

INCIDENCE AND SEVERITY OF COMMON SCAB OF POTATO CAUSED BY *STREPTOMYCES SCABIES* IN PUNJAB, PAKISTAN

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Abstract

In Pakistan, potato (*Solanum tuberosum*) is amongst the foremost cash crops for the farming community. Common scab (*Streptomyces* sp.) is one of major disease, which reduces the price, and market value of potato tubers. The present study was designed to determine the status of common scab in selected potato growing areas. This survey was directed to record the occurrence and intensity of common scab of potato in the major potato producing areas in districts of Punjab, namely Sialkot, Lahore, Kasur, Sahiwal, Okara, Pakpattan, tehsil Depalpur, Faisalabad Taxila and Hazro during the harvesting of potato in 2017-18. Overall, 456 potato fields were surveyed for data collection. It was noticed that the maximum common scab occurrence was recorded in Sahiwal followed by Okara, Kasur, Pakpattan, Faisalabad, Lahore, tehsil Depalpur and minimum in Sialkot and Hazro. Disease incidence was also checked on the current varieties of potato used for sowing, highest disease frequency was observed in var. 'Kuroda' and var. 'Santi' so these two varieties were highly susceptible while var. 'Diamant' and var. 'Cardinal' were detected as medium susceptible while var. 'Lady Rosita' and 'var. Asterix' were detected as highly resistant to common scab. The resistant and moderately susceptible varieties are therefore recommended for cultivation and in developing new resistant cultivars.

Key words: Common scab, Potato, Resistance, Susceptible, Distribution.

Introduction

Potato (*Solanum tuberosum* L.) is one of the most extensively produced and consumed tuberous crops in the world. Potato is also a common crop in Pakistan. Soil and climatic situations prevailing in Pakistan are extremely encouraging for the production of three crops of potato per year, i.e., autumn, spring, and summer crop (Zaheer & Akhtar, 2016). Several biotic and abiotic stresses limit potato productivity in the country. Among biotic constraints, viral diseases like potato leaf roll virus, (Solomon-Blackburn & Barker, 2001) Fungal diseases like black scurf, late blight, early blight, dry rot, *Fusarium* wilt, powdery scab (Arora & Khurana, 2004) and bacterial diseases such as bacterial wilt, ring rot, soft rot and common scab (Czajkowski, Perombelon, van Veen, & van der Wolf, 2011) have been widely reported for lesser production of potato while, common scab of potato is an economically important disease of potato and is categorized by the existence of cracks on the surface of potato tuber. The cracks can be on the surface or deep-seated (Loria *et al.*, 2008). Different species of *Streptomyces* are reported to cause common scab (Healy, Wach, Krasnoff, Gibson, & Loria, 2000). Among reported species, *Streptomyces scabies* is one of the important disease-causing pathogen in potato crop (Keinath & Loria, 1989). It arrived into potato cover through lenticels, in young tubers or through cuts and producing scab disease by creating thaxtomin (Lawrence, Clark, & King, 1990). Due to the incidence of common scab disease in the year 2006/07, almost 32% of the total produced potatoes were discarded because of the low-grade consignment (Gouws, 2013). Once the pathogen arrives in the field it is hard to eliminate (Dees & Wanner, 2012). In Pakistan (Punjab) the incidence of common scab, disease is reported as 65-66% (Rauf, Ashraf, & Ahmad, 2007) In Pakistan common scab of potato was firstly a negligible

disease but forthwith has developed the main potato disease. The severity of diseases is spreading constantly, as information regarding disease severity is not available. Therefore, an extensive survey was planned to study the occurrence and severity of common scab disease in different potato production areas of Punjab, Pakistan.

Materials and Methods

Survey: Survey was conducted at the time of harvesting in the major potato growing areas viz., Taxila, Hazro and districts of central Punjab Sialkot, Lahore, Okara, Depalpur, Sahiwal, Kasur, Pakpattan and Faisalabad. Different locations were surveyed, from each location randomly five fields were selected and from each field, five sampling spots were selected from each point twenty potato tubers were randomly collected thus making a total of 100 tubers field. Information regarding Agricultural practices was also recorded.

Disease Incidence (DI): Disease incidence was calculated using the following formula (Liu, Anderson, & Kinkel, 1995).

$$DI = \frac{\sum(n \times 1 + n \times 2 + n \times 3 + n \times 4)}{N \times 5} \times 100$$

- 0 = no symptoms on potato tuber
- 1 = 1 – 20% scabbed potato tubers
- 2 = 21 – 40% scabbed potato tubers
- 3 = 41 - 60% scabbed potato tubers
- 4 = 61 – 80% scabbed potato tubers
- 5 = High scabbed potato tuber

Disease severity (DS): The disease severity of common scab was measured as (Wang & Lazarovits, 2005).

$$DS = \frac{\sum(\% \text{ Coverage by Lesion} \times p \text{ Lesion type} \times \text{Number infected potato})}{(\text{Number fields visited} \times \text{Total number of potato evaluated})} \times 100$$

0 = No symptom, 1 = Very small lesions, 2 = Small superficial lesions, 3 = Periderm broken, 4 = Light pitted 5 = Deep pitted.

Varietal response to common scab was classified according to (Marais & Vorster, 1988).

< 08 comparatively resistant
 08 – 15 Low susceptible
 15 – 20 Medium susceptible
 20 – 24 High susceptible
 >24 Very highly susceptible

Isolation of *Streptomyces scabies* from infected potato:

Infected Potatoes were surface-disinfected in 70% ethanol for 20s and then washed numerous times in sterile distilled water (SDW). After that, from each potato a small piece of potato tissue was cut from under the surface of a single lesion, at the border between healthy and infected tissue, and then homogenized in 200 liter in distilled water (SDW) and incubated for 30 min at room temperature. A 100 ml aliquot of the homogenate was plated out on water agar and incubated at 28°C in the dark. Isolation was performed from each of a total of 957 independent tubers. From each plate, up to three single colonies, phenotypically characteristic of *Streptomyces*, were transferred to yeast, malt extract agar (YME) (Lambert & Loria, 1989). After approximately 6 days Subsequent transfer to the fresh medium was done to obtain pure cultures. *Streptomyces isolates* were grown on YME agar plates at 28°C and stored on YME agar plugs at 8°C in Eppendorf tubes.

Pathogenicity test of *Streptomyces scabies*: Potato (Kuroda, Lady Rosetta) tubers were obtained from the National Agriculture Research Centre, Islamabad, Pakistan, and were stored at room temperature. Some days later the tubers, which had sprouting eyes, were grown in sterilized soil in 15cm plastic pots in the greenhouse, under a regular watering regime. Inoculations were done at the time of sowing by applying 20ml of prepared scab suspension (108cfu/ml). Tubers were harvested after twelve weeks. The isolates which were capable of causing common scab in potatoes were selected for characterization.

Identification and biochemical characterization of isolates

Morphological characterization: Morphological studies were carried on Yeast extract-malt extract agar, Oatmeal agar, Inorganic salts-starch agar, and Glycerol-asparagine agar (Shirling & Gottlieb, 1966). All isolates were characterized biochemically for confirmation. The following tests were performed Gram staining reaction, potassium hydroxide test, catalase oxidase test, levan production from sucrose, Kovac's oxidase test, lipase activity. All these tests were performed as described by (Schaad, Jones, & Chun, 2001).

Statistical analysis

For quantitative analysis, the Statistical Packages for Social Science (SPSS) was used. The experiment results were evaluated through a univariate regression model. The

10 nested locations were considered in independent environments for two years. In fact, the crop varieties were different according to soil pH, which is reflected in the nested treatment structure. The approximation method for the demonstrated degree of freedom to test the fixed effect is efficient in this scenario (Kenward & Roger, 1997), which also has the specialty to analyze the effectiveness of plant disease incidence and disease severity within the treatment group efficiently. In addition, the data analysis was carried through separate crop varieties depending on F-stat results. Multiple comparisons were done following the Tukey test with the exception of interactions the significance level was $\alpha = 0.05$ in order to elucidate interactions significant at level $p < \alpha = 0.1$ effects were sliced into the single factors or factor combinations however results were only given for interactions not including locations. Difference between treatments combinations with the lowest value for $P < |t|$ were regarded as significant if F test revealed a significant difference between treatments even if the Tukey adjustment for multiple comparison did not reveal significance as within the varieties. The potato incidence and disease severity scab be considered as orthogonal and analysis was performed for variety crop rotation soil PH and seed status in order to analyses the effects of variety in more detail, however, this analysis was not carried for the other factors of variety because the effect of varieties within the locations were confounded with the effect of potato diseases incidence and diseases severity respectively.

Results

Survey: The survey in major potato growing areas in Punjab shows that the common scab of potato is a major problem found in every field. Total 66 locations from 10 areas and 5 fields from each location were surveyed (457 fields) it was recorded the disease incidence and severity in each location were variable. As shown in Table 1.

Disease incidence (DI) and disease severity (DS) of common scab of potato: The disease incidence and disease severity of common scab of potato were calculated. Maximum (DI) was recorded in Okara and minimum in Hazro while maximum (DS) was 62% in Okara and minimum 26% Taxila as shown in (Fig. 1).

The analysis of variance (ANOVA) for the whole data set is given in Table 2, for plant disease incidence and disease severity. Most of the significant effects on plant disease incidence correspond to the effects on disease severity. For both parameters, no significant effects of variety, location, crop rotation, seed status and the interaction of variety-crop rotation, variety- Soil pH, variety-seed status, crop rotation-soil pH, crop rotation-seed status, soil pH- seed status were observed except soil pH showed a significant effect. Additionally, the effect of variety-Soil pH was significant for plant disease incidence. Both plant disease incidence and disease severity were significantly influenced by the location. Disease incidence percentage ranged between 69% (Okara) and 28% (Hazro). Disease severity was significantly higher in Okara and Sahiwal compared with Hazro and Taxila. No general trend between plant disease incidence and disease severity was detected in Fig. 1.

Table 1. List of area and location visited during survey.

Area/ District	Location	DI (%) by number of infected potatoes	DS
Taxila (Rawalpindi)	Thatha	34%	1-3
	Usman Khattir	52%	1-3
	Ghari Sikandar	17%	1-3
	Khurum Paracha	23%	1.4
	Lab Tattoo	23%	1-3
Hazro (Attock)	Waisa	42%	2-3
	Mallah	25%	2-3
	Shamsabad	22%	2-3
	Sirka	32%	1-3
	Shadi khan	25%	2-3
	Kalu Kalan	24%	2-3
Sialkot	Kishan Garh	42%	2-3
	Hussain Pur	31%	1-4
	Randhir	44%	1-4
	Habibpur	52%	2-4
	Chicharwali	39%	2-4
Lahore	Mujaki	32%	2-4
	Mangaal	45%	2-4
	Lakhodair	59%	2-4
	Awan	58%	2-4
	Dehiwala	60%	2-4
	Kotha Pind	51%	2-4
	Jandiala	49%	1-5
	Melhi	53%	2-4
	Kot Chandi	47%	2-5
	Kalyan	45%	2-4
Kasur	Qutba	39%	2-4
	Baroom	49%	2-4
	Kasur	39%	2-4
	Faqeeriye Wala	48%	2-4
	Dolay Wala	61%	2-4
Okara and Depalpur	Baman Shah	57%	2-4
	Burj Jewy Khan	53%	2-4
	MozaAmeer Aman	67%	2-4
	Salwal	58%	2-4
	Qadirabad	67%	2-5
	47-3R	62%	2-5
	50-3R	48%	2-4
	49-3R	58%	2-5
	40-3R	67%	2-4
	48-3R	71%	2-4
	Fakhar town	52%	2-4
	Jait Pur	42%	2-3
	Kanti Pur	45%	2-4
	Awan Kalan	43%	2-5
Qadir abad	42%	2-5	
Basir Pur	42%	2-5	
Sahiwal	70-4R	72%	2-5
	82-6R	62%	2-5
	85-6R	69%	2-5
	87-6R	37%	2-5
	86-6R	65%	2-5
	44-12-L	83%	2-5
	9-14L	72%	2-4
Pakpatan	AsadUllah Pur	28%	2-4
	Boonga Hayat	45%	2-5
	Malik Pur	22%	2-4
	Malik Bahawal	56%	2-4
	Dolt Abad	52%	2-4
	33-SP	45%	2-5
	Balhoul Pur	41%	2-4
Fakhar town	29%	2-4	
Faisalabad	Karamsar	54%	2-4
	Ram Diwali	40%	2-4
	Thikriwala	59%	2-5
	Jaranwala	45%	2-5

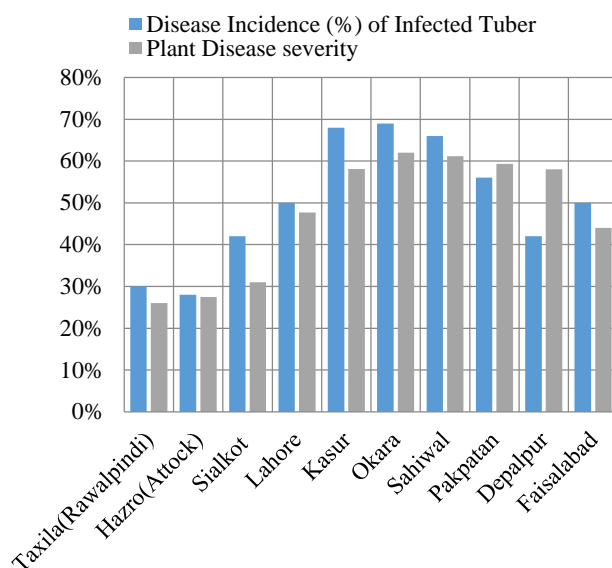


Fig. 1. Disease incidence and severity of common scab.

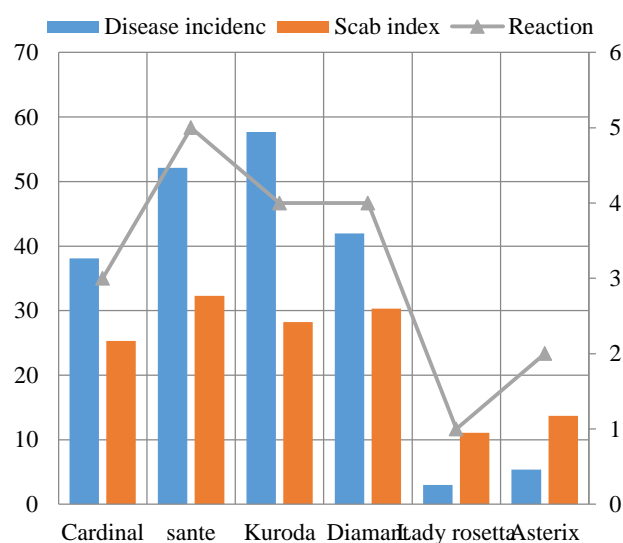


Fig. 2. Varietal response against Common Scab of Potato.

Table 3. Varietal response against common scab of potato.

Variety name	Reaction	Variety name	Reaction
Cardinal	LS	Diamant	MS
Sante	HS	Lady Rosetta	CR
Kuroda	HS	Asterix	R

Least susceptible (LS), Moderate susceptible (MS), highly susceptible (HS), comparatively resistant (CR)

The reaction of potato cultivar against common scab of potato: During survey reaction of famous potato varieties against common scab of potato was checked in Fig. 2 and Tables 3 and 4.

Isolation of *Streptomyces scabies* from infected potato: A total of 129 isolates were isolated based on the phenotypic character of *Streptomyces scabies* as shown in Fig. 2.

Morphological and biochemical characterization: White grey and yellow Colony colour indicated positive results (+) and negative results were recorded as – as shown in Table 5.

Table 2. Analysis of variance for effect of variety, crop rotation, soil pH, and seed status nested within variety on disease incidence and severity of common scab of potato.

Source	Plant disease incidence		Disease severity	
	F	Sig.	F	Sig.
Variety	1.881	0.083	1.619	0.140
Crop Rotation	0.201	0.654	1.806	0.180
Soil PH.	36.855	0.000	83.517	0.000
Seed status	0.131	0.717	0.033	0.857
Variety * Crop rotation	0.534	0.782	0.509	0.802
Variety * soil PH.	3.493	0.004	2.490	0.031
Variety * seed status	0.927	0.476	1.509	0.174
Crop rotation * soil PH.	1.448	0.229	5.138	0.024
Crop rotation * seed status	0.113	0.737	0.763	0.383
Soil PH. * seed status	4.210	0.041	0.034	0.854
Variety * Crop rotation soil PH.	2.451	0.063	0.439	0.725
Variety * Crop rotation * seed status
Variety * soil PH. * seed status	0.809	0.520	2.076	.083
Crop rotation * soil PH. * seed status	3.027	0.083	2.529	0.113
Variety * Crop rotation * soil PH. * seed status

Soil pH has significant effect and others have non-significant at $p < 0.001$, 0.01, and 0.05 respectively.

Discussion

The study aimed to investigate the incidence and severity of common scab of potato (Table 4). The highest common scab was recorded in Okara and Sahiwal while the incidence was low in Taxila and Hazro. The high scab incidence in Okara, Kasur, Sahiwal, pakpatan followed by Lahore and Faisalabad was due to high potato cultivation in these districts. Mono cropping cultivation is the main reason for maximum disease inoculum presence and normally Pathogen survives as spores within debris it remains viable in the soil for up to 20 years (Kritzman *et al.*, 1996). In this study, disease severity was also calculated and variation was observed from field to field and location to location within a district, while severity in Hazro and Taxila was low. The present findings are in line with those of (Naher *et al.*, 2013). Farmers adopting land follow a strategy or crop rotation and after crop rotation, the soil condition changes and become unfavorable for *Streptomyces scabies* is the main reason for low disease severity, whereas the cause of high scab incidence is soil pH (Lacey & Wilson, 2001). In Okara, Kasur, Sahiwal, and Pakatan high disease incidence are due to high soil pH which favors the disease. The soil pH of the understudied area is above 7. Alkaline soils with pH vary from 7-8 are usually deficient in Zn²⁺ due to clayey, alkaline, and calcareous soils in Pakistan (Tahir *et al.*, 1991). Framers are using fertilizer for macronutrients only while micronutrients ignored. Disease severity increase as the soil pH increases to 8.0 from 5.0. Meanwhile, crossing this maximum pH limit it has declined (Goto, 1985). Scab decreases at low pH, soil pH below 5.2 usually suppresses potato scab (Waksman, 1922) while in some places common scab

was not noticed in soils below pH 5.2 (Hussain *et al.*, 2017). The results from this study showed an important role for both soil-born and seed inoculum in the common scab disease (Keinath & Loria, 1989). Another cause of low disease incidence in Taxila and Hazro was the use of new seed tubers for sowing every year. While the ratio of using old potato seeds in central Punjab is maximum, However, some researchers have also reported that disease incidence increases when infected seed tubers are planted (Santos-Cervantes *et al.*, 2017). Early crop harvesting is common practice in Hazro and Taxila mostly farmers harvest potato crops after 90 days so minimum disease severity is another reason. These findings indicate that growers wishing to decrease common scab losses should minimize the period of the crop in the field after the tops die. Similar suggestions were recommended for decreasing tuber losses due to the previous presence of *Rhizoctonia solani* (Dijst *et al.*, 1986). Minimizing this period should decrease scab losses, but growers must not harvest too early as the risk problems with the green vines plugging the harvest machinery and damaging the skin of tubers. Early planting and early harvesting seem to apply to high yield avoiding excessive inoculum to common scab. The survey showed that common scab occurs in Punjab while its severity varied area to area and field to field. It was also noticed that the most famous variety in Pakistan is sante, which is highly susceptible to common scab disease while the cardinal is the least susceptible. No potato variety is completely resistant to common scab; these results indicate the need for the discovery of appropriate control methods for a disease like cultural, chemical control, and biological methods. It is therefore recommended to find long-term control of common scab disease.

Table 4. Analysis of variance for the effect of variety, separated by variety on disease incidence and severity of common scab of potato.

(I) Variety	(J) Variety	Plant disease incidence			Disease severity		
		Mean difference (I-J)	Std. error	Sig.	Mean difference (I-J)	Std. error	Sig.
Lady Rosetta	Kuroda	-12.69*	2.260	0.000	-0.44*	0.076	0.000
	Diamant	-6.05	3.575	0.622	0.08	0.121	0.993
	Asterix	-2.88	3.460	0.982	-0.17	0.117	0.762
	Cardinal	-18.71*	2.382	0.000	-0.54*	0.080	0.000
	Sante	-15.41*	2.356	0.000	-0.49*	0.080	0.000
	Mozica	-12.35*	3.640	0.013	-0.66*	0.123	0.000
Kuroda	Lady Rosetta	12.69*	2.260	0.000	0.44*	0.076	0.000
	Diamant	6.64	3.185	0.363	0.52*	0.108	0.000
	Asterix	9.81*	3.055	0.024	0.27	0.103	0.131
	Cardinal	-6.02*	1.743	0.011	-0.10	0.059	0.663
	Sante	-2.72	1.708	0.686	-0.05	0.058	0.974
	Mozica	.34	3.258	1.000	-0.23	0.110	0.387
Diamant	Lady Rosetta	6.05	3.575	0.622	-0.08	0.121	0.993
	Kuroda	-6.64	3.185	0.363	-0.52*	0.108	0.000
	Asterix	3.17	4.124	0.988	-0.25	0.139	0.530
	Cardinal	-12.67*	3.272	0.002	-0.62*	0.111	0.000
	Sante	-9.37	3.254	0.063	-0.57*	0.110	0.000
	Mozica	-6.31	4.276	0.759	-0.75*	0.144	0.000
Asterix	Lady Rosetta	2.88	3.460	0.982	0.17	0.117	0.762
	Kuroda	-9.81*	3.055	0.024	-0.27	0.103	0.131
	Diamant	-3.17	4.124	0.988	0.25	0.139	0.530
	Cardinal	-15.83*	3.146	0.000	-0.36*	0.106	0.012
	Sante	-12.54*	3.127	0.001	-0.32*	0.106	0.042
	Mozica	-9.48	4.180	0.263	-0.49*	0.141	0.010
Cardinal	Lady Rosetta	18.71*	2.382	0.000	0.54*	0.080	0.000
	Kuroda	6.02*	1.743	0.011	0.10	0.059	0.663
	Diamant	12.67*	3.272	0.002	0.62*	0.111	0.000
	Asterix	15.83*	3.146	0.000	0.36*	0.106	0.012
	Sante	3.30	1.865	0.570	0.04	0.063	0.992
	Mozica	6.36	3.343	0.480	-0.13	0.113	0.914
Sante	Lady Rosetta	15.41*	2.356	0.000	0.49*	0.080	0.000
	Kuroda	2.72	1.708	0.686	0.05	0.058	0.974
	Diamant	9.37	3.254	0.063	0.57*	0.110	0.000
	Asterix	12.54*	3.127	0.001	0.32*	0.106	0.042
	Cardinal	-3.30	1.865	0.570	-0.04	0.063	0.992
	Mozica	3.06	3.325	0.969	-0.17	0.112	0.716
Mozica	Lady Rosetta	12.35*	3.640	0.013	0.66*	0.123	0.000
	Kuroda	-.34	3.258	1.000	0.23	0.110	0.387
	Diamant	6.31	4.276	0.759	0.75*	0.144	0.000
	Asterix	9.48	4.180	0.263	0.49*	0.141	0.010
	Cardinal	-6.36	3.343	0.480	0.13	0.113	0.914
	Sante	-3.06	3.325	0.969	0.17	0.112	0.716

Lady Rosetta- Kuroda, Cardinal, Mozica, Sante, Lady Rosetta = Significant at $\alpha = 0.05$ Diamant- Cardinal = Significant at $\alpha = 0.05$ Cardinal-Diamant, Asterix, Lady Rosetta = Significant at $\alpha = 0.05$ Asterix- Cardinal, Sante, Lady Rosetta = Significant at $\alpha = 0.05$ Sante- Asterix, Lady Rosetta = Significant at $\alpha = 0.05$

Table 5. The morphological and biochemical characterization of isolates.

Isolate	MEA	OMA	Inorganic-salts- starch agar	Glycerol-asparagine agar	Gram staining	Catalase test	Levan production test	Starch hydrolysis test;	Hydrogen sulphide (H ₂ S) production test
CSOK1	White, Gray	Grey	White	White	+	+	-	+	-
CSSH5	White, Gray	Yellow	Grey	Grey	+	+	-	+	-
CSLAH9	White, Gray	Brown	White/Grey	White	-	+	-	+	-
CSTAX3	White, Gray	Grey	Grey	Yellow	+	+	-	+	-
CS7PAK15	White, Gray	Yellow	Grey	Yellow	+	+	-	+	-
CSPAK7	White, Gray	White	Grey	Yellow	+	+	-	+	-
CSSH12	White, Gray	White	Grey	White	-	+	-	+	-
CSOK5	White, Gray	Grey	White, Gray	Yellow	+	+	-	-	-
CSDEP9	White	Grey	Grey	White	+	-	-	+	-
CSLAH7	White	Grey	Grey	White, Gray	+	+	-	+	-
CSTAX27	Grey	Grey	Grey	White	+	+	-	+	-
CSOK45	Grey	Grey	Grey	White	+	+	-	+	-
CSKAS13	Grey	Grey	Grey	White	-	-	-	+	-
CSLAH25	Grey	Grey	Grey	Grey	+	+	-	-	-
CSPAK22	Grey	Grey	White, Gray	Yellow	+	+	-	+	-
CSDEP20	Grey	Grey	Grey	Grey	+	-	-	+	-
CSOK13	Grey	Grey	Grey	Yellow	-	+	-	+	-
CSLAH13A	Grey	White	Grey	Grey	+	+	-	+	-
CSHAZ19	Grey	White	Grey	White	+	+	-	+	-
CSKAS09	Grey	White	Grey	White	+	+	-	-	-
CSOK35	Grey	White	White, Gray	Yellow	-	+	-	+	-
CSPAK05	Grey	White	Grey	White	+	-	-	+	-
CSHAZ11	Grey	White	Grey	Grey	+	+	-	+	-
CSTAX02	Grey	White	Grey	Grey	+	+	-	-	-
CSLAH10	Grey	White	White, Gray	White	-	+	-	+	-
CSFAB05	Grey	White	White	Grey	-	+	-	+	-
CSOKA	Grey	White	White	White	-	+	-	+	-
CSDEP13	Grey	Grey	White	Yellow	+	+	-	-	-
CSOK52	Grey	Grey	White	Yellow	+	+	-	+	-

Conclusion

“Lady Rosetta” and “Asterix” are comparatively resistant as compared to other experienced varieties, so these are recommended for cultivation in Punjab to enhancing the market value of potato. Bacteria causing common scab of potato are an evolving threat to potato crop in Pakistan. Therefore, the need for new research is to grow environmentally friendly and sustainable agriculture. Therefore, it is important to find antagonistic organisms that perform against this pathogen.

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