J. Agric. Sci. Technol. (2004) Vol. 6: 139-145

X-Ray Microanalysis of Calcium Signal Transduction as a Defense Response in Spring Wheat to the Stripe Rust Disease Caused by *Puccinia striiformis* f. sp. *tritici*

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ABSTRACT

In this investigation levels of calcium were measured on the flag leaves of the susceptible spring wheat cultivar Thatcher and its near-isogenic line that has Yr-18 gene-mediated durable adult-plant resistance (APR). The results showed that crystals of various shapes were frequently present on the infected leaf areas in the spring wheat that has the APR to stripe rust. Crystals were characterized with an energy-dispersive X-ray microanalayser in conjunction with scanning electron microscopy (SEM). More calcium was mobilized into infected areas of the resistance line than into the susceptible genotype. The level of calcium in the non-infected areas of both resistant and susceptible hosts and on the infected areas of the susceptible genotype were nearly the same. The results indicated that calcium mobilization in the resistant near isogenic line upon infection with *P. striiformis* correlated with the elicitation of a defense response conditioned by the Yr-18 gene. This is the first report on the mobilization of calcium in a cereal-rust pathosystem as part of a resistance response.

Keywords: Calcium, Mobilization, Resistance, Rust stripe, Wheat.

INTRODUCTION

One category of resistance mechanisms involves the deposition of structural materials on and within the host cells as discussed by Ride (1986). Heath (1998) and Heath *et al*. (1997) examined cytocolic calcium levels during hypersensitive reaction (HR) in the epidermal cells of cowpea invaded by race 1 of the cowpea rust fungus (*Uromyces vignae*). The HR was observed in the resistant host but not in the susceptible cultivars complete fungal penetration and before other detectable cytoplasmic manifestations of the HR. Such a reaction is also reported to be involved in the deposition of lignin and other phenolic substances (Vance *et al*., 1980; Ride, 1983), suberin (Kolattukudy,1984), glycoprotein rich in hydroxyproline (Esqurre-Tugaye *et al*., 1979), silicon (Heath, 1979), and elements such as calcium (Bateman, 1964).

Calcium is an essential plant nutrient and a structural component of plant cell walls accumulated in appreciable amounts in higher plants (Demarty *et al*. 1984; Kirby and Pilbean, 1984). Calcium functions as a secondary messenger in cells that control many processes such as stomatal closure (Gilroy *et al*., 1991; Ward *et al*., 1995; Webb *et al*., 1996), tropism (Gehring *et al*., 1990; Williams *et al*., 1990; Sinclair *et al*., 1996) orientation of pollen tube growth (Malho and Trewaves, 1996; Hepler, 1997), acclimation (Knight *et al*.,1996), gene expression, and many calcium-dependent enzyme activities (Roberts and Harmon, 1992). The aim of this investigation is to study on the role of

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cytosolic free calcium during the hypersensitive response (HR) of resistant genotype of wheat to the stripe rust fungus.

MATERIALS AND METHODS

The distribution of calcium was studied in flag leaves of the spring wheat cultivar Thatcher upon infection with the stripe rust (*Puccinia striiformis*) and compared with its Yr-18 gene-containing near isogenic line having adult-plant resistance(APR) to this pathogen . These lines were originally developed by Dyck and co-workers and kindly provided to us by A. Ketuz, of Agriculture and Agri-Food Canada, Winnipeg, Manitoba, Canada. Thatcher carries the Yr-7 seedling resistance gene in addition but this did not interfere with the results since the pathotype used in this study was virulent to Yr-7.

A Canadian stripe rust pathotype namely the isolate SR99-UA, designated 70E128 virulent onYr2, Ba, 3c, 4a, 6, 7, 21, 22, and 23, was used in this study. The isolate was multiplied on seedlings of the susceptible spring wheat cv. Avocet. The urediniospores were collected, and kept in a desiccator until used.

For carrying out the experiment at the adult plant stage, 18-20 seeds of each genotype were sown in 12.5 diameter pots containing compost (Metro-Mix 292, Ltd, Terra). Inoculation was carried out when the flag leaf was fully expanded (8-9 weeks after sowing) using a mineral oil such as Soltrol 170. Sufficient spores (5 mg/5ml) were added to the oil to give it a bright yellow appearance. An atomizer was used to apply the spore suspension to the leaves of the hosts. A light coating of oil was enough to ensure good infection. The inoculated plants were left for at least one hour in order for the Soltrol to evaporate off the leaves. The plants were then lightly sprayed with water, covered with plastic bags in order to maintain high humidity, and placed in the dark at 10 C for one day. One day after inoculation, the plants were moved to a growth chamber set at 15 C with a 14h photoperiod and with a relative humidity within the range of 60-70%. The light intensity was approximately 8000 lux at seedling height.

For studies with the scanning electron microscope (SEM), segments of leaves from susceptible and resistant hosts were both sampled 18 days after inoculation. They were then mounted onto coverslips with a double-sided sticky tape. The samples were then vapor- fixed with 1% osmium tetroxide in water and air-dried in a fume hood at room temperature for about two days. Samples were then mounted onto SEM metal stubs using double-sided sticky tape and secured with Marivac collodial carbon paint. The specimens were then coated in a Nanotek, SEM Prep 22 with a gold layer of approx 15nm and observed in a Jeol JSM 6301 XV SEM operated at 5 to 20 kV. Energy- dispersive X- ray microanalysis was conducted using an XL energy dispersive X-ray system with a light element detector. Crystals on the surfaces of infected leaves were characterized based on their unique X-ray spectra. Spectra were obtained using a Bruker AM-300 NMR Spectrometer (300 Mhz).

RESULTS AND DISCUSSION

As an aim of this investigation we examined the role of free calcium levels during the HR of wheat to the stripe rust fungus. The HR of disease resistant plant cells to fungal invasion is a rapid cell death that has some features in common with programmed cell death.

We found crystals in various shapes frequently present on pustules and on the infected leaf areas in genotypes with Yr-18 gene-mediated APR to stripe rust but not on those of the susceptible genotype. Characterization of the crystals with an energy dispersive X- ray microanalyzer in conjunction with SEM showed that the resistant genotype contained calcium- rich crystals based on their unique X- ray spectra.

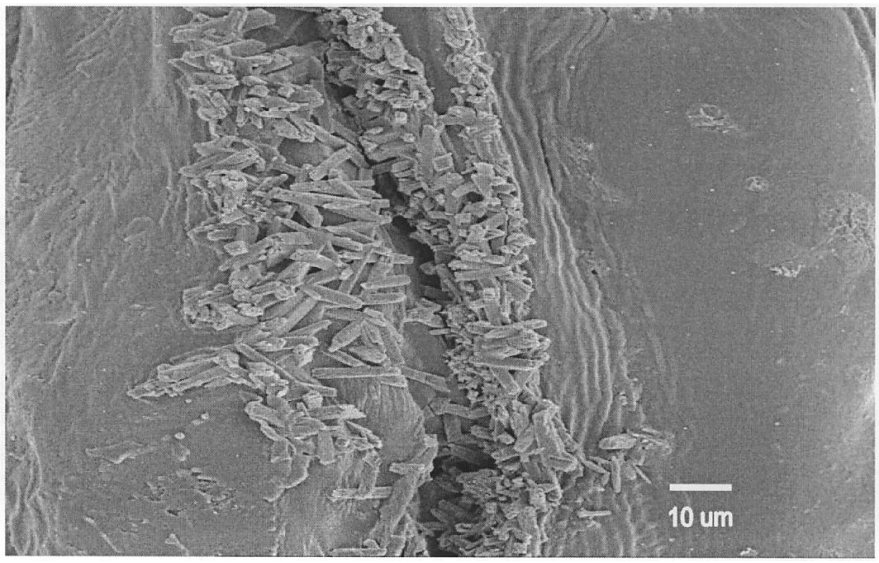


Figure 1. Electron micrograph of calcium-containing crystals blocking the pustule on the resistant near- isogenic line infected with *P. striiformis* 18 days after inoculation.

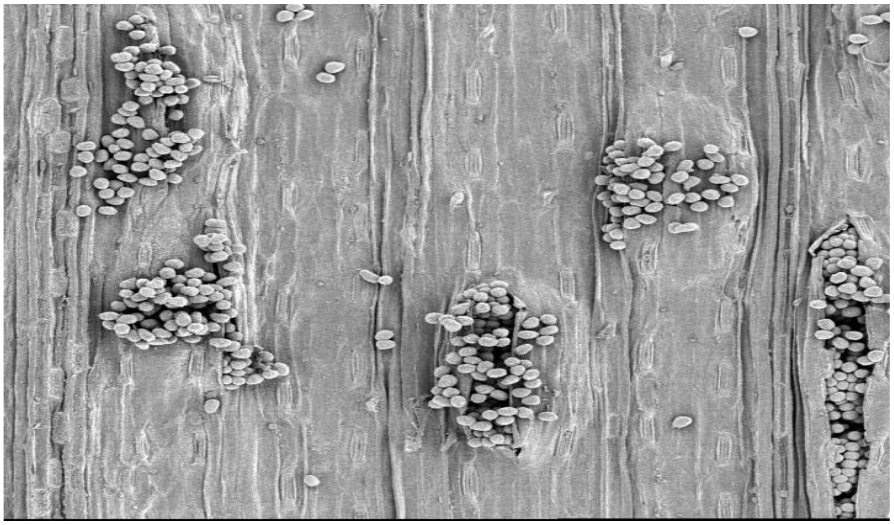
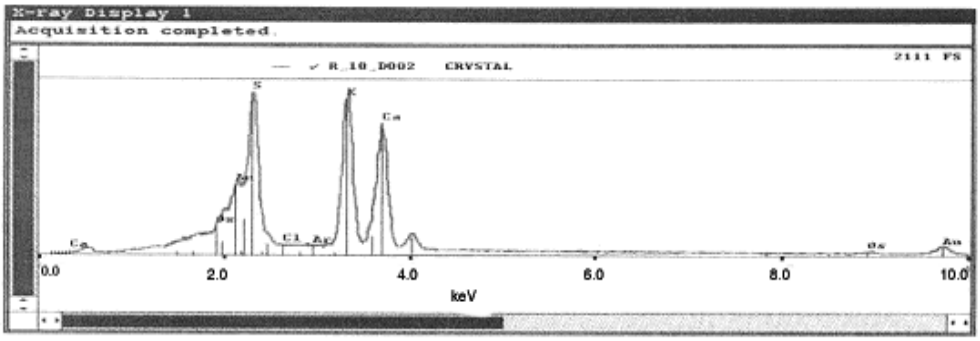


Figure 2. Pustules on the rust susceptible host showing spore production and lack of crystal formation 18 days after inoculation.

Calcium in used by plant cells as a secondary messenger to control many cell processes. In plants two kinds of calcium stores are believed to contribute to [ca2+] extracellular (apoplastic) stores in the sell wall and intracellular stores in the vacuole (cytocolic) or endoplasmic reticulum (Bush, 1995).

In this investigation we found that HR induced by fungal infection in intact plants was delayed by calcium in signal transduction similar to those in other studies involving plant materials (Ishihara *et al*,. 1996; Xu and Heath, 1998).

In our assessments, the level of calcium in and around the necrotic areas and pustules of *P. striiformis* was markedly higher in the resistant Yr-18 containing the isogenic line (Figure 1 and Figure 3) than in the suscepti-

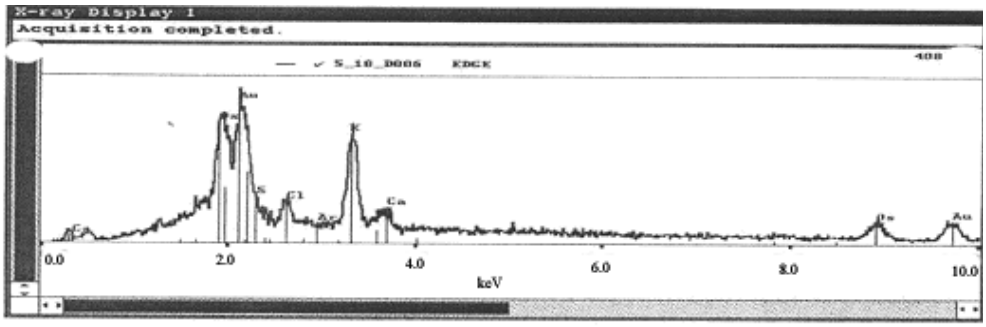


ELM	Rel.K	Norm wt%	Atomic%
Ca	0.4290	48.51	45.03
K	0.2192	22.76	21.66
Cl	0.0010	0.12	0.11
Ar	0.0000	0.00	0.00
S	0.0000	0.00	0.00
Au	0.28.44	28.61	33.19
Total		100.00	99.99
Goodness of fit		4.24	

Figure 3. Energy-dispersive X-ray microanalysis spectrum showing high calcium peak from crystals formed on pustules on the resistant genotype. Gold (Au) is present due to the specimen coating. Potassium (K), Sulfur (S) and chlorine (Cl) are detected from the background.

ble genotype (Figure 2 and Figure 4). Non-inoculated areas on the flag leaves of the control plants in both genotypes studied had lower levels of calcium that were similar to the levels found in the infected areas of the susceptible genotype. The reason for using adult (mature) plants for experiments rather than seedlings was that, in our preliminary seedling and adult plant stripe rust reaction evaluation experiments (unpublished data), both the isogenic and near-isogenic lines showed a compatible interaction with SR99-UAR at the seedling stage. However, at the adult plant stage the cv. Thatcher showed high disease severity while Thatcher- Yr 18 had a very low level of stripe rust severity.

These results are similar to those observed by Xu, and Heath, (1998) in the role of calcium in signal transduction during the hypersensitive response caused by basidiospore-drived infection of the cowpea rust fungus. In their calcium levels ratio analysis and imaging studies, they demonstrated a consistent increase in [ca2+] in the resistance near isogenic line but not in the susceptible spring wheat cells at the time the pathogen infected the plant cells. There are several reports on the role of calcium in the signal transduction leading to plant defense response such as those from cell cultures (cytocolic) treated with elicitor (Mahady and Beecher,1994; Messiaen and Van Custen, 1994; Suzuki, *et al*. 1995; Tavemier, *et al*. 1995; Ishihara, *et al*. 1996; and Levien *et al*. 1996). These results suggested that the increase in [ca2+] is related to the timing of the pathogen invasion and not to the timing of the HR. In addition, the results indicated that calcium mobilization exerts a major role in the mode of action of the Yr-18 gene in mediating stripe rust resistance in spring wheat. This is the first report on mobilization of calcium in a cereal-rust pathosystem as a part of a resistance response.



ELM	Rel.K	Norm wt%	Atomic%
Ca	0.0628	7.64	16.14
K	0.1236	15.45	33.38
Cl	0.0651	8.95	21.29
Ar	0.0000	0.00	0.00
S	0.0000	0.00	0.00
Au	0.5917	67.96	29.15
Total		100.00	99.96
Goodness of fit		1.67	

Figure 4. Energy-dispersive X-ray microanalysis spectrum showing low calcium peak from the surface of a pustule on the susceptible genotype. Gold (Au) is present due to specimen coating. Potassium (K), Sulfur (S) and chlorine (Cl) are detected from the background.

ACKNOWLEDGEMENTS

We would like to thank Shirley Brezden for her technical assistance and George Braybrook for his help in observations using scanning electron microscopy (SEM).

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میکروآنالیز کلسیم انتقال یافته ناشی از واکنش دفایی در گندم بهاره مبتلا به زنگ نواری (*Puccinia striiformis*) با استفاده از تابش اشعه ایکس

س. ع. الهی‌نیا و ج. پ. تواری

چکیده

در این بررسی مقدار کلسیم ناشی از واکنش دفاعی فوق حساسیت در سطح برگهای پرچم (flag leaves) و اطراف تاول‌های زنگ (Pustules) نواری (*Puccinia striiformis* f. sp. *tritici*) در گندم بهاره حساس به نام تاچر (Thatcher) و ایزوژنتیک لاین دارنده ژن عامل مقاومت Yr-18 در زمان بلوغ (APS) اندازه‌گیری شد. نتایج حاصله نشان داد که، کریستال‌هایی به اشکال مختلف و به فراوانی روی نواحی آلوده در گندم بهاره دارای مقاومت در زمان بلوغ (APS) نسبت به زنگ نواری تشکیل شدند. مقدار کلسیم موجود در کریستال‌ها با دستگاه Energy-ispersive X-ray microanalyser متصل به میکرسکوپ الکترونی اسکن (SEM) اندازه‌گیری گردید. نتیجه انتقال کلسیم از سلول‌های نواحی سالم به سلول‌های مورد حمله در لاین مقاوم بیشتر از ژنوتیپ حساس بود. مقدار کلسیم در نواحی سالم برگ میزبانهای حساس و مقاوم و همچنین در نواحی آلوده گندم حساس تقریباً مشابه بودند. این نتیجه مشخص نمود که انتقال کلسیم در میزبان مقاوم (near isogenic line) آلوده به زنگ نواری (*P. striiformis*) به افزایش عکس‌العمل دفایی ناشی از وساطت ژن Yr-18 بوده است.