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CALCIUM FERTILIZERS FOR REDUCING ERWINIA SOFT ROT OF POTATO

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ABSTRACT

Fertilisation of three potato cultivars i.e. Herms, Lady Rossetta and Lady Joy with Calcium chelate and Calcium nitrate decreased Erwinia sp. tuber rots, increased starch content and the cation content in the medulla. Greater effect was observed on Herms tubers compared to Lady Rosetta and Lady Joy. Weight loss due to soft rot incidence was significantly reduced by approximately 50% after 5 weeks of storage and by one and a half fold after 18 weeks.

The reduction in potato soft rot was inversely related to the content of tuber calcium in the field. *The tubers obtained from plots treated with calcium salts had calcium* contents in the medulla of tested cultivars ranging between 21.5 to 6.1ppm (on dry weight basis) with the greatest content in Herms tubers. When tuber calcium was increased, the decayed tubers due to Erwinia soft rot was significantly reduced and tuber starch content was enriched in the tissue.

The scanning electron microscopy (SEM) revealed compactness of cell structure and cell wall integrity due to calcium application. Calcium application preferentially enriched the outer cell layers of the treated tubers causing lessening in the internal diameters of parenchyma cells.

Keywords: *Potato- Calcium - Fertilization- Post harvest - Erwinia soft rot*

INTRODUCTION

Potato bacterial soft rot can cause heavy losses wherever potatoes (*Solanum tuberosum* L.) are grown. *Erwinia carotovora* pv. *atroseptica* is of particular importance on potato. *Erwinia carotovora* pv. *carotovora* exists in all climatic zones and attacks many other crops besides potatoes. It causes tuber loss during storage and transit in several regions of the world (Perombelon and Kelman, 1980).

Under post harvest conditions, a variety of factors influence the tubers resulting in a change that may affect the susceptibility to soft rot bacteria. Among such factors, the mineral nutrition of potato plant may influence soft rot development in tubers.

A large number of calcium-related disorders of plants have been reviewed (Bangerth, 1979, Millaway and Wiersholm, 1979 and Shear, 1975). Soils may contain adequate levels of calcium but most of what is taken up by the plants is distributed to the leaves and stems, frequently resulting in tubers that are low in calcium (Collier *et al.* 1980 and Dunn and Rost 1948). Recent findings indicated that increase in calcium deposition in cell wall of apple is correlated with the increased resistance to the infection by *Penicillum expansum* and *Botrytis cinerea*. Calcium was considered to induce the increased resistance of the tissue to maceration by pectic-enzymes of the fungi (Conway, *et al.*, 1994, McGuire and Kelman 1984, Biggs *et al.*,1997 and

El-Neshawy *et al.*, 2000). Since Erwinia soft rots of the Chinese cabbage and bean can be reduced by the calcium fertilisation (Park, 1969 and Platero and Jejernia, 1976). The possible role of calcium nutrition of potato tubers in the development of bacterial soft rot warranted investigation.

The objectives of this research were to increase the Ca^{2+} concentration of potato tubers by pre harvest calcium treatment and to determine whether this concentration of tuber calcium is related to the susceptibility of tubers to Erwinia soft rot and to elucidate the mode by which increased calcium may affect.

MATERIALS AND METHODS

Field Trials

Field experiments were designed to evaluate the uptake of calcium by tubers of the Herms, Lady Rossetta and Lady Joy potato cultivars cultivated in sandy loam soil. Calcium application in the form of $Ca\ (NO_3)_2$ and Ca chelate was made at northwest Egypt as a split- split plot design in randomised complete blocks with three replications.

At the first application, plants received $Ca\ (NO_3)_2$ at 50 Kg/fed as supplemental nutrients into sprinklers at tuberization one month after planting. A second application, fifteen days later, Ca^{2+} chelate at 1 kg/fed was applied as spray fertilizers onto plant foliar

The experiments were repeated twice at two summer seasons of the 2001 and 2002.

Soft Rot Evaluation

Cured tubers, 90 tubers total from three replications of each treatment, were evaluated for natural soft rot development. After 12 h in the mist chamber, potato tubers from each pre harvest treatment were transferred to storage room at 22 ± 2°C. The development of post harvest Erwinia soft rot after 5, 14 and 18 weeks of storage was determined.

Percentage weight loss (% wL)

Thirty potato tubers (10 per replicate) of each treatment were initially weighed at zero-time and were removed from storage room. Percentage of weight loss (%) was assessed by subtracting the net tuber weight at the end of each storage period out of the original weight at zero time and proportionately assessed in relation to the original weight.

Calcium Content Determination

Thirty tubers (10 from each replicate) were analysed 48 h after harvest for medullar calcium. Tubers were washed under a steady stream of water to remove any rotten tissue. Calcium content of the tuber tissue was determined by peeling a thin layer and cutting wedge of medullar tissue for a depth of 5 cm from two sides of the tubers. After removal from the tuber, the two segments from each of the ten tubers were mixed to make one sample and three samples from each treatment were analyzed. From each 0.250-0.001g was ashed at 500°C and then dissolved in 5ml of 6N HCL. The resultant was diluted and analyzed with Jarrell-Ash atomic absorption spectrophotometer and Ca^{2+} values were calculated on a dry matter basis (Conway *et al.*, 1987).

Scanning Electron Microscopy (SEM)

Herms and Lady Rossetta tubers treated with the two calcium forms, in addition to the untreated tubers, were were subjected to SEM observation (Harley and Fergusen, 1990). Tuber samples harvested at maturity stage were randomly chosen and immediately transported to the laboratory and stored at 22 ± 2°C.

Several scanning electron microscope observations were made to illustrate the possible accumulation of calcium on cell wall. Maximum diameters (length and width) of paranchyma cells were determined and compred.

Starch Development:

Four tubers of each treatment were taken 5 weeks after storage. Tubers were left at room temperature for 24 h, half cut and immediately inoculated with 100µL of freshly prepared iodine solution at either center zone (cz) or outer flesh zone(oz) of each half of treated or untreated tubers. After 12 hr of incubation at room temperature, the percentage of developed starch-stain area at two halves of each tuber was visually determined as a percentage of stained area compared with the control tubers according to a starch iodine index fo, determining the average starch staining in flesh tissue change.

RESULTS

Influence of pre harvest application of calcium nitrate $Ca(NO_3)_2$ and calcium chelate on soft rot development, and weight loss of potato tubers during storage:

Pre harvest application of calcium nitrate followed by calcium chelate reduced percentage of decayed tubers due to Erwinia soft rot in the treated Herms tubers by approximately 50%, 5 weeks after storage. This reduction was associated with prolonging storage period up to 18 week. Significant reduction was determined in treated tubers of cv. Lady Rossetta, while the reduction in treated tubers of cv. Lady Joy was non-significant (Table- Ia). In general, treatment with calcium compounds resulted in reduction of soft rot, the degree of response however varied with cultivar.

Accordingly, combined treatment of calcium salts was effective in reducing total weight loss (Table Ib). A significant reduction in percent of weight loss was recorded for Herms and Lady Rossetta - treated tubers with greater effect on Herms tubers.

The reduction in percentage of decayed tuber may be related to the decrease in tuber weight loss. As the percentage of decayed tubers reduced, the weight loss reduced.

Table 1a: Percent decayed potato tubers of tested potato cultivars treated with calcium content during the storage period at 22 ± 2°C.

Cvs	5 wk	14 wk	18wk
Herms (+)	3.5	8	10.5[b]
Herms (-)	9.5	11.8	26.6[a]
Lady Rossetta (+)	4.5	7.8	14.5[b]
Lady Rossetta (-)	10.5	12.0	23.5[a]
Lady Joy (+)	11.5	12.5	25.5[a]
Lady Joy (-)	11.8	13.5	28.0[a]
Significance	NS	NS	

(+) means treated tubers, (-) means untreated tubers, NS means not significant.
Within each calumn values followed by the same letters are not significantly different (P= 0.05 according to Duncan's multible range test.

Table Ib: Weight loss of potato tubers of tested potato cultivars treated with calcium during three storage periods at 22 ± 2°C.

	Weight loss (% wL)		
Cvs	5 wk	14 wk	18 wk
Herms (+)	6.0	18.8	18.8[c]
Herms (-)	14.0	21.5	33.5[a]
Lady Rossetta (+)	9.0	19.0	25.9[b]
Lady Rossetta (-)	15.0	21.5	35.0[a]
Lady Joy (+)	13.5	21.5	35.5[a]
Lady Joy (-)	15.0	23.5	35.5[a]
Significance	NS	NS	

(+) means treated tubers, (-) means untreated tubers Within each column values followed by the same letters are not significantly different (P= 0.05 according to Duncan's multiple range test.

Influence of pre harvest calcium application on starch change during storage:

Starch in tubers of the tested cultivars may be positively influenced by calcium fertilisation in the field. Visual rating of starch development in core zone (cz) of treated tubers (cvs. Herms or Lady Rossetta) was two fold compared to untreated ones. Visual rating of core zone (cz) starch of Herms and Lady Rossetta was full developed (100%). The respective rate of outer zone (oz) was 80%, 90% in treated tubers and 60%, 50 % of cvs. Herms and Lady Rossetta respectively.

Table 2: Effect of pre harvest Ca^{2+} application on tuber visual starch rate

Treated Tubers of Evaluated cvs.	Visual Rating of Tuber Starch VRTS (%)	
	CZ[X]	OZ[Y]
Herms (+)	100	80
Herms (-)	50	60
Lady Rossetta (+)	100	90
Lady Rossetta (-)	50	50
Lady Joy (+)	50	40
Lady Joy (-)	40	40

[X] CZ=central zone of tubers [Y] OZ = outer zone of tubers

The percent of starch in cz and oz of treated and untreated tubers of cv. Lady Joy were 50%, 40% and 40%, 40%, respectively.

Calcium content in the medulla of the tested cultivars:

The recorded increase in Ca content of the treated Herms tubers was approximately seven folds the calcium content of untreated ones. The content of calcium in treated tubers of Lady Rossetta or Lady Joy was one third more or less of that for Herms.

Table 3: Calcium content in tuber medulla after pre harvest application of Ca $(NO_3)_2$ and Calcium chelate in the field.

Treated Tubers of evaluated cvs.	Ca on tissue after fertilization (ppm) (% dry weight)
Herms	21.54[a]
Lady Rossetta	8.420[b]
Lady Joy	6.075[c]
Untreated (Herms)	3.840[d]

Samples of untreated tubers of lady Rossetta and Lady Joy are missed.
Within columns, numbers followed by the same letters are not significantly different (P = 0.05) according to Duncan's multiple range test.

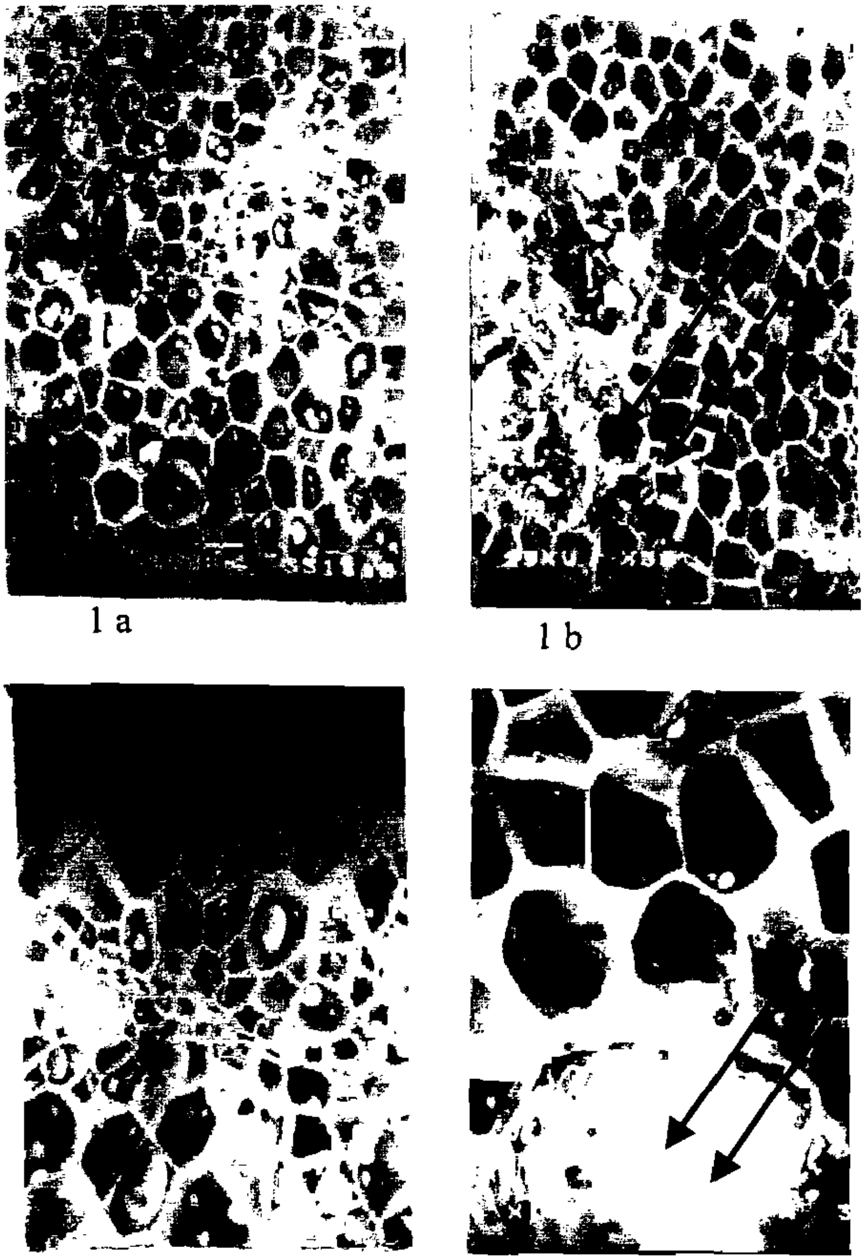
Electron - scanning observation:

Figures 1a, 1b, 1c and 1d illustrate the effect of pre harvest spray application of $Ca(NO_3)_2$ and Ca chelate on cell structure after harvest and storage for 14 weeks in cold storage room. It is obvious that cell structures of treated Herms and Lady Rossetta tubers are integrated and compacted. However, cell structure of untreated tubers of both cultivars are disintegrated (arrows refer to affected area). The accumulation of calcium on cell wall lessen the internal maximum diameter of parenchyma cells (length x width). Less internal diameters of Herms cells than which of Lady Rossetta were determined. The more calcium accumulation on cell wall refers to the less internal diameter of the cells.

Table 4: Maximum diameters (µm) of parenchyma cells of potato tubers upon calcium application in the field:

Treated Tubers of evaluated cvs.	Maximum diameters of parenchyma cells	
	Length (µm)	Width (µm)
Herms (+)	102.7	78.7
Herms (-)	166.3	132.6
Lady Rossetta (+)	132.8	106.9
Lady Rossetta (-)	148.8	132

(+) means treated tubers, (-) means untreated tubers



1 a

1 b

1 c

DISCUSSION

1 d

Figure 1a, 1b, 1c, and 1d illustrate typical photographs of cell wall structure of treated and untreated tubers of cvs. Herms and Lady Rossetta upon the pre harvest application, 1a (treated Herms), 1b (untreated Herms), 1c (treated Lady Rossetta) and

A field trial was designed to evaluate the role of calcium nitrate and calcium chelate in reducing Erwinia soft rot of potato after harvest. Tuber weight loss, tuber - starch and calcium content as well as cell wall integration were investigated.

Increasing Ca fertilisation of the growing potato plants of cv. Herms and Lady Rossetta resulted in a significant decrease in Erwinia soft rot. Potato tubers (cvs. Herms or Lady Rossetta) upon Ca treatment showed limited decay for up to 5 wk. followed by increased decay being more or less fifty percent after 18 wk under the same conditions. Tubers that received no calcium treatment appeared to be highly decayed. The obtained results conform to those of McGuire and Kelman 1984 who found that the severity of bacterial soft rot caused by *Erwinia cartovora* pv. *atroseptica* was linearly and inversely related to the concentration of tuber calcium in laboratory and field experiments. It is worth to note that Lady Joy did not show such relation indicating that the variety may be passive in this regard.

According to the noticeable positive effect of calcium on the Erwinia soft rot incidence, a considerable decrease in tubers (cvs. Herms and Lady Rossetta) weight loss occurred for up to 18 wks. The value of calcium content of treated potato tuber medulla (cv. Herms) was approximately seven folds of that the untreated ones. These findings are in accordance with those of McGuire and Kelman, 1984; and Arteca, 1982 who had proven that an inverse relation between bacterial soft rot susceptibility and calcium content of medulla tissue. It was recognized, however that other physical and physiological relationships exist that may be as important as the effect of calcium on pectolytic enzyme activity. Reduced respiration of tubers infiltrated with calcium has also been recorded. The obtained results were also confirmed by McGuire and Kelman, 1984 who proved that the increase in soil calcium resulted in potato tubers that had higher calcium content, and were more resistant to bacterial soft rot. They also explained that during the filling stage, tubers receive the greatest amount of their nutrients through the phloem. Because calcium moves throughout the plant almost exclusively in the xylem and preferentially to transpiring tissues, environmental factors such as high air temperatures and low humidity, which increase transpiration, will result in movement of greater amounts of calcium into foliage than with less extreme conditions. Such factors can also result in movement of water out of the tuber. With a return to cool night temperature, high humidity and ample soil moisture, the water deficit in the tuber can be restored with the solution comparatively high in calcium being carried directly from the roots through the xylem (Gandar and Tanner, 1976).

McGuire and Kelman, 1984 reported also that calcium nitrate was more effective than calcium chloride, calcium sulphate or calcium gluconate in increasing the calcium content of peel tissue up to five times and medullar tissue three times that of untreated tubers.

Concerning the development of tuber starch, it was noticed that increasing tuber calcium has resulted in increasing the development rate of tuber starch. A fifty percent increase of developed central zone-starch of treated tubers (cv. Herms) than of untreated tubers in addition to one and fourth increase of developed outer zone-starch. These findings can be explained by what was found by Christiansen and Foy, (1979) and Painter and Neukom (1968), who indicated that being calcium a divalent cation, it has the ability to bridge two glacturonases via their carboxylate groups and calcium pectate is a principle component of the middle lamella. In addition, binding of proteins to polysaccharides through phenolic acid and calcium bridges has been shown to have a strengthening influence on cell walls.

Scanning electron microscopic observations on potato tissue sections of tubers (cvs. Herms and Lady Rossetta) revealed that calcium improved the structural integrity of cell wall components and that the reduction in the severity of Erwinia soft rot in high-calcium potato tubers may be explained on the basis of the delay in the rate of maceration resulting from this structural enhancement. These findings are previously confirmed by Starr and Moran, 1962 who found same results in high-calcium potato tubers.

This study has concluded that calcium fertilisation has contributed to a minimal Erwinia soft rot incidence by lowering the severity of the disease caused by pathogen that is being macerated primarily with the pectolytic enzymes.

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التحكم فى الإصابة بالعفن الطرى فى البطاطس بزيادة محتوى الكالسيوم

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درس فى هذا البحث تأثير التسميد بنترات الكالسيوم $Ca(NO_3)_2$ مع كالسيوم مخلبى على نباتات البطاطس أصناف (هيرمز – ليدى روزيتا و ليدى جوى) من حيث اختزال مرض العفن الطرى المتسبب عن البكتيريا *Erwinia carotovora* spp. حيث وجد أن اختزال العفن الطرى يرتبط عكسيا مع تركيز الكالسيوم بدرنات البطاطس بعد الحصاد حيث يؤدى إلى اختزال معنوى لنسبة الإصابة بمرض العفن الطرى و كذلك لنسبة الفقد فى وزن الدرنات الذى يعزى إلى العفن الطرى بعد ١٨ أسبوع من التخزين للإصناف هيرمز و ليدى روزيتا .

كما وجد أيضا أن محتوى الكالسيوم بالدرنات المأخوذة من القطع المعاملة بأملاح الكالسيوم (على أساس الوزن الجاف) قد زاد ليصل ٧ أضعاف المحتوى بالدرنات الغير معاملة للصنف هيرمز – كما أدت معاملة الكالسيوم إلى زيادة مرئية فى معدل تكون النشا بالدرنات المعاملة و بنسبة أعلى فى الصنفين هيرمز و ليدى روزيتا مقارنة بالصنف ليدى جوى.

أوضحت صور الميكروسكوب الألكترونى اندماج جدر خلايا الدرنات كنتيجة للمعاملات بالكالسيوم قبل الحصاد على العكس من التحلل الذى يحدث لجدر الخلايا الغير معاملة ، و قد لوحظ أيضا أن هذه المعاملة تسبب زيادة فى سمك طبقات الخلايا الخارجية لدرنات البطاطس المعاملة مما ينتج عنه ضيق فى أقطار الخلايا البرانشيمية لجدر الخلايا المعاملة عنه فى الخلايا الغير معاملة لكل من الصنفين هيرمز و ليدى روزيتا.