCUSSION

Apical leaf curl disease in potatoes is caused by a variant of ToLCNDV (ToLCNDV) and is one of the most important diseases of potatoes in India. Due to the planting of potato, tomato and cucurbits at the same time and exposure of early sown potato to high temperature, the higher population of whiteflies transmit the virus to potato from tomato and cucurbits (Saha *et al*., 2014; Sohrab *et al*., 2013). The disease is now widely spread in all potato-growing regions of India (Jeevalatha *et al*., 2012, 2013, 2017a; Saha *et al*., 2014). In Indo-Gangetic plains, higher disease incidence with 40–100% infection has been observed resulting in heavy yield losses in susceptible varieties (Venkatasalam *et al*., 2011). It is important to identify resistant hybrids for this virus to sustain potato productivity in the country.

The genotypes under evaluation showed variable reactions to ToLCNDV infection. Initially, the symptoms appeared as chlorotic spots on leaves with light yellowing in susceptible genotypes. Later on curling, crinkling of apical leaves with distinct mosaic symptoms were observed. In severe cases, whole plant leaf curling and stunting was seen. Similar symptoms have been reported earlier in susceptible cultivar Kufri Pukhraj through agro-inoculation (Jeevalatha *et al*., 2017b). To screen the genotypes, it was necessary to first devise a scale based on the symptoms on the plant canopy. We observed the symptoms on inoculated plants in comparison to control plants and also the highly susceptible control plants maintained under controlled conditions. Based on the symptoms recorded throughout the season, a 0-5 scale was devised for the categorization of plant reaction to virus infection (Fig.1). This is the first report of the

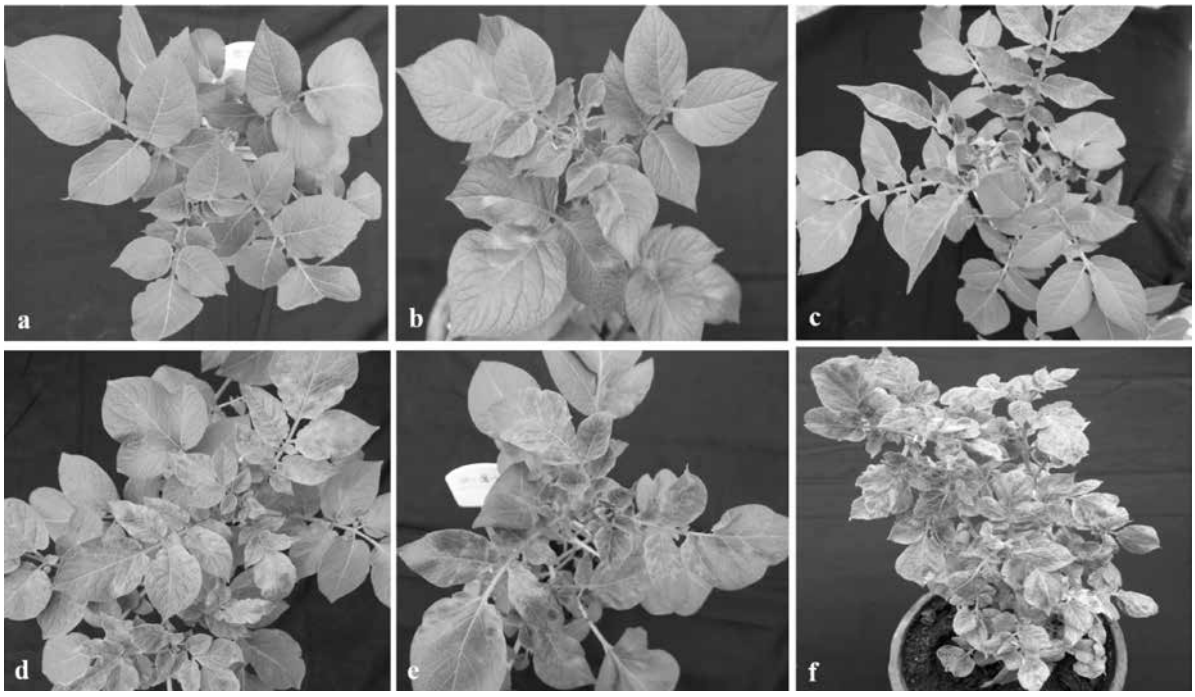

Fig. 1. Scale for apical leaf curl disease based on symptoms in potato plants

development of a scale for ToLCNDV resistance screening in potato genotypes. The symptoms and percentage of infection for classification have been provided in Fig. 1 & Table 1.

a) Highly resistant (Score 0) – No symptoms; b) Resistant (Score 1: 1- 10 % infection) - Yellow spots in few leaves; c) Moderately resistant (Score 2: 11- 25 % infection) - Mild mosaic in most of the leaves and small leaves d) Moderately susceptible (Score 3: 26-50 % infection) - Severe mosaic and crinkling e) Susceptible (Score 4: 51-75 % infection) - Severe mosaic, crinkling, small leaves & rosetting) Highly susceptible (Score 5: >75 % infection) - Severe mosaic, crinkling, small leaves & rosetting, shortening of internodal length (stunted growth).

On the 30th day after the first inoculation, no symptoms on advanced hybrids, SM/00-42, SM/00-120 (0 score) and yellow spots on a few leaves in VMT 5-1 (1 score) were observed. Advanced hybrid, SM/08-11 showed mild mosaic symptoms (2 scores), whereas remaining advanced hybrids and control varieties showed mosaic and puckering symptoms with a score of 3. At 45 days after the first inoculation, the score remained the same for advanced hybrids, SM/00-42, SM/00-120 and VMT 5-1, and there was no progression of symptoms indicating that these hybrids could resist the incidence of ToLCNDV. Hybrid, SM/08-11 showed mild mosaic symptoms, while the severity of symptoms increased in most of the advanced hybrids and control varieties. At 60 days after the first inoculation, yellow spots started appearing in SM/00-42 and SM/00-120 (1 score) also and the symptoms on SM/08-11 remained the same (mild mosaic).

The scale was used to screen the hybrids for their reaction to ToLCNDV. Among the nine hybrids, SM/00-42, SM/00-120 and VMT 5-1 showed a resistant reaction, SM/08-11 showed a moderately resistant reaction, while all others including control varieties were moderately susceptible to susceptible. None of the hybrids was highly resistant to ToLCNDV (Table 1). In an earlier study of field screening of potato genotypes at Hisar,

Table 1. Reaction of genotypes to ToLCNDV

S. No.	Genotype (s)	Symptoms	Mean score*	Reaction
	Hybrids			
1	LBY-18	Severe mosaic, puckering, small leaves, bunchyness	4.3	HS
2	SM/00-120	Few yellow spots	0.3	R
3	SM/08-12	Severe mosaic, small leaves and puckering	3.7	S
4	VMT 5-1	Few yellow spots	1.0	R
5	SM/08-11	Mild mosaic	2.0	MR
6	SM/00-42	Few yellow spots	0.3	R
7	SM/03-23	Sever mosaic, puckering, small leaves	3.7	S
8	SM/09-161	Severe mossaic	3.3	S
9	SM/05-75	Small leaves, bunchyness, mosaic	3.3	S
	Controls			
10	Kufri Jyoti	Severe mosaic, puckering	3.7	S
11	Kufri Pukhraj	Severe mosaic, puckering	3.7	S
12	Kufri Surya	Severe mosaic	3.0	MS
13	Kufri Himalini	Sever mosaic	3.0	MS

*Mean scores recorded after 30, 45 and 60 days after planting /first inoculation

eleven genotypes were found resistant while Kufri Pukhraj showed a highly susceptible reaction with a high white flies population (Mann *et al*., 2017). Several field studies conducted earlier under natural conditions for ToLCNDV resistance have resulted in the identification of resistant genotypes (Kumar and Gupta, 2015; Kumar *et al*., 2015; Lakhra, 2008), however, the plants were artificially inoculated and exposed to congenital conditions in the present study for evaluation and validation.

To support and validate the symptom expression in all genotypes the real-time PCR was performed to quantify the virus load. Among all the hybrids, VMT 5-1 (1.9×10^4), LBY 18 (3.7×10^4) and SM/00-42 (7.0×10^4) had the least virus load in inoculated plants. The highest virus load of $1.03 \times E^{12}$ was observed in Kufri Pukhraj while the other three controls varieties viz., Kufri Himalini, Kufri Surya and Kufri Jyoti showed values in the decreasing order i.e. $2.83\times E^{10}$, $2.56\times E^{10}$ and $8.15\times E^{6}$, respectively. Except for Kufri Jyoti, all other control varieties had higher virus load in comparison to advanced hybrids under evaluation. Three hybrids namely, SM/03-23, SM/09-161 and SM/08-12 recorded high virus load greater than $1.0\times E^{10}$ and their mean phenotype score reading was also >3.3. It reveals that these three hybrids do not have inherent resistance to stop the virus multiplication. In addition, the hybrids SM/08-11, SM/05-75 and SM/00-120 also observed lesser virus load in comparison to susceptible controls i.e. Kufri Pukhraj, Kufri Himalini, Kufri Surya and the three afore-mentioned advanced hybrids (Fig. 2). High viral load in susceptible variety, Kufri Pukhraj and a very low viral load in resistant cultivar, Kufri Bahar have been reported by Jeevalatha *et al*. (2017c). This indicates that although these three hybrids have the genetic resistance to restrict the virus multiplication but is not fully functional. The results observed through quantification using real-time PCR were almost similar to those of phenotypic evaluation based on disease symptoms in different genotypes. The only exception was LBY 18, where the symptoms were clear and showed high susceptibility but virus load was less in comparison to other susceptible genotypes.

Among the hybrids, SM/00-42 and VMT 5-1 showed resistance reaction both in the appearance of disease symptoms as well as quantification of virus load through real-time PCR of inoculated as well as uninoculated plants. Both of these advanced hybrids, SM/00-42 and VMT 5-1 appear ideal candidates for deployment in resistant breeding to ToLCNDV or as the direct release for cultivation based on other agronomic traits and adaptability traits. Quantification of virus load and disease scale corroborates the reliable selection of resistant genotypes. Moreover, the high similarity between symptom expression and virus quantification results indicate that the genetic resistance of potato genotypes can be easily identified using the proposed scale and the same can be effectively utilized for preliminary screening of hybrids for resistance to ToLCNDV in potato.

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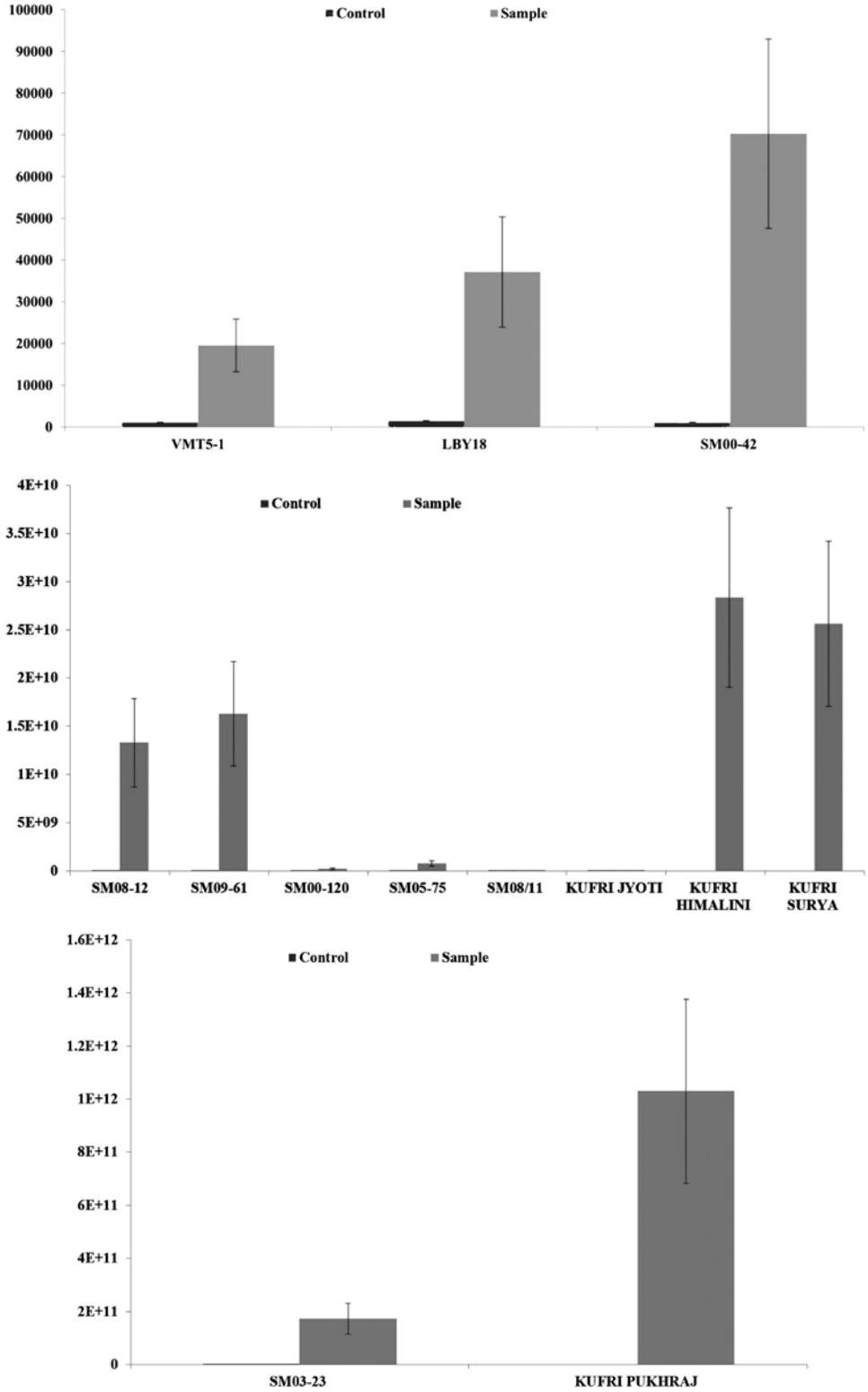

Fig 2. Virus load quantification through real-time PCR in control vs inoculated samples of hybrids.
a) low virus load genotypes; b) medium to high virus load genotypes; c) very high virus load genotypes

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MORPHO-MOLECULAR IDENTIFICATION OF POTATO SILVER SCURF CAUSED BY *HELMINTHOSPORIUM SOLANI* AND STANDARDIZATION OF SPORE INUNDATION TECHNIQUE

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ABSTRACT: Potato is considered as a future crop to secure food security in developing nations. Of many storage diseases that impede the trade value of potatoes, silver scurf is steadily rising. The causal fungus *Helminthosporium solani*, belongs to *Ascomycetes* group and it attacks the potato tubers in the field as well as in storage. During the year 2019 the tuber samples were obtained from cold stores of Madhya Pradesh which were symptomatic to silver scurf disease. The appearance of tuber comprised of silvery skin blemishes on the periderm of tubers. The inundation of spores was standardized from the silvery lesions by incubation of infected tubers at different temperatures (12, 18, 24 °C). The spores were observed in the form of whorls on the tuber surface and these whorls were also visualized in compound microscope using double adhesive tape method. The pure culture of fungus produced initial hyaline mycelium which turned blackish brown after 21 days. The fungus was observed as extremely slow in its growth. The conidiophores were septate having a thin apex and broader base and melanized conidia were observed in a basipetal succession. The conidia were recorded to have two to eight pseudosepta. The molecular identification of fungus was performed through internal transcribed region based universal primers. The obtained sequences were submitted to the NCBI *GenBank*. An attention towards the pathogenomics, infection process and management strategies of silver scurf disease is mandatory to strengthen the processing industries involved in potato production system worldwide.

KEYWORDS: Silver scurf; *Helminthosporium solani*; Mycelium

INTRODUCTION

As per the estimation of global consumption rate, the potato (Solanum tuberosum L.) comes first among the noncereal food crops (Devaux *et al*., 2019; Kumar *et al*., 2019, 2020; Lal *et al*., 2020a, 2020c, 2020b; Tiwari *et al*., 2020b). Moreover, the potato crop is also designated as future vegetable crop to ensure food security since 74% of total produce is utilized for consumption. The main reason behind such widespread importance is the short maturity period, diverse cultivation pattern, high calorific value and nutrition index of this wonder crop (Lal *et al*., 2021a). The leading vegetable crop occupies 19.23-million-hectare area with a global annual production of 390 million tonnes (Kumar *et al*., 2021). India comes after China in potato production and potato is grown on 2.13 million hectares with production and productivity of 44 million tonnes and 20.5 tonnes per hectare respectively (Kumar *et al*. 2019; Kumar *et al*., 2021). There is a consistent focus to increase the productivity of this crop but at the same time minimizing losses due to biotic and abiotic stresses is also critical (Tiwari *et al*., 2020a, 2020b). The biotic stressors such as fungi, viruses and bacteria are consistent threat in potato production system (Kumar *et al*., 2020d, 2021c). The late blight of potato

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which was the major cause of historical devastation on potato crop, still troubles the potato growers (Sharma *et al*., 2021). Likewise, there are more than 40 viruses which hampers the potato production worldwide (Naga et al., 2019). In addition to that, with globalization and trade and changing consumer preference the occurrence of storage rots such as soft rot, dry rot, silver scurf, black scurf and other blemishes are also emerging as major concern to potato growers (Tiwari *et al*., 2020b, 2020a). These storage rots not only infest the tubers during storage and cause direct loss but also appear in fields in the form of wilts and stem rots that leads to indirect loss to the crop. These blemishes and lesion were earlier considered as minor damage but with the rapid advancement of processing industries the demand of blemish free potato tubers has increased manifolds. Moreover, the infection on tubers not only cause quantitative damage but qualitative loss in term of disturbed nutritional profile of tuber has also been reported (Kumar *et al*., 2020a, 2021a; Lal *et al*., 2020d, 2021c, 2021b; Singh *et al*., 2020; Raigond *et al*., 2021).

Among the skin blemish disease, the potato silver scurf caused by fungus *Helminthosporium solani* is a rapidly gaining importance as major challenge to potato cultivation and storage system. The disease is of worldwide economic importance due to its appearance in field at harvesting and during storage. The incidence of this disease may reach up to 80% as previously reported (Cunha and Rizzo, 2004). A survey-based study by (Nærstad *et al*., 2012) reported the incidence of silver scurf in 93% of potato samples comprised of 247 potato lots for skin blemishes in different regions of Norway. The silvery lesions covered 11-13% of the tuber area which was highest among all the skin blemish diseases. Previously, Bradshaw *et al*. (2002) reported 98% incidence of silver scurf and cited it most severe among skin blemishes of potato in cold stores of England and Wales. Likewise, in USA 75% of asymptomatic tubers were reported to possess *H. solani* infection in ten leading potato cultivars (Mattupalli *et al*., 2013). These observation across the areas of potato cultivation increases greater concern that the visual evaluation of tubers is not adequate to conclude that the tuber is free from silver scurf. The processing purpose potatoes face more challenges due to the infection as these silvery lesions enhance the permeability of periderm of tubers that cause weight loss and shrinkage of tubers (Raigond *et al*., 2020; Tiwari *et al*., 2020c; Kumar *et al*., 2021b; Lal *et al*., 2021b). The coloured potato cultivars lose the pigmentation due to heavy infection and the market value of fresh produce is severely hampered (Jellis, 1975). The disturbed processing quality of tubers can be estimated by the report that chips made from severely infected tubers comprise of blackish burnt appearance (Errampalli *et al*., 2001). The emergence of thiabendazole resistant strains from potato growing regions areas of the world has created more awareness in the exploration of suitable management practices for this disease (Hide *et al*., 1992; Merida & Loria 1990; Holley & Kawchuk 1996; Kutuzova *et al*., 2017; Kutuzova *et al*., 2017).

The present study mainly establishes the aetiology of this disease through morphological and molecular identification techniques. The isolation of pathogen from the tuber periderm is a difficult due to slow growing nature of the fungi. But the initial identification and spore visualization was performed with the help of symptomatic tubers via incubation at an optimum temperature range in a moist cabinet. Moreover, after the initiation of spores the pure culture was obtained through subculturing on suitable media. Further, the

mycelial boll formation and DNA extraction was performed, and fungus was molecularly identified using universal ITS primers. The pathogen culture and developed spore inundation technique will assist in *in vivo* identification of the fungus and development of suitable management strategies.

MATERIALS AND METHODS

Pathogen isolation and pure culture

During the year 2019, symptomatic potato tubers were obtained from commercial sheds in Madhya Pradesh. Approximately, 60% area of the tuber surface was covered with silvery lesions. The pathogen isolations were made by washing infected tubers with distilled water and tissue fragments were excised from lesion margins, disinfested in 5% NaOCl for 2 minutes, dried on sterile paper towel and placed on either clarified carrot juice agar media (Himedia) amended with 20 ml/L of 0.25% streptomycin sulfate, or potato dextrose agar (PDA, Himedia) amended with 0.25% streptomycin sulfate. The plates were incubated at 25° C in the dark and examined every other day (Cullen *et al.*, 2001). As soon as the mycelia growth was evident from the fragments the hyphal tip was transferred to a new plate with similar conditions. These fresh plates were also incubated in dark at 25° C and periodic observations were made for the fungus growth.

The standardization of spore inundation

The on-tuber spore proliferation was standardized to select a suitable temperature. The infected tubers were washed with running tap water, surface sterilized with 1% NaOCl for 2 min followed by drying. Three different temperatures (12, 18, 24°C) were selected to observe the proliferation of spore from the diseased lesions. The diseased tubers were kept in a dark moist cabinet at respective temperatures to maintain the relative humidity (>90%) for 21 days. Using a stereoscopic microscope, the progressive emergence of fungal assemblies on lesions were periodically observed. This technique was also used for pure culture isolation directly from the developed fungal growth on lesions. A small hyphal growth was excised using a sterile blade and placed on antibiotic amended half strength PDA. The plates were agitated for spore dispersion in the media and incubated at 25° C in dark for 4 days. Again, with the help of stereoscopic microscope the geminated single spore was transferred to fresh carrot juice agar media plates and incubated for 3 weeks. Symptoms were visually assessed and the range of sporulating lesions per tuber recorded. Using the previously described isolation approach, re-isolations were performed on every treatment replica (Rodriguez *et al.*, 1995). Three replications were maintained at each storage temperature. The statistical analysis was performed using GraphPad software.

Morphological and molecular identification

The colonies developed after pure culture were assessed for colony morphology and colour, the morphology of conidia and conidiophores. The mycelial plugs were observed using a dissecting microscope for conidiophore structure and a compound microscope for conidia. Isolates were confirmed as *H. solani* based on the previously described keys (Olivier and Loria, 1998). To observe the proper spore attachments with the conidiophore the double adhesive tape technique was utilized. Once the morphology was established the mycelial plug was inoculated to Potato dextrose broth and kept at 200 rpm for 21 days. The developed mycelia mat was strained in laminar cabinet and the DNA extraction was performed as previously

described by the method which includes phenol chloroform extraction (Mattupalli *et al*., 2013). The concentration of DNA was checked on nanodrop and it was stored at -20°C for further experimentation. Universal primers targeting internal transcriber spacer (ITS) region for the detection of *H. solani* were used. The primers ITS1, 2 amplified a fragment of 550-bp for detection of *H. solani* in through mycelium (Pérez *et al*., 2011). For ITS, initial denaturation was performed at 95°C for 3 min followed by 40 amplification cycles of 95°C for 45 s, 68°C for 45 s, and 72°C for 90 s. After 40 cycles, amplicons were incubated for another 8 min at 72°C for a final extension and a 10°C soak. To estimate the concentration and quality of PCR, 1 µL of amplicon from the PCR reaction was run on a 1.5% agarose gel with 0.5 µg of ethidium bromide ml–1 and 0.5 × Tris borate EDTA buffer for 45 min. DNA bands were visualized on a UV trans-illuminator. PCR amplicons were sequenced using the BigDye Terminator V2.0 ready reaction Kit (Applied Biosystems, CA, USA) and 0.5 × reactions.

RESULTS AND DISCUSSION

Pathogen isolation pure culture and symptomatology

The characteristic symptoms on the infected tubers were observed as blemishes or grey lesions on the periderm of tubers that appeared silvery when the tubers were moistened and incubated (Figure 1a, b). Discoloration of the lesions on periderm subsequently observed due to suberin deposition and cell desiccation. The silvery lesions were observed nothing but erumpent mass of conidia and conidiophores. The dispersed lesions later merge to develop into larger shiny silvery patches having a whorl of conidiophores bearing conidia in a typical basipetal fashion observed under a stereomicroscope. The pure culture plate of fungus has been provided in Figure 1 (c, d). Initially, the fungus appeared as hyaline mycelia which turned blackish brown on artificial media over the period of one month. The growth of mycelia was extremely slow, and it took more than three weeks to cover the petri plate (90mm diameter).

Spore proliferation on infected tubers

Three different temperatures were chosen to liberate the spores from the silvery lesions under 90-95% relative humidity conditions. The periodic observations revealed that maximum spore inundation occurred at 24°C as compared with 12°C or 18°C (Figure 2). At 12°C there were minute black dots which took 15 days in initiation and spore liberation was extremely low when counted using haemocytometer. The spore count was observed as 1.2×10^2 at the specified temperature. However, at 18°C the minute black dots appeared within 10 days and the hyphal tip initiation and spore mass was evident on the lesions after 20 days of incubation. The spore count was also elevated as it was observed up to 3.2×10^4.

Figure 1 a, b: the typical symptoms of potato silver scurf showing co-leased lesions and spore proliferations on tubers; c, d: colony growth of Helminthosporium solani.

Interestingly, at 24°C the initial of black blemishes occurred within a week and an erumpent mass of conidia and conidiophores were observed after two weeks on incubation. There was an extensive sporulation near the crevice and other depressions nearby stolon. The spore count had reached 2×10^5. The tuber eyes were also heavily colonized from the spore mass. These observations emphasize that the processing purpose tubers which are kept at 12°C are relatively safer from this disease as minor infection may not cause severe damage due to lack of sporulation. However, the tubers stored above that temperature and moreover the frequent fluctuation in the cold store that leads to build-up of heat may initiate the infection in other healthy tubers from the infected ones. Previous reports also highlight that table purpose potato stored at 4°C were less vulnerable than processing potatoes. Previously it was also reported that the conidial count on infected tuber surface was higher at 10-18°C as compared to 4°C (Rodriguez *et al.*, 1995). It was also recorded that the asymptomatic tubers developed scattered sporulation and shiny lesions after four weeks of incubation at 15 °C temperature and 90% relative humidity.

Morphological and molecular identification

The conidiophores were septate and had thin apex and broader base on which melanized conidia were developed in basipetal succession. The conidia possessed two to eight pseudosepta which arise in whorls on conidiophores (Figure 3 b, c). Conidiophores displayed the Christmas tree-like appearance, with the first conidium on the terminal position and 7 to 12 conidia arranged in a basipetal succession on the conidiophore (Figure 3c). The conidia were club shaped, cylindrical, melanized, 3–8 celled (Figure 3a). The size varied from 12.0 to 57.0 µm in length to 4.0 to 9 µm in width. Similar morphological features were depicted earlier and based on the characteristic appearance of conidiophores and conidial dimensions the fungus was identified as *H. solani* (Barnett and Hunter 1998). Similar pattern of conidiation, shape and size of conidia and pigmentation were also previously reported from semi-arid U.S. Pacific Northwest and western Washington (Inglis *et al.*, 2019; Miller *et al.*, 2015). The pathogenicity was proved on cultivars 'Kufri Pukhraj'.

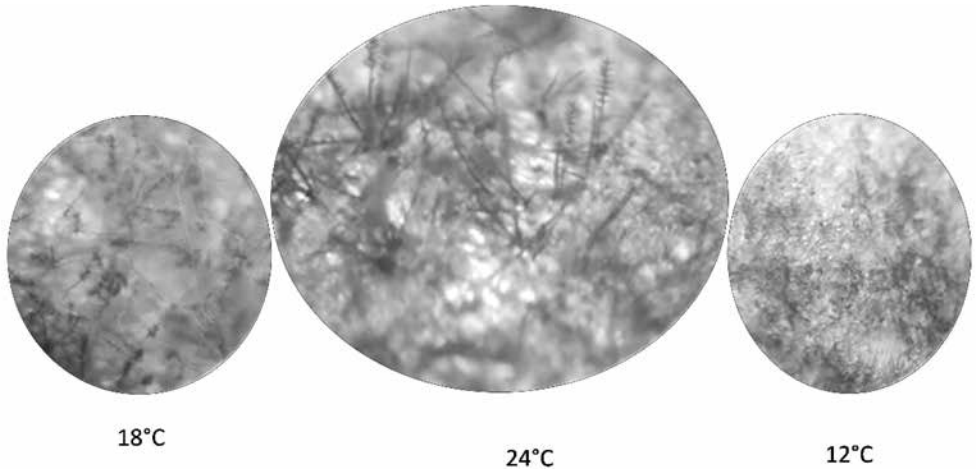

Figure 2: The pattern of sporulation at different temperature regimes

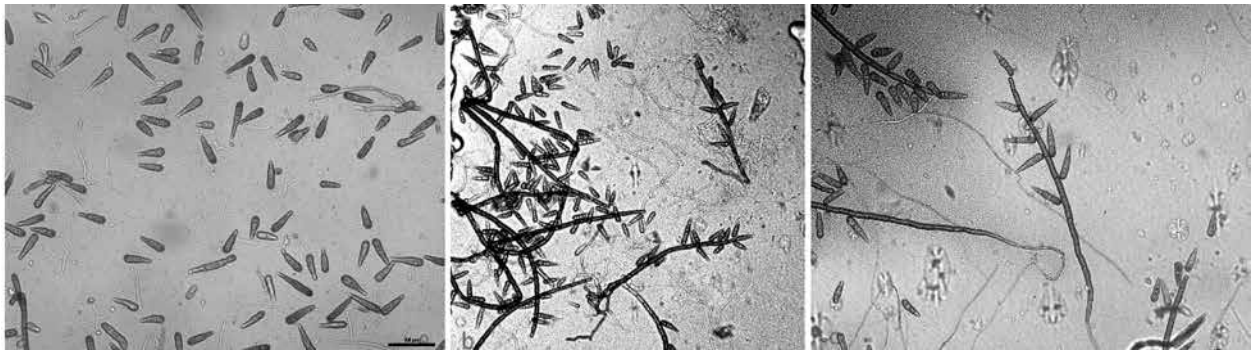

Figure 3: The morphological features of H. solani observed under compound microscope, a: solitary melanized conidia; b: the conidiophore bearing conidia at 20X; c: conidiophore and conidia at 40X

The morphological identification was further supplemented with molecular identification using ITS based PCR primers. The primer pair ITS1, 2, directed the amplification of a 550-bp target sequence in genomic DNA from the pure culture of fungus. The amplified bands were excised from the gel and purified GeneJET TM Gel Extraction Kit (Thermo Fisher Scientific). The products were sequenced using forward and backward primer (Eurofins Genomics India Pvt. Ltd). BLASTn analysis of the ITS sequence (Accession no. OK035227) showed more than 99% similarity (e-value: 0.0) to *H. solani* isolate CBS 365 (KY984341.1) and other isolates across the world. The phylogenetic tree was also constructed to analyse the evolutionary relationship (Figure 4). Previously the causal agent of silver scurf has been reported to be *H. solani* by using ITS based primers (Errampalli *et al.,* 2001). Mattupalli et al. 2013 utilized similar set of primers to identify the fungus from the asymptomatic organic potatoes.

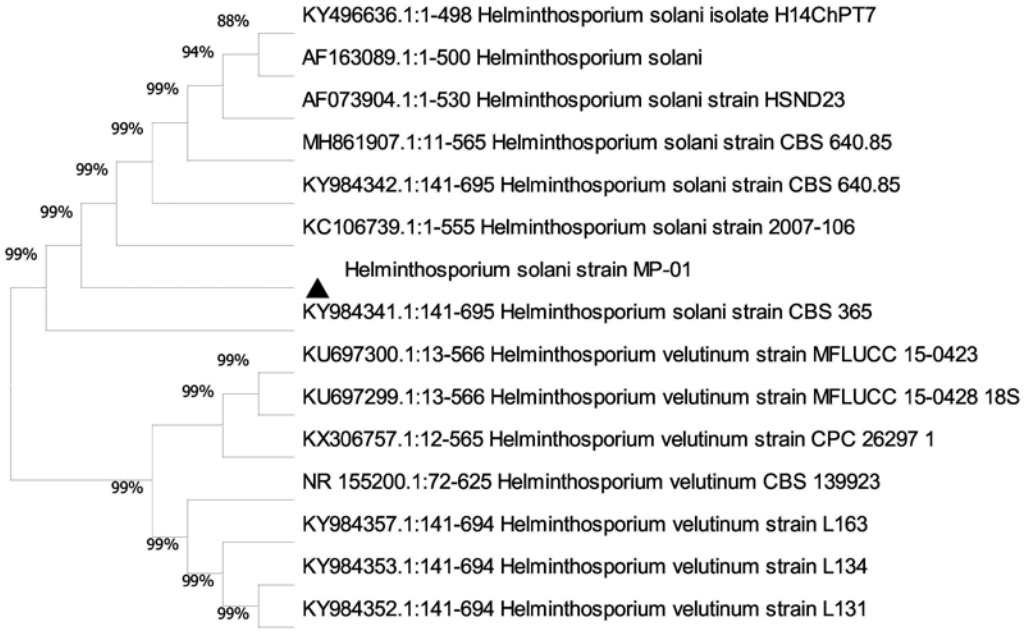

Figure 4: The phylogenetic tree of the isolate MP-01 constructed using neighbour-joining method using 1000 bootstrap value.

Subsequently the same group of researchers sequenced the whole genome of *H. solani* in the year 2014.

CONCLUSION

The potato silver scurf is a skin blemish storage disorder of potato having a worldwide concern. The diseased samples were obtained from a cold store and depicted typical silvery lesions scattered on the tubers. Initial observation and incubation gave an indication of *H. solani* infection. The spore proliferation was evaluated at a range of temperature, and it was observed that 24°C is the best sporulation temperature that produces the huge conidia mass in less time interval. The isolation and pure culture of pathogen was subjected to morpho-molecular identification and the aetiology of the disease was established. This information will assist in studying the epidemiological aspects of this disease coupled with designing of suitable management strategy.

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COLD-INDUCED SWEETENING IN TUBER ENDS OF POTATO (*SOLANUM TUBEROSUM* L.) GENOTYPES

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ABSTRACT: It is a known fact that cold-induced sweetening (CIS) is produced more in tuber ends that rest of tuber parts in potatoes. But most of the studies so far have been directed to whole tuber while tuber end studies are reported very rare. In the present study, CIS is measured in tuber ends of potato genotypes, "Lady Rosetta" and "Kufri FryoM" (processing), "Kufri Sindhuri" and "MS/8 1148" (non-processing) during storage at 4° (cold) and 25°C (control) in four-way interaction experiment. Tubers were harvested after 30 d of haulm cutting and hardened in field for 10 d and then stored. Tubers were analysed at 0, 20, 40, 60 and 90 d after storage. Results found that amylases increased, and starch decreased at 4°C in non-processing genotypes only and changes were more marked in basal than apical ends. Starch decreased at earlier stages (20 and 40 d), but amylases increased throughout the storage, at 4°C. Soluble sugars increased at 4°C where glucose/ sucrose increased relatively earlier (at 20-40 d) than fructose (at 20-60 d). Increase of glucose and fructose at 4°, was higher in non-processing than processing genotypes and in basal than apical ends. However, increase of sucrose at 4°, was higher in "Lady Rosetta" and "MS/8 1148". Principal component analysis (PCA) related fructose, more closely than glucose, to amylolysis. Results indicated that sucrose synthetic/breakdown activities may be involved in CIS, and sucrose synthetic activity may relate to CIS-resistance of "Lady Rosetta".

KEYWORDS: Amylase, Fructose, Glucose, Invertase, *Solanum tuberosum* L

INTRODUCTION

Cold storage of potatoes at less than 9°C effectively extends storage life by reducing tuber respiration, fresh weight loss, disease pressure, and sprouting, but this quality is compromised by the induction of cold-induced sweetening (CIS) (Herman *et al.* 2016). CIS occurs due to the catabolism of starch to reducing sugars, glucose and fructose (Glc + Fru) (Herman *et al.* 2016). These sugars during frying serve as substrates for Maillard reaction, which gives rise to off-flavor, dark pigments, and acrylamide (probable human carcinogen) (Herman *et al.* 2016).

In the present study, four genotypes varying in CIS resistance were studied by taking tissue from tuber ends. Tuber ends are known to produce high CIS compared to other tuber parts, where basal (stem) end defect is reported to be the most common defect (Thompson *et al.* 2008). However, tuber end studies are reported less in the literature. Among four potato genotypes, "Lady Rosetta" and "Kufri FryoM" are the known processing varieties, while "Kufri Sindhuri" and "MS/8 1148" were non-processing genotypes. Work was planned at two storage temperatures, 25°, and 4° C, to know the cold-induced effect over average room temperature.

MATERIALS AND METHODS

Plant materials

Tubers of potato (*Solanum tuberosum* L.) genotypes, "Lady Rosetta", "Kufri FryoM", "Kufri Sindhuri", and "MS/8 1148", were obtained from the fields of the Department of Vegetable Science, Punjab Agricultural University, Ludhiana. Tubers were harvested

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30 days after haulm cutting and hardened in the field for the next 10 days then heaped for each genotype. From the heap of each genotype, average-sized tubers (i.e. neither too big nor too small in size) were picked randomly by observing with naked eye and packed in jute bags (about 100 tubers per bag) and stored at 4° and 25°C. Tubers were analyzed at 0, 20, 40, 60, and 90 d after storage, where 0 day was the storage day. For each measurement, four tubers per genotype were selected randomly, washed under tap water, then in distilled water, and tubers were then sliced at apical end and basal ends (approximately 1 to 1.5 cm from each end). These basal (stem) ends or apical (bud) ends were consolidated into a single sample and used for measurements in three replicates (Dhillon *et al.* 2021).

Biochemical measurements

Extraction and estimation of sugars and starch was done as given (Kaur and Zhawar 2021). Tissue (0.1 g) was extracted twice with 80% methanol, and the extract was filtered through Whatman filter paper 1. The supernatant was kept at 50-60° C to evaporate methanol, and volume was made with distilled water. This was used to estimate glucose, fructose, and sucrose. Residues were dried and used to estimate starch. Free glucose was assessed by an enzymatic method where the extract was incubated in the total volume of 2 ml containing 1.8 units of glucose oxidase, 0.6 units of peroxidase, 0.015% o-dianisidine, and 22.5% glycerol at 30° C for 90 min. Then the reaction was terminated in 1N HCL. The content was read at 540 nm and calculated using glucose standard (0.1 to 0.5 µmol). For sucrose estimation, the extract was first incubated with commercial invertase (0.25U mL^{-1}) in 0.1 M sodium acetate buffer pH 5 for one hour at 37° C, and then reaction stopped in boiling water bath for 10 min and glucose estimated as above. Free glucose value was subtracted from the invertase-treated value to calculate sucrose. Fructose was estimated by subtracting bound fructose from total fructose. Total fructose was assessed by incubating extract in 0.025% resorcinol, 23.75% ethanol, 22.5% HCL at 80° C for 20 min, and then reading at 540 nm. Standard fructose (0.1 to 0.5 µmol) was used to calculate the amount. Bound fructose was estimated after destroying free fructose in 15% NaOH at 100° C for 10 min. For starch estimation, the dried residue was incubated twice in 30% perchloric acid at 4° C, then centrifuged at 10, 000 × g for 20 min then glucose was estimated from supernatant by anthrone method (0.2% anthrone in 95% ice-cold H_2SO_4) and the value was multiplied by 0.9 to get starch value.

Amylases were estimated as given (Srivastava and Kayastha 2014). Amylases were extracted in 100 mM sodium acetate buffer pH 5.5 with 1 mM beta-mercaptoethanol and 3 mM $CaCl_2$ and 20% glycerol. Amylases were assayed in 0.8% starch, and 50 mM sodium acetate buffer pH 5.5 at 30°C where the reaction was stopped with DNS reagent, thereafter, reducing sugars were estimated by the DNS method where maltose (1-5 µmol) was used as standard.

Statistical analysis

Data were subjected to four-way factorial ANOVA in a randomized complete block design in DSAASTAT-XLX (Onofri and Pannacci 2014) to determine effects of genotype, storage temperature, duration, tuber end and their interaction. Data in the interaction were analyzed with least-squares means and separated with Fisher's least significant difference (L. S. D.) at $p < 0.05$. Multivariate analysis was done with principal component analysis (PCA). The least-square means and PCA were determined using emmeans and ggbiplot packages respectively in RStudio (ver. 1.4.1717, Boston, MA).

RESULTS AND DISCUSSION

ANOVA result (Table 1) showed the significant effect of storage temperature, duration, genotype, and tuber ends and their interaction on sugar attributes.

Storage at 4° C is compared to 25° C (control). Only statistically significant results are presented.

Starch and amylases

Starch levels (Figure 1A) decreased at 4° by 50% in apical and 80% in basal ends of "Kufri Sindhuri" and 50% in basal ends of "MS/8 1148" at 20 d, thereafter, starch increaserd to the level of 25° in both genotypes. In "Lady Rosetta" and "Kufri FryoM", starch was not reduced rather increased 4° except in the basal end of "Kufri FryoM" at 40 d where starch decreased by 20%. Starch levels were higher in apical than basal ends at many stages in all genotypes, at 4° C. During cold storage, trend of starch was decreasing.

Amylases (Figure 1B) increased at 20 d in both ends of "Kufri Sindhuri" and "MS/8 1148" and basal end of "Kufri FryoM" while at later stages (60 and 90 d), amylases increased in all genotypes, at 4° C. The highest amylase was seen in the basal end of "MS/8 1148" at 60 d at 4° C. Amylase levels were low in "Lady Rosetta" compared to other genotypes, at 4° C at 40, 60, and 90 d. Amylase levels were higher in basal than apical ends at many stages in all genotypes at 4° C. During cold storage, amylase increased to 60 d then decreased.

The process of cold sweetening in tubers is reported to be based on starch mobilization in amyloplasts shown by activation of amylolytic enzymes (Zhou and Solomos

Table 1. Analysis of variance for the effect of storage duration, storage temperature, genotype and tuber-ends and their interaction on sugar attributes of potato during post-harvest storage

Source of variation	df	MS					
		Starch	Amylase	Glucose	Fructose	Sucrose	Sucrose/Hexose
duration (D)	4	109713.7**	11908.76**	74.45**	89.44**	784.89**	5.99**
temperature (T)	1	470.8**	7802**	125.84**	67.5**	1772.67**	1.72**
T × D	4	19742.8**	1594.05**	11.06**	14.77**	139.58**	2.58**
genotype (G)	3	31973.8**	1988.74**	30.71**	1.25**	1321.24**	14.99**
G × D	12	9513**	277.3**	4.87**	6.99**	105.16**	4.9**
G × T	3	42692.9**	292.04**	0.57**	0.88**	102.87**	0.05
G × T × D	12	12131.4**	121.78**	0.49**	1.11**	26.77**	0.7**
tuber ends (E)	1	17845.2**	3.4	8.86**	0.49*	68.81**	9.07**
E × D	4	26830.4**	303.15**	5.66**	6.97**	41.94**	5.81**
E × T	1	2928.5**	547.63**	0.65**	8.7**	11.78**	2.92**
E × G	3	10575.3**	219.21**	0.28**	1.22**	7.34**	1.49**
E × D × T	4	1543.2**	93.92**	0.2**	2.09**	4.25**	0.44*
E × D × G	12	20398.5**	148.4**	0.41**	3.5**	12.27**	1.44**
E × T × G	3	1968.7**	164.94**	0.23*	2.25**	0.62	0.89**
E × D × T × G	12	986.1**	67.99**	0.22**	1.3**	2.47**	0.3**
Residual	80						
Total	239						

*, ** significant at p <0.05 or p < 0.01 respectively.

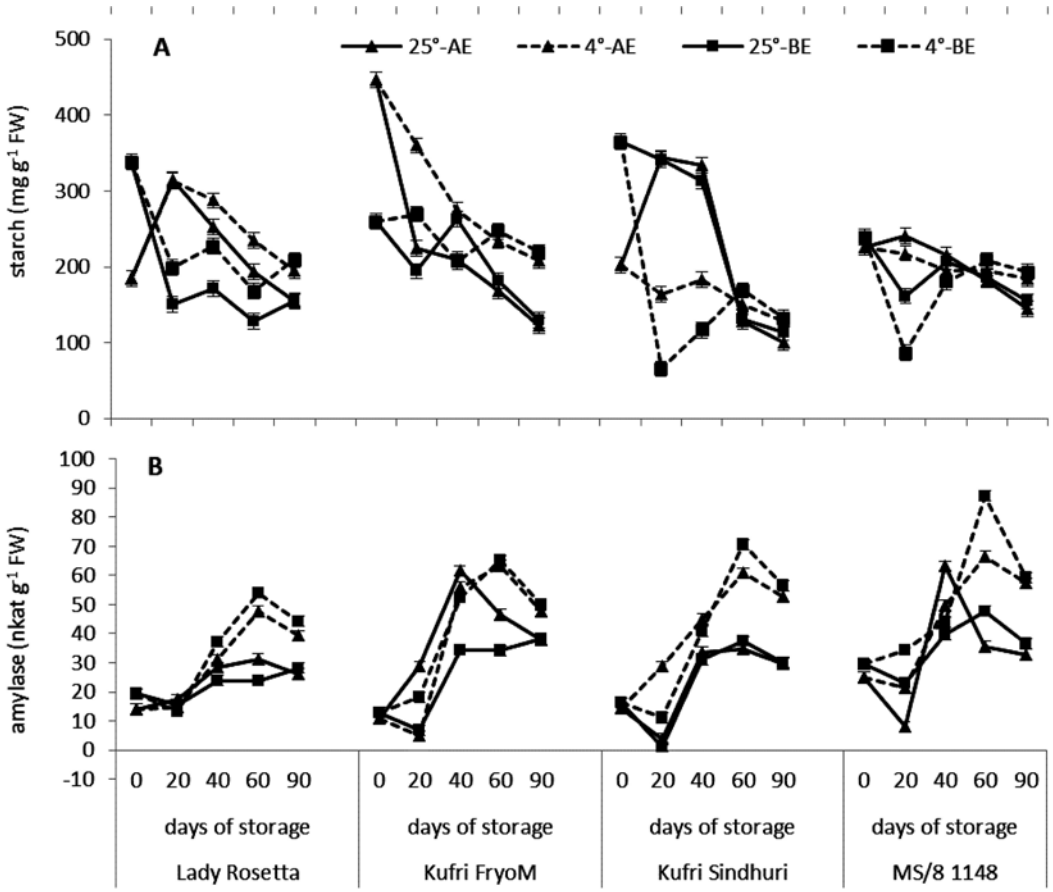

*Figure 1. Change of starch (mg g^{-1} FW) (**A**) and total amylase activity (nkat g^{-1} FW) (**B**) in tuber apical end (AE) and basal end (BE) at 0, 20, 40, 60, 90 d of storage at 25° and 4° C in potato genotypes, "Lady Rosetta", "Kufri FryoM", "Kufri Sindhuri" and "MS/8 1148". Data points are the least-square means of replicates (n = 3), and vertical bars indicate standard errors. In some instances, standard errors are small enough to be obscured by the line width.*

1998). Conversion of starch to reducing sugars by amylases is considered one of the main pathways in cold-induced sweetening in potato tubers (Wiberley-Bradford *et al.* 2016, Hou *et al.* 2017). The present results related cold-induced susceptibility of the genotype with starch degradation due to the gain of amylase during the early stages of cold storage. However, during late cold storage, amylases were gained by all genotypes, but starch was not reduced. Thus, starch re-synthesis might be operational during late cold storage (Sergeeva et al., 2012).

Liu *et al.* (2016) suggested that starch degradation and active transport of carbohydrates remained high in the basal ends during storage, to transport assimilates to other tuber parts during early, while sprouts during late storage. The present results found starch catabolic activity high in basal compared to apical ends during cold storage.

CIS (glucose and fructose) levels

Glucose (Figure 2A) levels were higher at 4° than 25° C at 20, 40, 60 and 90 d in all genotypes. Increase of glucose at 4°C at 20 d, was 2 to 2.5-fold in "Kufri Sindhuri" and "MS/8 1148" while 1.2 to 1.3-fold in "Lady Rosetta" and "Kufri FryoM" in basal ends. In apical ends, fold increase was 1.5 to 1.7-fold

in other three genotypes while 1.3-fold in "Lady Rosetta". During cold storage, glucose trend was the increase between 0 to 20/40 d then decrease to 90 d. Glucose levels at 90 d were higher in "Kufri Sindhuri" and "MS/8 1148" than "Lady Rosetta" and "Kufri FryoM" at 4° C. The highest glucose level was seen in the apical ends of "MS/8 1148" at 20 d at 4° C. Glucose levels were seen higher in basal than apical ends at many stages of cold storage in genotypes. Results showed that glucose increased during cold exposure but decreased during acclimation. Cold induced increase of glucose was higher in non-processing than processing genotypes and in basal than apical ends.

Fructose (Figure 2B) levels were higher at 4° than 25° C at many stages in all genotypes. Increase of fructose at 4°C at 20 d, was higher in "Lady Rosetta" and "MS/8 1148" than "Kufri Sindhuri" and "Kufri FryoM" in basal ends while in apical ends, fold increase was higher in "Lady Rosetta" and "Kufri Sindhuri" than "MS/8 1148" and "Kufri FryoM". However, at 40 and 60 d, fold increase was higher in other genotypes than Lady Rosetta in basal ends. Highest fructose level was seen in the basal ends of "MS/8 1148" at 40 d at 4°C. During cold storage, fructose trend was increase till 40/60 d then decreased to 90 d. At 90 d of cold storage, fructose levels were higher in "MS/8 1148"

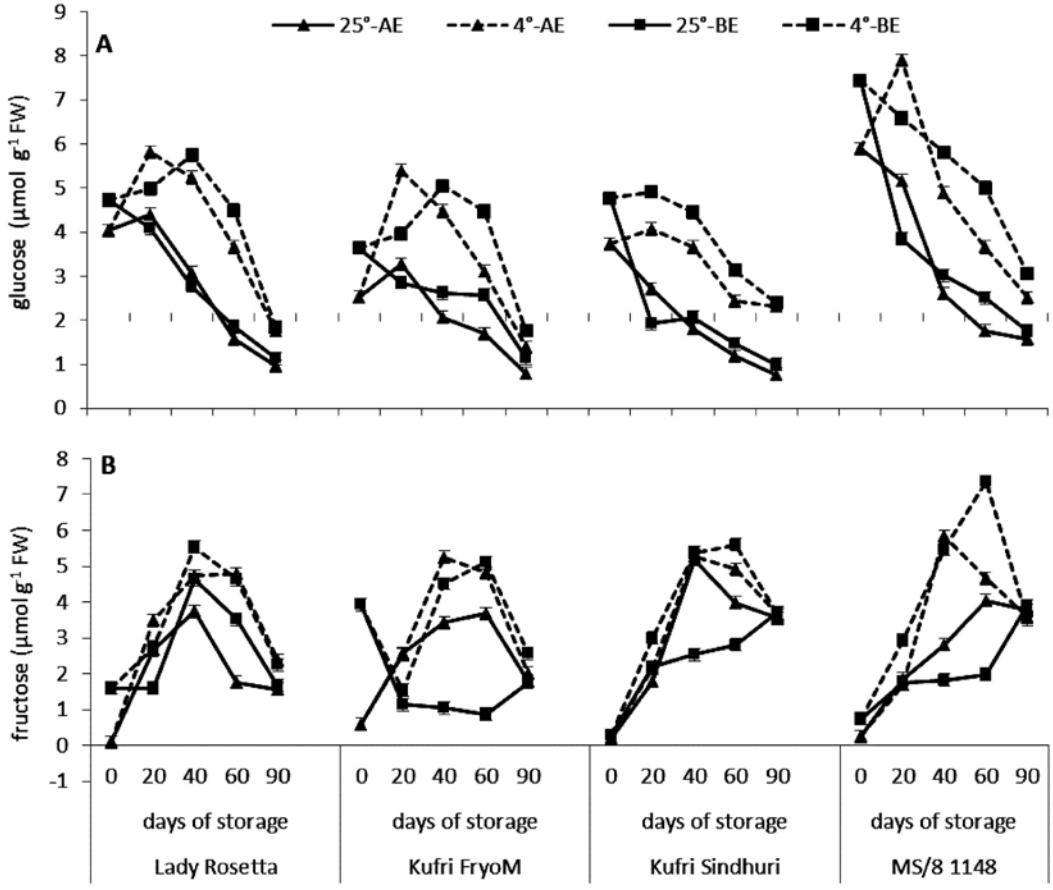

*Figure 2. Change of glucose (µmol g^{-1} FW) (**A**) and fructose content (µmol g^{-1} FW) (**B**) in tuber apical end (AE) and basal end (BE) at 0, 20, 40, 60, 90 d of storage at 25° and 4° C in potato genotypes, "Lady Rosetta", "Kufri FryoM", "Kufri Sindhuri" and "MS/8 1148". Data points are the least-square means of replicates (n = 3), and vertical bars indicate standard errors. In some instances, standard errors are small enough to be obscured by the line width.*

and "Kufri Sindhuri" than "Lady Rosetta" and "Kufri FryoM". Fructose levels were higher in basal than apical ends at many stages of cold storage in genotypes. Results showed that fructose followed glucose accumulation during cold storage.

As reviewed (Thompson *et al*. 2008), CIS occurred higher in tuber basal than apical ends. The increase of amylase, glucose and fructose under cold was higher in basal than apical ends in "Kufri Sindhuri" and "MS/8 1148".

Fold increase of glucose was seen high in "Kufri Sindhuri", while CIS levels were highest in "MS/8 1148". Glucose increased by 2.6-fold in the basal end of "Kufri Sindhuri" while 1.2-fold and 1.4-fold respectively in the basal ends of "Lady Rosetta" and "Kufri FryoM" at 20 d at 4°C. "MS/8 1148" was the least dormant while "Lady Rosetta" was the most dormant genotype among eleven genotypes studied (unpublished data). Thus, high levels of sugars including sucrose can be due to the high metabolic state in "MS/8 1148" (Dhillon *et al.* 2021). "Kufri Sindhuri" was found lowest in antioxidant activity during cold storage (Dhillon *et al.* 2021), thus, sugars might be utilized at a high amount to produce stress protectants under deficiency.

Sucrose and sucrose to hexose ratio

Sucrose levels (Figure 3A) were higher at 4° than 25° C at all four stages, 20, 40, 60 and 90 d in all genotypes except basal end of "Kufri FryoM" at 20 d. Increase of sucrose at 4°, was found much higher in "Lady Rosetta" and "MS/8 1148" than "Kufri Sindhuri" and "Kufri FryoM" at 20 and 40 d. Sucrose increased by more amount in the apical end of "Kufri Sindhuri" than of "Kufri FryoM" at 20 d. Sucrose levels were seen higher in apical than basal ends in genotypes at many stages of cold storage. During cold storage, sucrose increased to 20/40 d then decreased to 90 d. Thus, sucrose accompanied glucose during cold storage.

QTL analyses have indicated the involvement of sucrose synthase (SUS), sucrose phosphate synthase (SPS), in CIS resistance (Xiao *et al.* 2018). Starch can be synthesized directly from sucrose (Baroja-Fernández *et al.* 2003). Thus, the synthesis of starch from sucrose may be the mechanism of CIS resistance in "Lady Rosetta". However, the CIS resistance of "Kufri FryoM" related to starch metabolism but not to sucrose metabolism. Low sucrose levels in "Kufri FryoM" showed that this genotype might be using sucrose breakdown direction to synthesize starch (Geigenberger 2003). Thus, the CIS resistance of "Lady Rosetta" may differ from that of "Kufri FryoM".

In potato tubers, total sugar concentrations are primarily constituted by reducing sugars (glucose and fructose) and non-reducing ones (sucrose) but unlike glucose and fructose, the role of sucrose in the browning of fried potato products is limited (Xie *et al.* 2018). Sucrose levels in potato tubers play an essential role during storage. Lessening sucrose levels through transgenic approaches had led to increased starch catabolism and respiration inside tubers during storage (Hajirezaei *et al.* 2003). A decrease in sucrose may be a signal for starch mobilization (Hajirezaei *et al.* 2003) and the onset of sprouting (Ferreira *et al.* 2017). In the present study, sucrose was seen high in "Lady Rosetta" during storage at both 4° and 25°C.

The sucrose to hexose ratio (Figure 3B) decreased at 4° C, by a large amount at 20, 40, and 60 d in the basal ends of all genotypes except "Lady Rosetta" where the ratio decreased by a small amount at 60 d only. In apical ends, the ratio was not altered at 4° C by a large amount in all genotypes. Ratio levels at many stages were

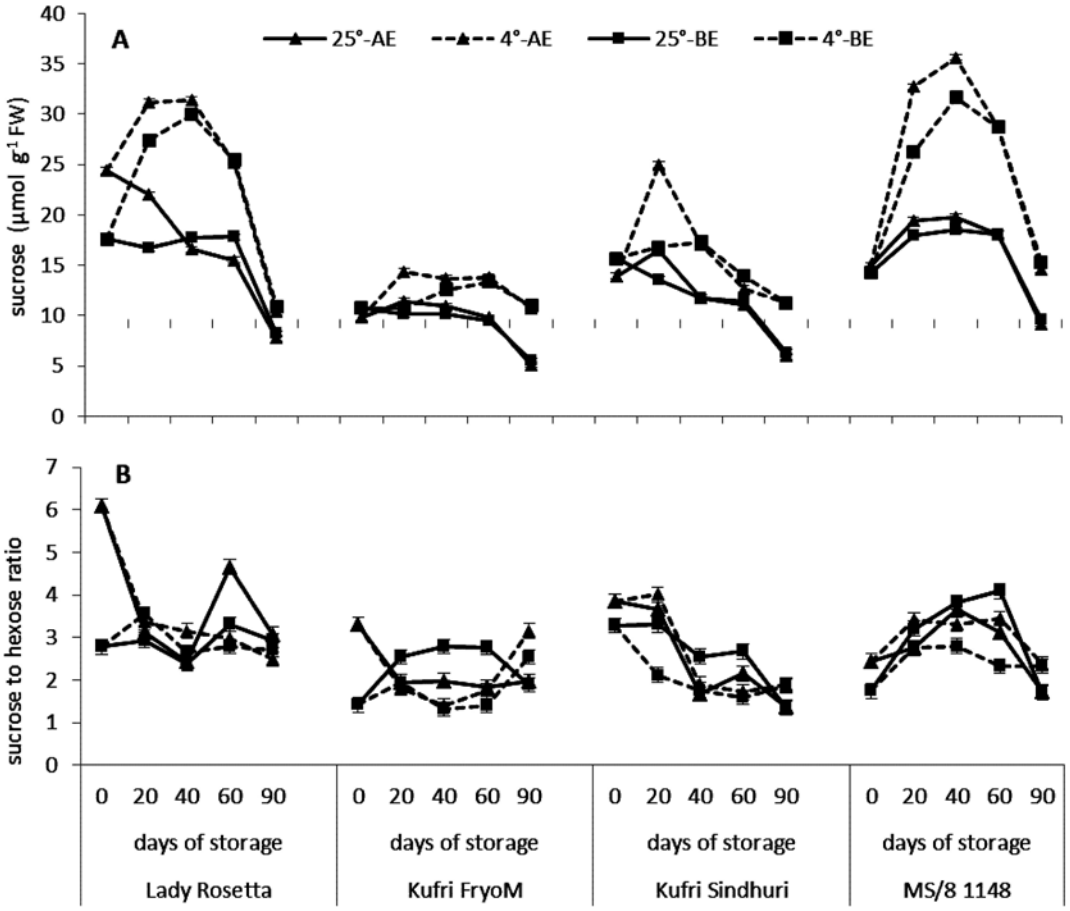

*Figure 3. Change of sucrose (µmol g^{-1} FW) (**A**) and sucrose to hexose ratio (**B**) in tuber apical end (AE) and basal end (BE) at 0, 20, 40, 60, 90 d of storage at 25° and 4° C in potato genotypes, "Lady Rosetta", "Kufri FryoM", "Kufri Sindhuri" and "MS/8 1148". Data points are the least-square means of replicates (n = 3), and vertical bars indicate standard errors. In some instances, standard errors are small enough to be obscured by the line width.*

higher in apical than basal ends in genotypes at 4° C. Results showed that hexoses were oriented towards sucrose in "Lady Rosetta".

Principal component analysis (PCA)

PCA (Figure 4) explained 65.2 % variation on PC1 and PC2. Quite distinct clusters for 4° and 25° C storage were found. The cold (4° C) cluster carried high values of sucrose, glucose, fructose, amylase while the 25° C cluster had high values of starch and sucrose/hexose ratio as expected. Amylase and fructose, but not glucose, related inversely to starch. Amylase related more positively to fructose than glucose. Fructose, but not glucose, related inversely to sucrose to hexose ratios. Starch related positively to sucrose to hexose ratio. Thus, results showed that sucrose synthase (breakdown) activity might be involved in producing fructose where glucose was in bound form (UDP-glucose). And fructose accumulation might be the end-product of CIS.

Acid invertase

Acid invertase activities were performed (data not shown) where enzyme activities were largely decreased at 4° compared to 25° C and only an increase was observed in the

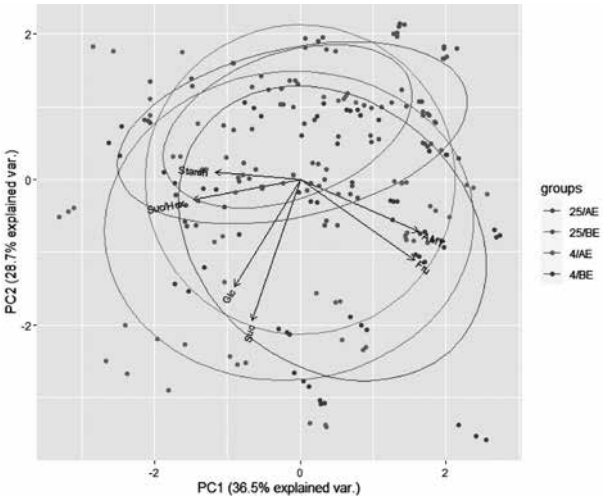

Figure 4. Principal component analysis (PC1 and 2) performed with storage temperatures (25° and 4° C), apical and basal ends (AE and BE) of all four genotypes of all storage days (0, 20, 40, 60, 90 d).

basal ends of "Kufri FryoM" and "MS/8 1148" at 20 d. A decrease in activities might be due to low metabolic activity at 4°C. Acid invertase activities were higher in basal compared to apical ends in genotypes and so was the CIS. However, activities pattern could not explain differences observed in the fructose levels among genotypes at 4°C. Invertase activities were found higher at 4° compared to 12°C storage and related to CIS-susceptibility in potato genotypes (Bandana *et al.* 2016).

This study concludes that cold-induced sweetening (CIS)-susceptibility of the genotype relates to starch degradation due to the gain of amylase during cold storage. Tuber basal ends produce more CIS than apical ends. Between tuber ends, the starch catabolic activity of the basal end relates to CIS. CIS resistance of "Lady Rosetta" may differ from that of "Kufri FryoM". Sucrose as well as starch synthetic activities may be involved in CIS-resistance of "Lady Rosetta". In the future, sucrose/starch catabolic and anabolic activities can be studied in detail in these genotypes to know more about CIS resistance.

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EFFICACY OF RICE STRAW MULCH FOR THE CONTROL OF VECTOR AND VIRUS INCIDENCE IN POTATO

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KEYWORDS: Aphids, PVY, virus transmission, straw mulch, virus incidence, plastic mulch, vector, whitefly

The vector-virus complex in potato is managed mainly with the use of synthetic pesticides in addition to growing of seed crops during the low vector activity period and use of disease-free seed (Shah et al., 2020a). Other than environmental concerns, pesticides do not provide satisfactory control of the non-persistent viruses like *Potato Virus Y* (PVY), therefore alternatives for efficient management of the vector-virus complex are being continuously explored (Shah *et al*., 2019; 2020b). Among the alternatives is mulching of potato fields with cereal straw after planting. The efficacy of straw mulching against aphid and whitefly transmission of viruses has been reported in a wide range of crops, including potatoes (Saucke and Doring, 2004; Johnson *et al*., 2004; Kirchner *et al*., 2014; Dupuis *et al*., 2017). In India, potato is mainly cultivated in the Indo-Gangetic plains under short day conditions during winter following rice (Shah et al. 2020a). Therefore, there is ample quantity of rice straw available at potato planting.

Studies on the use of straw mulch have shown that fewer winged aphids are captured in mulched plots compared to non-mulched ones which could potentially lead to lower virus spread. Therefore, this study was undertaken to test how efficiently the rice straw mulch reduces the landing rate of aphids and whitefly on potato plants in comparison to non-mulched control and whether the incidence of potato viruses decreases in mulched plots. For this purpose, field experiments were conducted at ICAR-Central Potato Research Station, Jalandhar (Punjab) during *Rabi* 2020-21 in Randomised Complete Block Design, with seven treatments and three replications. Potato variety *Kufri Khyati* was grown in the experimental plots with recommended package of agronomic practices without any crop protection measures. Plating was done on 13th October, 2020 and haulms were cut on 08 Jan., 2021. Plot size of 3 × 3.2 m^2 was used for evaluating the mulches. Paddy straw was used either as chopped (5 – 7.5 cm) or un-chopped (30 - 45 cm) at 5 and 10 tons per ha, along with silver reflective mulch, black plastic mulch and un-mulched control. Mulches were applied two weeks after planting just before anticipated crop emergence.

The landing rate of aphids and whitefly was assessed using the weekly trap catch on yellow sticky traps (YST) throughout the crop growth period. Traps (22.8 × 15.2 cm) were installed within all the plots and

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replaced at weekly interval. Observations on the incidence of adult whiteflies and winged aphids on potato plants were taken from ten randomly selected plants per plot at weekly interval. The numbers were counted from three leaves on each plant, one each from upper, middle and lower strata. The total tuber yield in all the experiments was recorded on whole plot basis. Percent virus incidence (expressed as percentage of infected plants over total number of plants/plot) was noted one week before the haulms were cut. The data on trap catch and incidence of aphids and whiteflies was analysed with GLM using Poisson distribution and the treatments means were separated with Tukey's HSD test. All statistical analyses were done in R-software 4.0.0 (R Core Team, 2021).

The trap catches of aphids and whitefly, used as a measure of flight activity and landing rate on potato plants, indicated significant difference between the mulched and un-mulched plots. The trap catch of aphids was highest at crop emergence and decreased gradually afterwards while as that of whitefly increased for initial couple of weeks and decreased afterwards in the control plots (Fig. 1). The whitefly and aphid trap catch were lowest for silver reflective mulch, followed by rice straw mulch and black plastic mulch on most of the sampling dates. No significant difference was found between the chopped and non-chopped rice straw mulch plots or rice straw mulch plots applied @ 5 or 10 t/ha for both aphid and whitefly trap catch. Further, no significant difference was noted between the mulched and non-mulched plots after 4th week of deployment of the mulches.

The incidence of aphids and whitefly on potato plants indicated non-significant effect of mulches on most of the weekly samplings (Table 1). No significant difference was found among the treatments for the number of

Table 1: Effect of mulching on the incidence of aphids (various species) and whitefly, *Bemisia tabaci* on potato plants

Weeks after crop emergence	Parameters	Whitefly	Aphids
04-11-2020	*F* value	44.83	4.53*
	d.f.	6, 12	6, 12
	p (0.05)	< 0.01 (Significant)	0.01 (Significant)
11-11-2020	*F* value	3.16	1.84
	d.f.	6, 12	6, 12
	p (0.05)	0.04 (Significant)	0.17 (Not significant)
21-11-2020	*F* value	2.53	6.51
	d.f.	6, 12	6, 12
	p (0.05)	0.08 (Not significant)	< 0.01 (Significant)
28-11-2020	*F* value	1.27	2.35
	d.f.	6, 12	6, 12
	p (0.05)	0.33 (Not significant)	0.09 (Not significant)
05-12-2020	*F* value	0.79	1.41
	d.f.	6, 12	6, 12
	p (0.05)	0.59 (Not significant)	0.28 (Not significant)
11-12-2020	*F* value	0.74	1.09
	d.f.	6, 12	6, 12
	p (0.05)	0.62 (Not significant)	0.41 (Not significant)
26-11-2020	*F* value	3.49	0.61
	d.f.	6, 12	6, 12
	p (0.05)	0.03 (Significant)	0.71 (Not significant)

*Based on Robust regression.

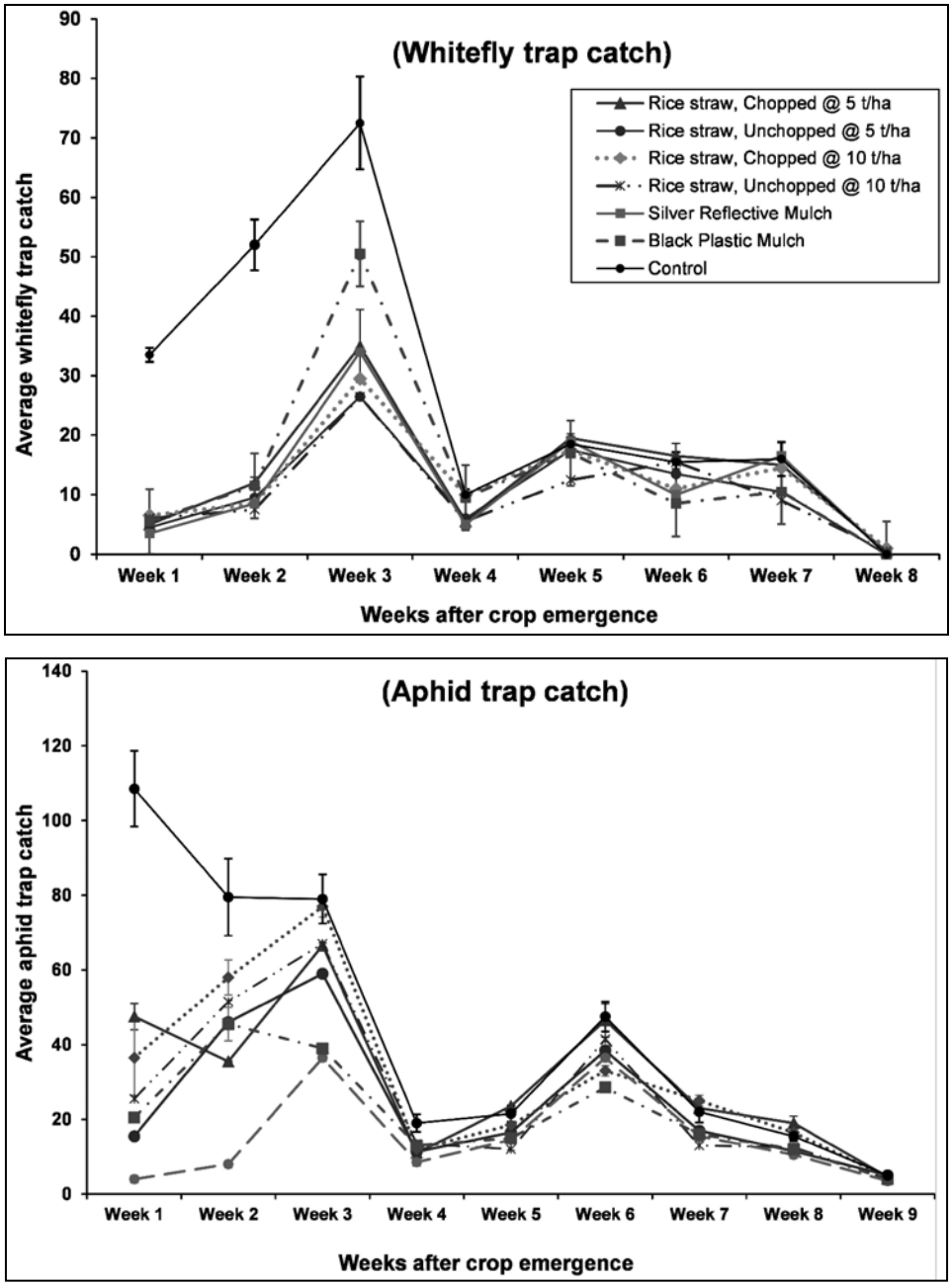

Fig. 1. Effect of different mulches on the landing rate of whitefly, Bemisia tabaci, and aphids (various species) in potato

aphids and whiteflies per plant although the incidence was close to half in the mulched plots as compared to non-mulched plots. The total tuber yield was found to vary from 32.53 to 34.30 t/ha with no significant difference among the treatments (Table 2). Considerable reduction in the incidence of virus symptomatic plants in the mulched plots was noted with significant effect of mulches (Table 2). The virus incidence reduced by 36.10 to 44.43 % in the mulched plots as compared to control (average virus infection 8.89 ± 1.57 % in control). The reduction in virus incidence was on par

Table 2: Effect of mulches on total tuber yield and virus incidence in potato

S. No.	Treatment	Tuber yield (ton/ha)	Reduction in Virus Incidence (%)
1.	Rice straw, Chopped @ 5t/ha	32.77	36.10 (6.03)
2.	Rice straw, Unchopped @ 5t/ha	32.53	38.90 (5.45)
3.	Rice straw, Chopped @ 10t/ha	33.70	44.43 (6.71)
4.	Rice straw, Unchopped @ 10t/ha	34.30	41.66 (6.46)
5.	Silver Reflective Mulch	32.87	36.10 (6.03)
6.	Black Plastic Mulch	32.77	36.13 (5.84)
7.	Control	32.73	0.00 (1.00)
8.	SEm	1.75	0.88
9.	CD (p = 0.05)	NA	2.74

Values in parenthesis are square root transformed as ×x + 1

among the mulched plots. Similar results were obtained from testing of random samples for the presence of viruses through DAS-ELISA and RT-PCR (data not shown).

The mode of action of cereal straw mulches is primarily attributed to the manipulation of the host finding behaviour of aphids by the visual properties of straw (Doring *et al*., 2005). According to a hypothesis proposed by Doring *et al*. (2005), aphids land indiscriminately on either the straw or the plant as a consequence of the low contrast between the plant and the straw background. However, once an aphid has landed on the straw and probed on it, it will take off again and leave the plot, starting the so called 'rejection flight'. Straw mulching is not effective during the whole growing periods; it is primarily effective from the emergence of the plants until the closure of the canopy (Heimbach *et al*., 2004; Saucke and Doring, 2004; Kirchner *et al*., 2014; Dupuis *et al*., 2017), as during this time the straw is visible for the winged aphids and thus has an effect on their landing behaviour. Afterwards, when the crop canopy covers the soil, the effect is lost. In the present study, we found that the effect of mulches on landing rate of the aphids and whitefly was non-significant after 4th week of crop emergence which coincides with the canopy closure of potato crop in the plains. Of particular interest is the non-significant variation in the incidence of aphids and whitefly on potato plants among the treatments. It seems like mulches reduce the number of insects landing on the plants however, the within plot spread is not affected.

Saucke and Doring (2004) used 3.5 t/ha of straw mulch and obtained an average efficacy of 31% (a 3-year trial in Germany) while Kirchner et al. (2014) used 5.5 t/ha with an efficacy of 44% (a 3-year trial in Finland). It is to be expected that a higher cover of the soil would better control aphid landings early in the season and better protect the plots against PVY spread. It is reported that 2.5 t/ha straw covers 60% of the soil at potato emergence and that it is the minimum quantity needed to reduce aphid captures in the crop (Dupuis *et al*., 2017). No significant difference in the aphid and whitefly trap catches was detected in this study between the plots mulched with rice straw at 5 and 10 t/ha; which is in coherence with the earlier reports. A virus reduction of 36.10 to 44.43 % in comparison to control was found in this study. Straw mulches have proven to be efficient in controlling PVY spread (Heimbach *et al*., 2004; Saucke and Doring, 2004). Kirchner *et al*., (2014) reported that 50-

70% lower incidence of PVY by using straw mulch, compared with untreated crops.

It has been shown that the initial colonization of potato fields by aphids tends to be concentrated on the margins of the fields (Carroll *et al*., 2009). We observed that the plants at margins of the mulched plants harboured higher number of aphids and whitefly (data not shown). It is suggested that the strong visual contrast provided by the fallow ground and the crop canopy might be more attractive to aphids than a homogeneous surface with relatively little contrast (Doring and Chittka, 2007). Therefore, it is advisable to mulch a plot 1 to 2 m beyond the plant line for best results.

It is concluded that rice straw mulch @ 5 t/ha can reduce the landing rate of aphids and whitefly considerably in potato crops. The mulches can provide protection to the crops for at least 4 weeks from crop emergence or till the canopy closure. Therefore, mulching can protect the crops from virus infection during early crop growth period when other methods of pest management are not very effective. Also, straw mulches can help to reduce the number of insecticide sprays needed and thus helps to protect the environment. It is suggested that rice straw mulching should be evaluated at multiple locations and on large scale over a number of seasons. Rice straw mulch has the potential for integration in to pest management program of seed potato crops, particularly where high value seed material is grown on small scale. Also, the use of rice straw mulch is compatible with organic potato cultivation.

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AN EMERGING CONCEPT OF PHOSPHORUS NUTRITION IN POTATO UNDER ELEVATED CARBON DIOXIDE [CO_2] CONDITION

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ABSTRACT: Potato (*Solanum tuberosum* L.) is a cheap source of carbohydrate and nutrition which is grown in different soil conditions. Phosphorus (P) is the crucial macronutrient that is involved in the metabolism in the plant such as glycolysis, respiration, formation of ATP and other catabolic and anabolic processes. Potato plant requires high P for optimum growth and development. However, under P deficiency the growth and yield of potatoes might lead to a significant reduction due to its disturbed metabolic activities. P deficiency might lead to a reduction in biomass and production, photoassimilates translocation, photosynthesis, flower formation, starch synthesis, source-sink activities and other physio-biochemical processes. The rising CO_2 due to anthropogenic intervention has significantly affected plant growth and development which has ultimately affected its yield and nutritional attributes. However, high CO_2 conditions might positively ameliorate the detrimental effect of P deficiency in potato plants. Improved root growth and increase in the total amount of nutrient uptake under elevated CO_2 conditions might also lead to enhancement in P use efficiency even when the P acquisition efficiency declines. Overall there is an array of the physiological, biochemical and molecular mechanism and interactions work under phosphorus stress with elevated CO_2 condition.

KEYWORDS: Phosphate transporters, Photosynthetically active radiation, Photosynthesis, Phosphorus acquisition, Nutrient uptake

INTRODUCTION

Phosphorus (P) is a non-renewable source of plant fertilizer and an essential nutrient for all life forms on the earth and especially for plants that are the primary producers. The concentration of P in the soil ranges from 0.6 to 11 µM and is poorly mobile in soil. This P as fertilizer we mainly get from rock phosphate, which is excavated from mines. This non-renewable resource is projected to get depleted by 2050 from the major reservoirs (Vance *et al*., 2003). Most of the P in soil remains unavailable for plant uptake as it is fixed in soil colloids. P is primarily involved in the production of the energy currency of the cell, ATP for high energy bonds, biomolecules and membranes. It is an essential nutrient that intervenes in the cellular energy transfer, photosynthesis, respiration and component of nucleic acid-like DNA RNA and phospholipids (Chourasia *et al*., 2021; Kumar *et al*., 2021a; Lal *et al*., 2021d; Raigond *et al*., 2021; Tiwari *et al*., 2021b). This is also an integral component of metabolic pathways and signalling cascades (Cordell *et al*., 2009). It is, therefore, an extremely essential macronutrient that profoundly influences plant growth and development, ultimately affects the production and productivity of potatoes. The

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plant undergoes several adaptive mechanisms that help to cope up long term P starvation. The modifications by the plant to adapt certain kind of stress includes alteration in root system architecture, efflux of organic acid and hydrolysing enzymes like acid phosphatase and induction of high-affinity transporters (Raghothama and Karthikeyan, 2005; Changan *et al*., 2020; Raigond *et al*., 2020; Kumar *et al*., 2021b; Lal *et al*., 2021b). Mycorrhizal symbiosis increases P acquisition by enhancing the volume of soil explored by increasing the root surface area of plants. Phosphorus Solubilizing Bacteria (PSB) also play major role in availability to plants by realizing of some enzymes or acids so increase the available form of phosphorus to plant from the non-available form of P. The kinetic properties of phosphate transporters that enhance P uptake have little effect on its mobility in soil solution. Hence, attempts to select/engineer efficient genotypes for increased P acquisition must consider the properties of both plant and soil microorganisms

Global climate change is a major challenge of the present scenario and also for future agriculture. The climate change has affected the weather patterns, heat, drought, frequent snowfall and frost in high altitudes (Stocker *et al*., 2013). Global climate change and global warming is delivering a negative impact on plant growth, survival and crop yield. Atmospheric CO_2 concentration [CO_2] has increased from 280 ppm during pre-industrial times to almost 405 ppm at present (IPCC, 2013). Continued burning of fossil fuels and changing land-use patterns threaten to cause further increment in [CO_2]. The present atmospheric [CO_2] does not saturate photosynthesis in a majority of terrestrial plants. Hence elevated [CO_2] would have profound effects on plant growth like increased rate of carbohydrate synthesis and net photosynthesis coupled with inhibition of photorespiration, ultimately increasing biomass and yield. Climate scientists have projected that the current ambient CO_2 concentration of 400 will nearly double to 700 ppm by the end of the century (IPCC 2007), which is likely to be coupled with a rise in global atmospheric temperature by 0.3 to 4.8 (IPCC 2014). This predicted increase in air temperature will also increase the evapotranspiration water loss causing soil water limitation and agricultural drought in the field which affects the production and productivity of tuber crops (Vandegeer *et al*., 2013). High atmospheric [CO_2] will certainly increase the CGR (Crop growth rate) which in turn leads to increase in demand of nutrients from soil. This enhancement in the growth and development of the crop under high CO_2 will lead to nutrient deficiency. Such disparity is attributed to efficient nutrient utilization under elevated as compared to ambient CO_2 level. Elevated [CO_2] may increase acquisition efficiency and uptake patterns of essential nutrients such as P and S. Modern high yielding cultivars have higher nutrient requirement, which is expected to rise further with rising atmospheric [CO_2] (Lal and Pandey, 2015; Pandey *et al*., 2018; Lal *et al*., 2021d). Elevated [CO_2] enhances growth mainly through increased leaf area, photosynthesis and nutrient use efficiency, which are all compromised during P or S deficiencies as reported in soybean (Prior *et al*., 1998). Elevated [CO_2] in the atmosphere is beneficial to plants through its enhancement of crop growth, but detrimental to human life (Devi *et al*., 2021; Raigond *et al*., 2021; Tiwari *et al*., 2021c).

P deficiency reduces leaf area, photosynthesis, nitrogen (N) fixation, seed yield and quality in soybean plants (Cure *et al*., 1988). P deficiency decreased biomass, photosynthesis and stomatal conductance of

soybean plants. Such negative influences on growth may be compensated for by elevated [CO_2] mainly by increasing nutrient use efficiency, leaf area and photosynthesis. High [CO_2] led to higher soybean yield, through its positive effects on number of pods and seeds, rather than seed size (Salvucci and Crafts-Brandner, 2004). Studies have shown that P deficiency coupled with elevated [CO_2] may alter nutrient dynamics and biomass partitioning in plant organs (Lal *et al*., 2021c; Tiwari *et al*., 2021a). Moreover, the positive influence of [CO_2] on plant growth may be beneficial even under nutrient deficiency owing to the increased carbohydrate synthesis and accumulation under high [CO_2] conditions.

Potato is the world's fourth important staple food after rice, wheat and maize which is an underground tuber crop (Kumar *et al*., 2020c, 2020b; Lal *et al*., 2020b, 2020c, 2020d; Thakur *et al*., 2020). It requires high P for its optimum growth and development for better production and productivity. Potato cultivation in the P deficient soil leads to a considerable loss in yield and production (Dechassa *et al*., 2003). Along with the deficiency of P, biomass reduction is also attributed with amount of PAR (photosynthetically active radiation) absorbed by the plant (Tiwari *et al*., 2020a, 2021b; Kumar *et al*., 2021b; Lal *et al*., 2021d, 2021a). The different traits have been affected under P deficiency along with interaction with high CO_2 are as follows. Under elevated CO_2 condition potato exhibit positive growth and development (Lal *et al*., 2020a, 2021a; Singh *et al*., 2020; Tiwari *et al*., 2020a, 2020b). However, this may influence the nutrient uptake and utilization. The combined interaction data of CO_2 and phosphorus stress in potato is very scarce, hence we tried an attempt to put forward all the parameter related to it in this article.

We must identify the best cultivation and practices under climate change in order to tackle both biotic and abiotic stresses like heat stress, drought, insect and pathogen. By taking these factors into consideration the newly developed cultivars of potato will best adapt the changing environment.

Effect on Photosynthesis

It was reported that Pi-regeneration capacity and not rubisco is involved in the acclimation of potato plants to elevated CO_2 (Sage, 1994). It was clear that acclimation of photosynthesis occurs in potato when the plant is exposed to elevated CO_2 for the long term. However, the analysis of A/Ci curves of potato leaves under high CO_2 shows inconsistent results. The symptoms of Pi deficiency can be visually observed by observing the plant which possesses reduce the growth of the plant, acute leaf angles, prolonged dormancy and a decrease in the size and number of flowers (Mengel *et al*., 2001). There was also the development of the dark green or blue-green foliage which shows the symptoms of Pi deficiency. Red, purple and brown pigment development along the veins is due to anthocyanin production along with the increased leaf sucrose concentrations (Kumar *et al*., 2017, 2020a; Lal and Tapas Ranjan Sahoo and Lopamudra Nayak, 2017; Sahoo *et al*., 2017; Pandey *et al*., 2018).

There was no change in the activation of Rubisco but a reduction in total protein content was observed in leaves grown in elevated CO_2 (Stitt & Schulze, 1994). Reduction in the stomatal conductance is another observation in response to elevated CO_2 due to partial stomatal closure. The reduction in the conductance for CO_2 diffusion into the leaf leads to the decrement of the photosynthetic rate. The photosynthetic rate was found to be decreased under elevated CO_2 (700 ppm) conditions and also in super-elevated conditions (1000 to 10,000 ppm).

Effect on pigment system

The P deficiency decreases the electron transport rate in the photosystem and chlorophyll fluorescence parameter. This deprivation of P inhibits photosynthesis by inhibiting rubisco activation and regeneration of ribulose-1, 5-bisphosphate (RuBP). The Rubisco content was found to be decreased under P deficient conditions (Pandey *et al*., 2018).

Starch/Carbohydrate Content

The potato plant when exposed to elevated CO_2 for long-term there was an increase in the leaf starch contents. The high level of starch due to elevated CO_2 leads to accumulation of high starch in potato leaves which ultimately damage the chloroplasts. However, it was found that the concentration of soluble carbohydrates was unaffected under elevated CO_2 condition (Barnaby *et al*., 2019). The affects the phosphate nutrition where ultimately the deficiency of P leads to accumulation of starch in the leave. The interactive effect of both P deficiency and elevated CO_2 leads to drastic increase in starch content in the leaves of plant (Lal *et al*., 2019). This increase in leaf carbohydrate coincides with photosynthetic acclimation.

Effect on growth and development

The effect of elevated CO_2 on plant growth and development can also be observed. Under elevated CO_2 conditions there is an overall increase in the total biomass production. Leaf area of potato was found to be increased when CO_2 concentration was increased from 500 µmol mol^{-1} to 1000 µmol mol^{-1}. Leaf area index (LAI) was found to be increased under elevated CO_2 conditions in potato. However, the rate of decline of LAI was observed at the end of the growing season (Finnan *et al*., 2005). Above-ground biomass and tuber dry weight was found to be increased in under elevated CO_2 and P deficient condition. This show that CO_2 ameliorate the deficiency of P stress in potato. In Changing climate and potential Impacts on Potato yield and quality project (CHIP) experiment it was also reported that above-ground biomass, tuber number and root biomass were found to be increased at an intermediate harvesting time (Craigon *et al*., 2002). The biomass accumulation is also highly affected by P supply and light interception. P deficient potato plant possess poor root growth, haulm growth and delayed maturity as compared to well fertilized plant. During stressed situations, plants allocate more photosynthetic assimilates toward the growth of organs that have direct role in acquiring the limiting resource (Pandey *et al*., 2015). Higher root-to-shoot ratio observed under nutrient deficiency and/or elevated [CO_2] aids in increasing nutrient uptake by providing more root surface (Pandey *et al*., 2013). Soil exploration by enhanced root surface area is a preferred adaptation to changing climatic scenarios wherein elevated [CO_2] would increase carbon supply for the production of finer roots. In contrast, Maestre and Reynolds (2006) reported that root proliferation of a Brachypodium increased with nutrient availability and was not influenced by atmospheric [CO_2]. It was suggested that belowground biomass increased with elevated [CO_2] only when sufficient nutrient requirement of the plant was met.

Effects on crop yield

Earlier it was reported that tubers and root crops are identified as responsive crops to elevated CO_2. Moreover, it was also suggested that potato being the underground crop possess the large below the ground sink for carbon and along with the apoplastic mechanism of phloem loading it is the

best crop that responded to elevated CO_2 condition under nutrient stress (Kimball *et al*., 2002). Application of phosphorus fertilizer in potato crop shown to an increase in marketable tuber yield per hectare. Maximum tuber yield was recorded at a rate of 135 kg ha^{-1} with 98 % yield advantage over control treatment. Niguse *et al*., 2016 has conducted an experiment in Tigray, Ethiopia showed that the application of phosphorus at a rate of 89.50 kg ha^{-1} had the highest marketable and total tuber yield. (Misgina, 2016).

Effects on tuber quality

In the above section we concluded that the elevated atmospheric concentration of CO_2 increases the yield of tuber, but also improves the quality of tuber. Potato provides substantial amount of carbohydrates, protein, vitamin and other mineral. Any change in the quality parameter of potato will hamper the and will have an effect on human nutrition. Under elevated CO_2 there is a report of increase in the vitamin C content and reduction in nitrogen content. However, as the tuber yield is increased there is also an increase in the tuber starch and viscosity of the starch paste (Vorne *et al*., 2002). High dry matter content will benefit the potato processing industries like those involved in making potato chips and fries. Moreover. High dry matter content will prevent excessive fat absorption (Tiwari *et al*., 2020a). Major abiotic stress viz., high temperature, drought, salinity and nutrient stress like P stress will adversely affects the process of distribution of photo assimilate and substantially restrain the plant growth, development, tuberization, tuber bulking and finally the tuber yield and quality (Minhas, 2012). However, the magnitude of the yield increase will depend on agronomic practise, cultivar choice and growing conditions.

Interactive effects of CO_2 and P on nutrient concentration

Elevated [CO_2] leads to increased P acquisition to sustain growth under limited P supply to plants when the internal P is used judiciously for biomass production. Enhanced P uptake and utilization was reported in soybean plants exposed to elevated rather than ambient [CO_2] (Cure *et al*., 1988). In cereals, total P acquisition increased by 70% at sufficient P and elevated [CO_2], whereas 26% enhancement of P utilization efficiency was observed at low P. Improved root growth and increase in the total amount of nutrient uptake led to enhancement in P use efficiency even when the P acquisition efficiency declines. Elevated [CO_2] resulted in higher biomass and net photosynthesis owing to enhancement in P and N uptake per plant, which was closely associated with greater utilization efficiency. Similarly, elevated [CO_2] increased uptake of N, P and potassium in *Agrostis capillaris*, with no significant enhancement in biomass. Excessive P application reduces the micronutrient like zinc which ultimately leads to an increase in the rate of senescence in plants.

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DIPLOID F_1 HYBRID TPS POTATO BREEDING - PIPELINE AND PROSPECTS

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ABSTRACT: Potato is the third most important food crop worldwide. Till date, the potato breeding has been carried out at the tetraploid level, which hampered the genetic studies to decipher the trait genetics. Diploid inbred based hybrid breeding in potatoes provide the opportunity to take advantage of modern breeding methods, tools and genomics resources. The first step in the diploid inbred based hybrid breeding strategy involves identification of elite diploid lines and di-haploids of adapted varieties, followed by introgression of self-compatibility gene (*Sli*) for advancing the lines through selfing for few generations. A number of inbred lines from different lineages with more than 95% homozygosity will be generated through selfing. The best heterotic hybrids will be selected through evaluation of inbred lines for general and specific combining ability for tuber and quality traits. Finally, the best hybrid combinations will be evaluated along with commercial tuber-based tetraploid varieties for comparative analysis. Although, the methodology of F1 diploid hybrid TPS based potato breeding has several advantages over conventional tetraploid tuber based approach, a number of challenges need to be overcome to bring this technology at the forefront in the farmers field.

KEYWORDS: Diploid potato, Inbreds, *Sli*, Heterosis breeding, F_1 hybrid TPS

INTRODUCTION

Potato is propagated clonally through tubers to maintain the purity of cultivars due to high heterozygosity and polyploid genome. The true potato seeds (TPS) i.e. botanical seed could not be used for propagation of varieties due to genetic non-uniformity i.e. each TPS is genetically different. TPS however is easy to maintain, easy to transport, easy to store, amenable to genetic manipulations and is free from diseases and pests inoculums (Sood et al., 2020). Potato breeding through inbred diploid hybrid TPS is a completely new breeding method (Lindhout et al., 2011), being adopted in all potato growing countries including India (Sood et al., 2021). The new diploid hybrid breeding technology works by sexual propagation in each generation like cereal crops. The method is based on identification, evaluation of diploid cultivated species and di-haploids of *Solanum tuberosum* for selection of desired clones with acceptable tuber and other plant traits. Diploid potatoes are naturally self-incompatible. *Sli* is the key gene for self-compatibility in diploid potatoes and now offers a path forward for the inbred-based diploid F1 hybrid potato breeding program (Ma et al., 2021). First identified in *Solanum chacoense* clones 'chc 525-3' by Hosaka and Hanneman (1998), the gene has been recently fine mapped and introduced in diploid tuberosum lines for inbreeding and generation of inbred lines (Eggers et al., 2021). Different sources of self-incompatibility identified in other species also have been found to be regulated by the *Sli* gene (Clot et al., 2020). Recent studies have unraveled the structure and function of the *Sli* gene which has been narrowed down to a region of 12kb on chromosome 12 (Eggers et al., 2021). It was further found that the *Sli* gene encodes an F-box protein which is expressed in the pollen of self-compatible clones only.

The introgression of the *Sli* gene in self-incompatible diploid clones made inbreeding

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possible in hitherto self-incompatible lines. However, the accumulation of deleterious genes in the recessive phase over a hundred years of clonal selection in cultivated tetraploid potatoes is another major hindrance in inbred lines development. High inbreeding depression is evident from decreased fertility and vigour in selfing generations. To overcome the problem of deleterious genes, Zhang et al. (2021) suggested the genome design pipeline for the development of inbred lines where they emphasized to use of the starting material with low heterozygosity and few deleterious mutations. To identify inbred lines with beneficial alleles, the genetic analysis of S_1 inbreeding generation was carried out to identify beneficial alleles and purge large effect deleterious mutations. The diploid inbred lines selection following this breeding pipeline would be highly homozygous and remain true to type on selfing. The inbred lines originating from different lineage could be crossed to develop F_1 hybrids TPS for evaluation. This sexual crossing of the two inbred parent lines results in thousands of true potato seeds per plant instead of only a few potato tubers. So multiplication of a new variety can occur much faster. Since the offspring of a sexual cross is a pristine true seed, these are completely free of diseases and therefore make excellent seed material for potato growers around the world.

Diploid inbred F_1 hybrid methodology

Diploid F_1 hybrid potato breeding, and producing varieties from true potato seed, has been getting a lot of attention worldwide. Since the potatoes have been multiplied from tubers and people are unaware of the botanical seeds-based hybrid system, the basics workflow of the development of diploid inbred lines and hybrid TPS is explained below (Fig. 1).

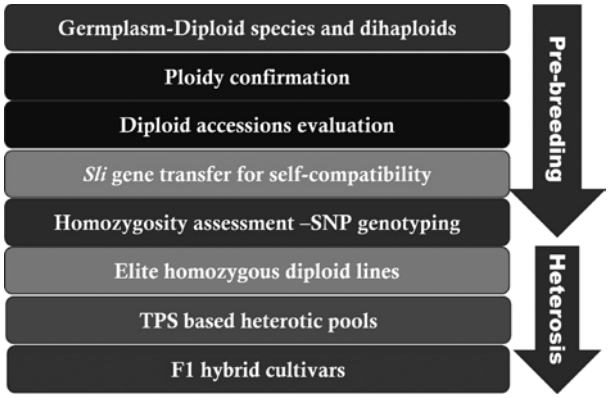

Fig. 1: Work flow of inbred based diploid hybrid potato breeding

Diploid germplasm lines: The first and foremost requirement of diploid F_1 hybrid potato breeding methodology is elite diploid germplasm lines in the cultivated background. To do so the tetraploid cultivated potatoes are crossed with haploid inducer lines (HI) and di-haploids are developed, which originate from the female parent only i.e. we get exactly half the genetic constitution of tetraploid variety. The di-haploids are weak and mostly self-incompatible. The weak plants with deleterious alleles are removed and strong plants will be selected and maintained.

Self-compatibility and inbreeding: The healthy plants will be made self-compatible by introgressing the *Sli* gene from elite *Sli* donor lines to fix the genetic constitution upon selfing in inbred lines. The diploid lines will be selfed for few generations to achieve >95% homozygosity. Robust, vigorous and fertile plants are selected in each selfing generation for advancement to the next generation to accumulate beneficial alleles in inbred lines.

Heterosis: Selfing will generate many inbred lines originating from different di-haploid parents i.e. lineage. The inbred lines from different lineages will be evaluated for general and specific combining ability

and hybrid vigour for tuber yield, quality and processing traits. The best hybrid combinations will be evaluated along with commercial tuber-based tetraploid varieties for comparative analysis.

Advantages

- The technology offers numerous advantages over tuber-based potato breeding and cultivation methodology.
- The seed requirement will be drastically reduced due to TPS being the propagation material. Transport will be very easy to far off locations.
- The degeneration of tubers due to virus accumulation will not be a problem anymore. Less than 5% of the pathogen are seed-borne which means the significantly reduced transmission of many of the pathogens causing diseases in potato seeds.
- Genetics will be simple at the diploid level and it will become easy to track and map genes for important traits in potatoes, which at present is difficult to do due to tetraploid inheritance and heterozygosity.
- The trait stacking will be very easy as backcrossing can be used to introduce the desired trait in true breeding lines without altering the genome of the pure lines.
- The genetic gain will be higher over time due to the sexual breeding cycle and accumulation of desired alleles in inbred lines.

Challenges

- The inbred lines produced to date in diploid potatoes are not completely homozygous for all the loci in the genome, which means the F_1 hybrids may lack uniformity for some traits governed by these loci. This could be a major deterrent to the acceptance of technology.
- The approach involves a complete transformation of potato breeding methodology, hence it will require rigorous evaluation in various agro-ecologies to convince the stakeholders on the adoption of this technology. The trials in African countries have shown at par or less tuber yield of F_1 TPS in comparison to tuber raised tetraploid varieties. At par tuber yield and quality to tetraploid varieties is a must for the adoption of diploid F_1 hybrid TPS technology.
- The TPS is the starting material for growing potatoes, which will require additional time and resources to raise the crop in the nursery and transplanting seedlings in the field. The crop duration of TPS based approach needs to match with tuber raised crop to fit in the cropping system in India.
- Both scientists and farmers are accustomed to growing potatoes from tubers i.e. conducting trials or raising crops. It will be important to standardize the agronomy of TPS-based crop as a complete package and practice of the diploid F_1 hybrid TPS.

Preliminary reports of productive diploid F_1 hybrid TPS and its advantages as an inbred-based crop (Jansky et al., 2016) made it one of the most exciting approaches in potato breeding. The inbred diploid potatoes would allow for systematic genetic studies and incorporation of new genes and traits through back-crossing in these fixed inbred lines. Over time, the identification and stacking of beneficial alleles in inbred lines would show substantial yield gains between defined heterotic groups. The possibility of combining complementary traits from the parents, obtaining heterosis from hybridization of inbred parents, avoiding pathogen load of seed tubers, and facilitating the transport

of true seeds leaves no doubt that hybrid varieties will attract a lot of interest across the globe including India.

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Gram	g	Minute	min
Hectare	ha	Nanometre	nm
Kilogram	kg	Second	s
Litre	l	Square cm	cm^2
Metre	m	Square km	km^2
Mega gram	Mg	Tonne	t

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