



RESEARCH ARTICLE

SCREENING ADVANCED POTATO MUTANT GENOTYPES AGAINST BACTERIAL WILT DISEASE UNDER DIFFERENT AGRO-ECOLOGICAL ZONES OF KENYA

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ABSTRACT

Host plant resistance is a key tool in the management of plant diseases. Mutant lines (IP1, IP2, IP3 and IP4) were tested against other potato varieties (Shangi, Sherehekea, K. Mpya and Unica) in different agro-ecological zones to ascertain their performance under potato bacterial wilt disease pressures. An experiment was set in an RCBD design with three replications in four different sites, namely, Nyandarua, Elgeiyo Marakwet, Burnt Forest and Moiben. Assessment was done on potato bacterial wilt disease prevalence and severities, as well as host plant status, which were rated on a scale of 1-5. Data obtained were subjected to analysis using statistical software GenStat 16th Edition VSN International Ltd. Different response levels and uniqueness were expressed by each test line across all AEZs, where variety, sites and time intervals (DAS) as well as their interactions had significant effects on disease severity, with different tolerance levels to the pathogen experienced in all the lines. The severity mean of four mutant lines was below 2.0 as per the days after sowing (DAS). Moreover, IP1 (2.0) and IP2 (2.1) had an overall mean across all sites; therefore, they proved a high level of tolerance to the bacterial wilt pathogen, while IP3 (2.4) and IP4 (2.5) were less than the resistant check (2.8) across all the sites and the susceptible check Kenya Mpya (3.2). Hence, there is a need for regular screening of the available genotypes for *R. solanacearum* management in potato production. The existence of the most tolerant varieties could form a baseline in integrated disease management for sustainable potato production.

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth major cash crop grown in Kenya after rice, wheat and maize that is mainly grown as a source of both processed and raw products (Muthoni & Shimelis, 2023). In terms of nutritional composition, potatoes are a rich source of vitamins, proteins (1.8 g), carbohydrates (17 g), fat (0.1 g) and fibre (1.2 g) as well as other minerals and micronutrients. Potatoes are well known for their undisputed source of fibre, especially when consumed with the skin on, thus contributing to a sense of fullness and good digestive mechanisms (Singh et al., 2023). Over the years potato demand has been on the rise due to factors such as urbanisation, changes in people's lifestyles and rapid population growth. However, the production trend has been affected by fluctuating environmental conditions, pests and diseases and poor adoption of technological advances of new varieties (Chindi et al., 2021; Sunitha et al., 2024). Major constraints to potato production are biotic factors and socio-economic factors, as well as abiotic factors that are reported to reach up to 80-100% losses in yield in susceptible conditions

(Chepkoech, 2022). Major constraints to potato production are biotic factors and socio-economic factors, as well as abiotic factors that are reported to cause up to 80-100% losses in yield in susceptible conditions (Divya et al., 2021). Bacterial wilt has been reported to cause yield loss ranging between 50-100%, hence causing negative and significantly huge socioeconomic impacts on small, medium and large-scale farmers in Kenya. Based on its severity and potential damage, this disease is considered to be the second most important after bacterial canker caused by *Pseudomonas syringae* (Charkowski, et al., 2020) and a major point of research in Kenya based on the role of potatoes in human nutrition and contribution to GDP. Among the factors hindering effective management of this disease is the presence of a wide host range of over 200 plant species in the Solanaceae family together with the ability to undergo rapid mutation when a new host plant is introduced. In the past, several chemical control methods have been widely used by potato farmers. However, this disease is still a constant threat in potato production, and the gap in sustainable management techniques still exists in its appropriate. The present study attempted to address the

contribution of the choice of varieties and prevailing environmental conditions as one of the factors responsible for the prevalence of potato bacterial wilt diseases in Kenya that has resulted in low production rates over the recent years. Multi-locational performance is essential in the developed varieties to boost farmers' knowledge of the varieties as well as boosting the varieties' adoption rates.

MATERIALS AND METHODS

Site of experiment and variety selection: This study was carried out at four sites, namely Nyandarua, Elgeiyo Marakwet, Burnt Forest and Moiben. These sites were selected based on their past reports on bacterial wilt disease prevalence and also the history of high potato production over the years. The site characteristics areas are described in Table 1 below (Akenga *et al.*, 2020; Cheruiyot *et al.*, 2023; Muthoni *et al.*, 2014; Szpunar-Krok *et al.*, 2022).

Eight potato varieties were selected and used in this study. These genotypes were the four newly developed lines (IP 1 to IP 4) that were developed at the University of Eldoret and the four commercial varieties, such as Sherehekea (susceptible check), UNICA (resistant check), Shangi (resistant check) and K. MPYA (susceptible check), and these varieties were sourced from ADC. The site characteristics are as shown in Table 1 below:

Planting and experimental design: This experiment was set during the long rains of the month of May of the year 2020 in a randomised complete block design with three replications. Planting was done at a spacing of 75 by 30 centimetres and a plant density of eight plants per line in a single line with eight hills representing a plot. Four inner plants were tagged for data collection. The plot was measuring 3 m plot length, and the inter-row spacing was 1 m. During planting, DAP fertiliser was applied at the rate of 150 kg per acre to supplement the nutritional status of the soil.

Assessment of the bacterial wilt disease: Quantitative information on the response of potato lines to the pathogen was recorded basing on the observed symptoms on the plants either on the leaves withering or of the whole plant wilting using the scale of 1-5 for diseases assessment: where 1 = high resistance, 2 = above 10% infection on leaves 3 above 25% lesion on leaves, 4 = over 50% extended to necrosis, 5=defoliation and plant dead.

Bacterial wilt disease on the other hand was scored using a scale of 1=resistant (one leave partially wilted) 2 = 2-3 leaves wilted, 3 = leaves wilted except the upper leaves, 4 = all leaves wilted, 5 = plant dead. The assessment begun at the pre flowering stage, 45 days after planting until full maturity and the data collected at an interval of 7 days whole plot was assessed for severity assessment.

Statistical analysis: Data on disease severity and the response of the selected potato lines under different agro-ecological zones were subjected to analysis using GenStat statistical software, where analysis of variance was established, while means were further separated using the Duncan multiple range test at the 5% level of significance, where results were presented in the form of a table of means, plates, and biplot correlation matrices.

RESULTS

Response of improved potato mutants to *R. solanacearum* under fiend condition: Sites, variety, and time intervals, as well as the interactions among these three main factors, expressed a significant effect on severity levels of bacterial wilt under field conditions ($p < 0.05$). Additionally, the main effects of variety, site and days after sowing significantly influenced the level of infection, hence the severity levels among the screened potato mutant lines ($p < 0.05$). However, the three-way interaction among the three factors, namely site, variety and days after sowing, was not significant ($p > 0.05$). Overall, most of the mutant lines (candidates) expressed more tolerance to bacterial wilt under field conditions than their parental checks. For instance, lines IP1 and IP2 were the most tolerant to bacterial wilt, with an average severity rating of 2.0 on a 1–5 severity rating scale. Also, IP3 and IP4 lines were ranked tolerant to the disease with an average score of 2.4 and did not differ significantly under DMRT mean separation.

Contrary to the previous reports, the resistant and susceptible checks recorded higher disease severities compared to the improved mutant lines under field conditions. In terms of disease progress for every genotype, disease severities were directly proportional to time. This was from days 60-105, when IP1 and IP2 had the lowest disease progressive growth, with the highest recorded a 3.2 severity level, while IP3 and IP4 recorded 3.5, which was basically lower than that of the check lines, which recorded above 4.0 at the pre-flowering stage, unlike their counterparts (candidates), which showed many symptoms at the post-flowering stage (Table 2). On the other hand, different agro-ecological zones showed significant variations in terms of severity levels by different potato genotypes. In this respect, potato genotypes were more affected by this pathogen at the Moiben site, hence higher severity, while Burnt Forest conditions did not show much severity (Table 2). Phenotypic expression among the checks and improved mutant lines indicated that against the known traits of resistant checks (Shangi and Unica), Shangi, which was bred for resistance, showed a high level of susceptibility to *Ralstonia solanacearum*, which led to the total loss of batches even before physiological maturity. However, the test lines (IP1, IP2, IP3, and IP4) showed more resistance to *Ralstonia solanacearum* across different agro-ecological zones (Plate 1).

Genotypes and environment Interaction (GEI) effect on *R. solanacearum* severity: The screened mutant lines alongside commercially grown potato varieties (checks) showed varied responses to *R. solanacearum*, with some showing stable responses with respect to sites. Specifically, the scatter plot correlation matrix gave a total variability of 96.41%, which results from the sum of PC2 4.91% (dependent factors) and PC1 91.50% (independent factors). Other than the significant contribution by the independent factors to the total observed variability in terms of severity to *R. solanacearum*, the agro-ecological zones where screening was done formed two mega-environments which separated the Burnt Forest zone from Marakwet, Nyandarua and Moiben, which also formed a bigger mega-environment (Figure 1). In terms of genotype adaptability to the two mega-environments, the responses by Unica and K. Mpya varieties showed higher stability and hence adaptability towards Nyandarua and Moiben agro-ecological zones.

Table 1. Environmental characteristics of the study area

site	Altitude (M)	Temperature (oC)	AV Rainfall (mm)	Relative humidity (%)	Soil pH	Soil Type
Moiben (UOE)	2154	16.8-22	800-1000	43.9-71.7	4.5-5.5	Rhodic ferrasols
Marakwet (Chabera ATC)	2448	15.8-21	900-1200	43.9-71.7	5.0-5.5	Humic Nitisols
Oljororok	2246	13.8-17	975-1200	41.7-78.9	3.9-6.2	Andasol/planosol
Burnt forest	2390	14-18	1000-1019.4	43.9-75.5	4.5-5.0	Ferasol

VARIETY	DAS	EXPERIMENTAL SITE				MEANS		DMRT
		Burnt forest	Marakwet	Moiben	Nyandarua	DAS	Variety	
IP1 (A57)	60	1.0	1.0	1.5	1.0	1.1	2.0	a
	70	1.5	2.2	1.8	1.5	1.8		
	80	1.5	2.0	2.0	2.2	1.9		
	90	2.0	2.7	2.7	2.5	2.5		
	105	2.0	3.3	3.0	3.2	2.9		
IP2 (S33)	60	0.7	0.7	1.5	1.5	1.1	2.1	a
	70	1.2	1.5	2.2	1.8	1.7		
	80	1.7	2.0	2.7	2.5	2.2		
	90	1.8	2.7	3.2	2.7	2.6		
	105	1.8	3.2	3.0	3.2	2.8		
IP3 (S15)	60	1.3	1.5	1.5	1.7	1.5	2.5	b
	70	1.8	2.2	2.5	2.0	2.1		
	80	2.5	2.3	2.8	2.5	2.5		
	90	2.8	3.2	3.5	2.8	3.0		
	105	3.0	3.5	3.3	3.3	3.3		
IP4(M15)	60	1.2	0.8	1.8	1.0	1.2	2.4	b
	70	1.8	1.5	2.2	1.8	1.8		
	80	2.5	2.5	2.7	2.5	2.5		
	90	2.8	2.8	2.8	2.8	2.8		
	105	3.0	3.7	3.7	3.7	3.5		
K. Mpya	60	2.0	2.2	2.8	1.7	2.2	3.2	e
	70	2.5	2.7	3.3	2.2	2.7		
	80	3.2	3.2	3.8	3.0	3.3		
	90	3.5	4.0	4.2	3.5	3.8		
	105	3.7	4.5	4.5	3.8	4.1		
Shangi	60	1.8	1.8	2.0	1.5	1.8	3.0	d
	70	2.3	2.5	2.5	2.5	2.5		
	80	3.2	3.0	3.2	2.8	3.0		
	90	3.8	3.7	3.5	3.2	3.5		
	105	4.0	4.5	4.0	3.5	4.0		
Sherekea	60	2.0	1.5	2.5	1.7	1.9	3.0	d
	70	2.2	2.8	2.8	2.0	2.5		
	80	3.2	3.3	3.5	2.2	3.0		
	90	3.5	4.0	3.5	3.2	3.5		
	105	4.5	4.5	4.2	3.7	4.2		
Unica	60	1.5	1.5	2.0	2.0	1.8	2.8	c
	70	1.8	2.0	3.0	2.5	2.3		
	80	3.2	3.2	3.2	2.8	3.1		
	90	2.8	3.3	3.3	3.3	3.2		
	105	3.5	3.5	3.5	3.8	3.6		
MEAN		2.4	2.7	2.9	2.5	2.6		
DMRT		a	c	d	b			
Statistics	Variety	DAS (D)	Site	V x D	V x S	D x S	V x D x S	
Probability	<.001	<.001	<.001	0.419	<.001	0.001	0.963	
S.E	0.0551	0.0436	0.039	0.1233	0.1103	0.0872	0.2466	
S.E.D	0.078	0.0617	0.0551	0.1744	0.156	0.1233	0.3488	
% CV	16.3							

Also, Shangi and Sherekea were stable and adapted towards the Burnt Forest and Marakwet zones of production. However, despite recording lower disease levels on average, all the mutant lines had varied responses towards *R. solanacearum* and did not show any adaptability to a specific agro-ecological zone (Figure 1).

With reference to the ranking biplot, the Unica variety proved to be the most stable genotype across all agro-ecological zones where screening was done; hence, it was positioned closer to the tip of the arrow (centre of comparison) than the rest of the genotypes screened. However, despite being a resistant check,

this genotype still recorded some level of considerable severity towards bacterial wilt disease. For the candidates, though IP3 and IP4 expressed considerably more severity than IP1 and IP2, they were the most stable among the candidates and hence positioned closer to the arrow (centre) (Figure 2). In the comparison biplot for the genotypes, the centre of the concentric ring depicts where the most ideal genotype (resistant or susceptible to the disease) is located, and in this note, Kenya Mpya, which expressed susceptibility across all sites, was the most ideal in terms of susceptibility to bacterial wilt. However, all the mutant lines appeared further from the centre compared to all parental checks, indicating that they

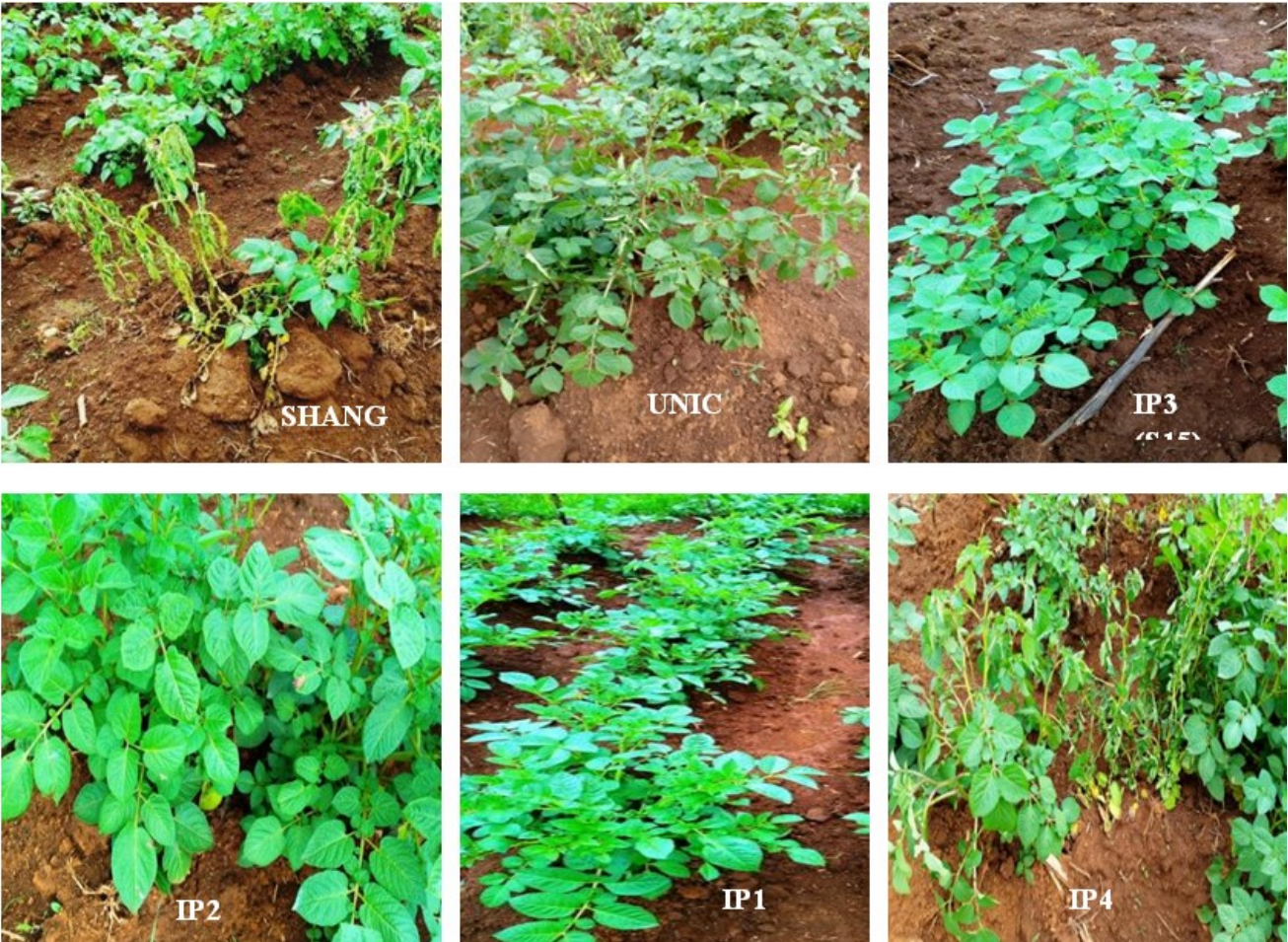


Plate 1. Severity and resistant expressions by improved potato mutant lines across different agro-ecological zones The improved mutant lines (IP1 – IP4) were more tolerant to *R. solanacearum* than checks (Shangi and Unica)

were less affected by the disease. For instance, among the candidates, IP1 and IP2 appear at the furthest end of the concentric ring compared to IP3 and IP4, hence the most tolerant to bacterial wilt under field conditions (Figure 3).

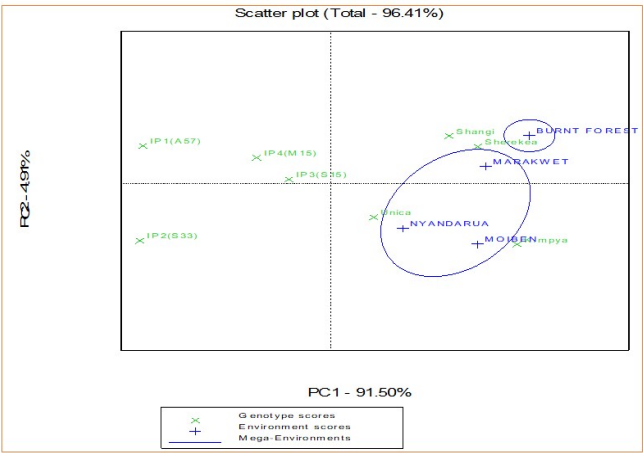


Figure 1. Scatter plot correlation matrix between potato mutant lines and environmental (sites) interactions in response to *Ralstonia solanacearum*

DISCUSSION

The significant difference in the response of tested potato genotypes to bacterial wilt disease under different agro-ecological conditions could be attributed to the presence of

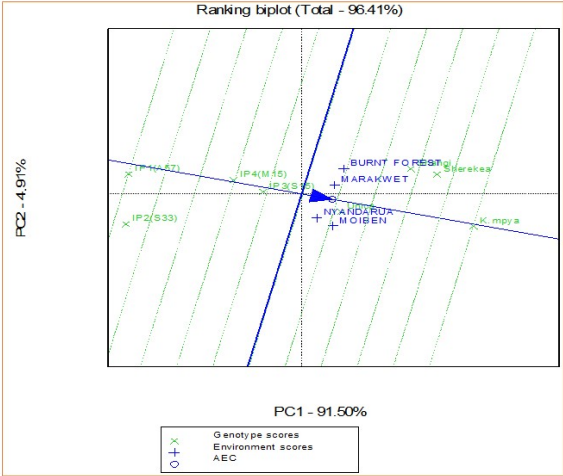


Figure 2. Ranking biplot correlation matrix between potato mutant lines and environmental (sites) interactions in response to *Ralstonia solanacearum*

trapping substances in the test lines that stop the penetration of bacterial pathogens through the parenchyma cells; therefore, there is no multiplication of pathogen cells in the host plant, resulting in very low disease pressures in the mutant lines as compared to their counterpart varieties (Yadeta & Thomma, 2013). The interaction with the environment and time interval (DAS) enhanced effective results in that the disease severity was severe with an increase in time and the prevailing environmental factor (Charkowski *et al.*, 2020). An increase in

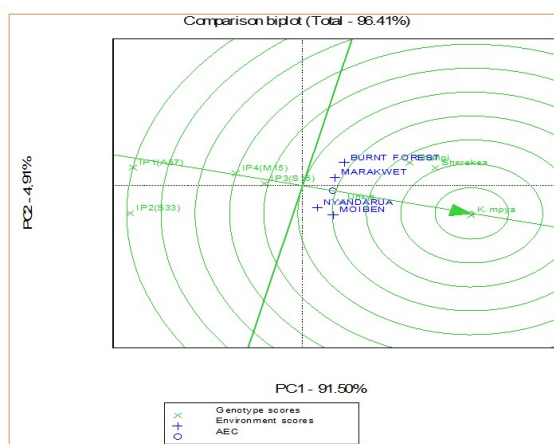


Figure 3. Comparison biplot correlation matrix between potato mutant lines and environmental (sites) interactions in response to *Ralstonia solanacearum*

time intervals resulted in an increase in disease severity across all the sites. Therefore, the older the plant, the more susceptible they become, especially for the checks. This agrees with the earlier report that host plants have specific growth stages where infection occurs, which are the seedling stage or adult stage (pre-flowering or pod stage depending on the plant), and once the stage is passed, then the host plant has escaped. Another report clarified that the host plants have a signalling pathway for defence proteins synthesising that they are modified by introducing new effectors for bacterial management without distorting the natural pathway (Hamuel, 2015). Additionally, the similarity matrix of the test lines showed that mutant lines had two different ranking levels that might have been as a result of different genetic makeup and adaptability, while check lines had their ranking levels due to race-specific genes to specific pathogens. The above result showed an opposite report to that of (Yuliar *et al.*, 2015) where there was 70 % wilt resistant at a seedling stage and early vegetative phase in tomato cultivars (Nivas *et al.*, 2015). The above needed to be confirmed by the new mutant lines from the University of Eldoret, where the reverse occurred, with severity being fully expressed at the advanced stage rather than the seedling stage. Generally, the interaction between genotype and environment is known to determine the pathogen virulence level, but they had a low impact with a lower PC2 % as compared to PC1 %. This result can be looked into as per a study conducted in the past where a decrease in temperature up to 10°C enhanced pathogen survival on tubers, while a temperature range of 12-15°C in the soil enhanced its survival, thus resulting in a low infection rate of bacteria in lower temperature zones and in highland Kenya even though the soil is infected (Musah *et al.*, 2023; Muthoni *et al.*, 2014). The above can only be explained by the fact that the test line's genetic makeup determined the level of severity in different sites and regulated the pathogen entry to enhance invasion into the crop intercellular space to trigger a signal through the hypersensitive gene in potato, which in turn triggers the molecular syringe secretion system to produce regulatory genes. Thus suppress defence genes interfering with plant signal pathway functions (Kannan, *et al.*, 2015). In the large mega-environment, the Marakwet, Moiben, and Nyandarua sites, respectively, were at the same level, while the burnt forest was different. This would be due to external factors that were present in the area and not in others. For example, a soil pH of 4, like in the four sites, played a major role in high infection and disease stability. This agrees with a study where

an increase of soil pH up to 4.5 (acidic), *R. solanacearum* expressed very high severity in a number of potato varieties, but the same reduced at a pH of 7.5 (slightly alkaline condition), whereas low severity expressions with few bacterial colonies on soil samples have been reported (Musah *et al.*, 2023). In other reports, virulent genes can be expressed at pH 5.5, restricting the host plant in expressing genes for resistance. This further explains the effect of acidic conditions on microbial activities and the ability of bacterial pathogens to cause disease in host plants. Such findings could be responsible for the varied responses among various potato varieties to bacterial wilt due to their varying levels of tolerance to acidity and nutritional status (Li *et al.*, 2017). In other research nutrient availability in the soils can determine the level of pathogen severity, i.e., potassium (K) inducing host plant resistance to pathogens and its reduction influencing molecular-weight organic compound synthesis; hence, disease infections occur. Calcium is known to assist in pathogen recognition immediately the invasion occurs by doubling cytoplasmic Ca, hence host plant defence (Tripathi *et al.*, 2022). Moreover, high calcium (Ca) concentration in acidic soil increases pathogens. In another study, the effect of nitrogen concentration in soil and its influence on host-pathogen interaction justified that nitrogen concentration in the soil and its utilisation either increase or reduce disease severity in the host plant. On the other hand, phosphorus has also been reported to suppress bacterial pathotype growth, especially in potatoes; this depends on phosphorus concentration in soil and on potato periderm (Gumiere *et al.*, 2019).

CONCLUSION

Potato mutants (IP1, IP2 and IP3) expressed high tolerance to *R. solanacearum* under different agro-ecological zones than commercially grown varieties. These varieties should be adopted by farmers and research institutions to improve potato production.

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