EVALUATION OF POTATO VARIETIES FOR RESISTANCE TO SOFT ROT BACTERIA IN BANGLADESH

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ABSTRACT

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Thirteen potato varieties collected from Tuber Crop Research Center (TCRC) of Bangladesh Agricultural Research Institute (BARI), Gazipurwere screened against soft rot bacteria. There were significant differences among the potato varieties in soft rot disease severity ($p<0.05$). BARI Alu 25 exhibited the least soft rot disease severity compared to other potato varieties. Calcium ($p=0.018$) but not dry matter percentage of tubers was correlated with soft rot ranking in these varieties. A regression analysis involving both calcium percentage and dry matter percentage showed that rotted tissue percentage varied with the increase or decrease of calcium percentage ($p<0.05$) but not with the changes of dry matter percentage.

Key words: BARI, Calcium, dry matter, *Pectobacterium*, potato, soft rot.

INTRODUCTION[1]

Conventional screening could be the best way to identify sources of resistance to soft rot to incorporate into potato breeding programs in Bangladesh. There are many factors that influence the resistance of potato to soft rot (*Pectobacterium carotovorum*s sp. *carotovorum*), including water potential, ambient oxygen status, reducing sugar content, electrolyte leakage, dry matter content, age of tubers and storage conditions (Gnanamanickam 2006). Dry matter content is an important factor that determines the resistance to soft rot bacteria in tubers (McGuire and Kelman 1984). Potato tuber with higher dry matter content easily get bruised and make entry for bacteria penetration. So the tuber with higher dry matter content is probably susceptible to soft rot bacteria. However, Biehn *et al*. (1972) reported that potato varieties with a relatively high amount of dry matter were generally less susceptible to soft rot than those with lower dry weights. Earlier studies showed that increased calcium content reduced the probability of potato soft rot incidence in storage (Tzeng *et al*. 1990). Calcium is an integral part of the cell wall and membrane of potato tubers and tubers with higher content are less susceptible to pectolytic bacteria than those with low calcium content (McGuire and Kelman 1984). Soft rot *Pectobacterium* infiltrates host cells through the action of pectolytic enzymes that hydrolyzes pectin between individual cells resulting the separation of cells and loss of cell structure. Calcium content of plant cell wall gives strength to the cell wall. As a result, pectolytic enzymes secreted by *Pectobacterium* cannot easily disintegrate the tissues of potato and delay in the rate of tissue maceration. However, little is known about the resistance of Bangladeshi potato varieties and there are no soft rot-resistant commercial varieties available in the markets of Bangladesh. This research was aimed to identify the degree of resistance in potato varieties currently available in Bangladesh to bacterial soft rot bacteria. Potato breeders can then include moderately resistant or resistant potato germplasm in varietal improvement programs. The objective of this study was to determine the correlation of tuber susceptibility with different concentration of calcium (Ca) and dry matter content of tuber.

MATERIALS AND METHODS

Previously characterized *Pectobacterium carotovorum*s sp. *carotovorum* strain Pki2 (GenBank acc. no-KX098357, collected from Plant Pathology division, BARI, Gazipur) was streaked on LBA medium and incubated at 28°C for 48 hours. The concentrations of bacterial suspensions were adjusted to 10^8 CFU/mL (0.2 OD at 600 nm). Tubers of 13 BARIpotato varieties harvested at the same time in February 2018 were collected from the storage of Tuber Crop Research Center (TCRC), BARI where the potatoes were kept at 25°C. Twelve randomly selected tubers from each cultivar were washed with 0.5% sodium hypochlorite. Bacterial inoculum was stirred with a magnetic stirrer to avoid sedimentation. Potato tubers were cut in half and two wells, 5 mm in diameter x 5 mm deep, were made in each site on each half tuber using a sterilized metal screw. A 40µL aliquot of *P. carotovorum*s sp. *carotovorum* Pki2 inoculum was placed in each well and the wells were wrapped with para film. Three randomly selected tubers from each cultivar mock-inoculated with sterilized distilled water served as a negative control. After inoculation, tubers were placed in moistened plastic containers in the dark for 3 days at 28°C. After incubation tubers were weighed, rotted tuber tissue was separated from healthy tissue with a spatula and the fresh weight of the rotten portion was measured in grams and later converted to percentage (Pasco *et al*. 2006). The experiment was designed in randomized complete block design (RCBD) with three replications.

Four tubers from each potato cultivar were peeled, cut into thin slices, weighed and dried at room temperature. Potato slices were oven dried at 65°C for 2 days and mixed all the replications sample of each cultivar together to make sufficient sample. Samples were digested with H_2SO_4 and H_2O_2 (Thomas *et al*. 1967) weighed. Dry matter percentage was calculated following the formula:

Dry matter % = (dry weight/fresh weight) x100

Dried potato samples were sent to the Department of Soil Science, BARI, Bangladesh to measure the percentage of calcium in medullar tissues using flame atomic absorption spectrometry following a wet digestion method (Habib *et al*. 2004).

Data analysis. R statistical software was used for the analysis of variance (ANOVA), correlation and multivariate regression. Differences in means were assessed using Least significant difference (LSD) with an error rate of α=0.05.

RESULTS AND DISCUSSION

All the 13 potato varieties showed tuber soft rot damage 3 days after inoculation. There were statistically significant differences among the potato varieties in soft rot disease severity ($p<0.05$) (Figure 1). Mean soft rot of potato tuber tissue ranged from 27.5% (BARI Alu 25) to 97.5% (BARI Alu 40). BARI Alu 25 exhibited significantly less damage than four other most susceptible varieties BARI Alu 40, BARI Alu 70, BARI Alu 64 and BARI Alu 66, whereas the percentage of soft rot was statistically similar for all these four observed potato varieties (Table 1). In this experiment, variation was observed in the susceptibility of potato tubers of ten different varieties released from the BARI breeding program. This variation in susceptibility is supported by the previous work of Reeves *et al*. (1999) who reported that different level soft rot incidence and weight reduction in potato tubers when they were inoculated with soft rot pathogens. In our study, no varieties were fully resistant to soft rot. Naturally soft rot-immune potato varieties are not known; rather some partially resistant potato cultivars are available (Lyon 1989). This may be due to a narrow range of genetic variation in the tubers (Tzeng et al. 1990). According to Hijmans and Spooner (2001), a large amount of useful genetic material present in more than 200 wild *Solanum* species of Europe, and North and South America, which includes resistance to soft rot bacteria, could be used to develop soft rot resistant potato cultivars. Therefore, a wide range of potato varieties and potential resistance sources should be included in future screening programs. In addition, disease resistance of potato varieties varies season to season (Perombelon and Salmond 1995). Field evaluations over several consecutive years may be needed to identify potato varieties with meaningful levels of resistance to soft rot (Pasco *et al*. 2006). The ability to identify soft rot resistance reliably depends on many factors such as inoculation method, presence or absence of oxygen, and laboratory and field conditions, as well as weather conditions in different seasons (Czajkowski*et al*. 2011).

The percentage of tuber calcium ranged from 0.57-1.31% in the thirteen potato varieties and differences among varieties were significant ($p<0.001$) (Table 1). Percent calcium content was the highest in BARI Alu 25 followed by BARI Alu 40 (1.06) and was lowest in BARI Alu 70 followed by BARI Alu 56 (0.68). In an analysis, the correlation coefficient ($r=-0.374$) for the relationship of rotted tissue and calcium was statistically significant ($p=0.018$). Calcium is an integral part of the cell wall and membrane of potato tubers. In this study, a significant negative correlation was found between tuber susceptibility and calcium percentage in tubers. This observation is supported by Pagel and Heitefuss (1989), who indicated that there is a consistent relationship between calcium content of tissues and soft rot susceptibility of potato cultivars. Other factors such as sugar content, oxygen level (McGuire andKelman1984; 1986) and starch content should also be studied to determine their relationship to resistance to soft rot.

Percentage tuber dry matter ranged from 24.56 (BARI Alu 61) – 36.90 % (BARI Alu 70), and differences among varieties were significant ($p<0.001$). BARI Alu 28, BARI Alu 40, BARI Alu 45, BARI Alu 64 and BARI Alu 66 had relatively high dry matter content, whereas the BARI Alu 13, BARI Alu 37, BARI Alu 56 and BARI Alu 61 were relatively low in dry matter content (Table 1). However, the correlation coefficient ($r=0.237$) for the relationship of rotted tissue and dry matter was not statistically significant (p=0.144). The regression model considering both calcium and dry mater percentage was significant ($p<0.05$) at 5 % significance level. The regression model is:

Rotted tissue (%)= 58.74+1.85 Dry matter (%)-51.02Ca

Dry matter percentage of potato tubers may be a source of variability in the evaluation process of potato varieties for resistance to soft rot (Tzeng*et al*. 1990). In a study, Wright *et al*. (2005) reported that soft rot severity increased with increasing dry matter content in the tuber.In our study, there was no significant correlation between dry matter content and severity of soft rot was recorded. Although this type of information is helpful for breeders, and the varieties that show partial resistance could be used in varietal evaluation programs. Information on additional factors such as water status on the tuber surface, high relative humidity and temperature, anaerobic condition of storages are needed (Bourne *et al*. 1981). A multiple regression analysis was generated including both percentage of calcium conc. and percentage of dry matter. Here we found that percent calcium conc. has negative significant association whereas percent dry matter has positive non-significant association with percent rotted tissue.

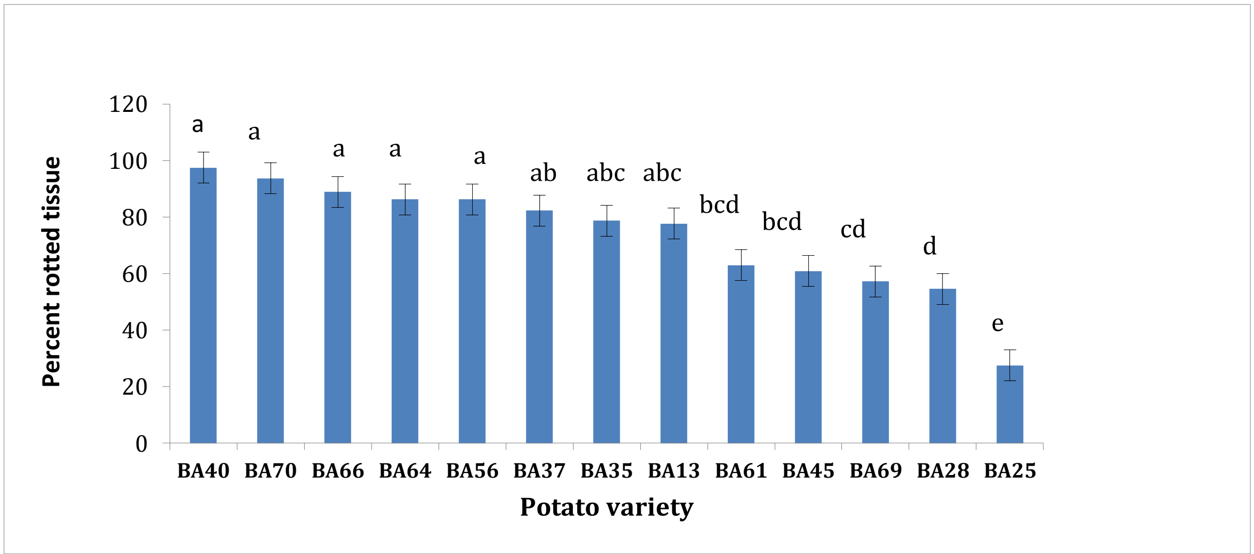

Figure 1. Percent rotted tissue of Bangladesh Agricultural Research Institute (BARI)-released potato varieties after inoculation with *Pectobacterium carotovorum* subsp. *carotovorum* strain Pki2. The error bars indicate standard errors for three replications. Values for means followed by the same letters are not significantly different in LSD

Table 1. Percent rotted tuber of Bangladesh Agricultural Research Institute (BARI)-released potato varieties after inoculation with *Pectobacterium carotovorum* ssp. *carotovorum* strain Pki2, and the percentages of calcium and dry matter for each cultivar. Values for means followed by same letters are not statistically different in LSD method

Potato Variety	Percent rotted tissue	Percent dry matter	Percent tuber calcium
BARI Alu 40	97.51983 a	35.91061 c	1.068667 b
BARI Alu 70	93.73685 a	36.9054 a	0.658 i
BARI Alu 66	88.90577 ab	31.42093 e	0.781333 g
BARI Alu 64	86.29331 abc	31.58708 e	0.872333 d
BARI Alu 37	82.29814 abc	29.54982 h	0.787 fg
BARI Alu 35	78.72671 abcd	30.94234 f	0.792667 f
BARI Alu 56	78.18518 abcde	28.63897 i	0.682667 h
BARI Alu 13	77.67946 abcde	25.63816 j	0.783333 g
BARI Alu 45	69.02721 bcde	32.05854 d	0.828333 e
BARI Alu 61	63.04515 cde	24.56048 k	0.823333 e
BARI Alu 69	57.2253 de	28.73836 i	0.572333 j
BARI Alu 28	54.58335 e	36.26929 b	0.919667 c
BARI Alu 25	27.58679 f	30.08235 g	1.319667 a
p value	<0.001	<0.001	<0.001

CONCLUSION

This study was conducted twice in one season with a limited number of potato varieties. To get a conclusive result, another experiment would be conducted in the field with these selected potato varieties to see the performance against soft rot disease.

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