Determining drought stress resistance mechanisms in potato plants using GIS

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Abstract

Potato plants are very sensitive to drought stress, but field potato crops undergoing mild water deficits may acclimate to subsequent severe water deficits through physical and physiological mechanisms. To examine resistance and sensitivity mechanisms adopted by potato plants through leaves and stem, drought stress experiments were conducted with three potato genotypes (Fv12246-6, 'Vigor' and 'Russet Burbank') in a greenhouse. The measurement of key drought stress responses such as changes in cuticular thickness, platelet size, stomatal size, stomatal pore aperture, and xylem diameter is time consuming and challenging. Out of four techniques, a geographical information system (GIS) most easily and reproducibly measured cuticular layer relevant responses and facilitated data acquisition for the above factors. Under intense drought conditions, the more adapted potato plants reduced the leaf cuticle platelet area and increased the thickness of the cuticle layer which likely resulted in the observed reduced water loss.

Background and Relevance

Potato is the third most important food crop after wheat and rice with a worldwide production of 360 million tonnes in 2012 (http://faostat.fao.org/). Potato plants are sensitive to drought (van Loon, 1981) and have evolved a number of adaptive responses to overcome drought stress. Stomatal closure (Yordanov et al., 2000) and changes in leaf cuticular layer (Boyer, Wong & Farquhar, 1997) are key adaptive drought stress resistance factors. Typical examination of ultrastructural changes in the cuticle layer (Bargel et al., 2006) by atomic force microscopy and, transmission and scanning electron microscopy is tedious and expensive. are typical methods to examine ultrastructural changes in the cuticular layer. An initial quick and inexpensive evaluation of cuticular layer responses would enable researchers to assess whether further in-depth microscopy techniques would be valuable and would facilitate screening of more resistant genotypes in plant breeding programs. The use of the Suzuki SUMP disc system to acquire leaf imprints (Tanaka et al., 1985, Arve et al., 2011) combined with geographical information system (GIS)-based approach, described for the first time here, effectively distinguished drought adapted and stressed (DAS) from non-adapted and stressed (NAS) potato genotypes. The specific objective of this study was to examine the changes in the leaf cuticle layer platelet area and thickness during drought acclimation and stress. Attempts were taken to measure these parameters by: (a) hand sections, (b) embedding in LR white medium and sectioning to 10 micron thickness, (c) 3D confocal microscopy, and (d) GIS software (Table 1). Of all the approaches, evaluating cuticular thickness and platelet size using GIS software was the easiest, least expensive and a reliable technique.

Table 1. Advantages and disadvantages of using different techniques to determine potato leaf characteristics work.

Technique	Advantage	Disadvantage
Hand sectioning of potato leaf.	Simple, inexpensive technique.	Time consuming. Getting a thin leaf section is difficult. Difficult to identify cuticular layer by fluorescence microscopy due to other interfering fluorescence.
Embedding potato leaf sections into LR white medium and sectioning by rotary microtome.	Systematic laboratory technique.	Time consuming and laborious. Potato leaf cells were damaged through the embedding process. Requires expensive chemicals.
3D confocal microscopy.	Three dimensional view of leaf imprint or fresh leaf sample is clearly visible.	The bottoms of all cuticle platelets are not on the same base platform which then underestimates the relative cuticular thickness. Many platelets are found broken and cannot be considered.
GIS.	Easy to implement, inexpensive, reliable technique.	Original images were two dimensional and did not have thickness or elevation information. To calculate the thickness of the leaf cuticle platelet, we assumed that the brightness of the images was not affected; thus the calculated thickness is the relative thickness between treatments and not the absolute thickness.

Methods and Data

Establishment of plants

In a previous study, three potato genotypes 'Vigor' (V), 'Russet Burbank' (RB) and Fv12246-6 (Fv) were examined to determine their resistance to drought stress. Harvested tubers were used to grow plants in a greenhouse. Cuttings were taken from 6 week old plants and were dipped in rooting hormone. Approximately 50 cuttings were potted into a soilless mix (SM#4, Sunshine Mix No. 4) on a polystyrene tray. The trays were then placed on a heated mist bed. Three week old cuttings were transplanted into SM#4 in 11 L pots. Plants were watered as needed and fertilized twice a week. When plants reached 5cm above the pot rim, additional SM#4 Mix was added up to 2.5 cm below the rim. Pots were placed randomly into 60 cm × 40 cm × 21.5 cm white plastic trays. Four TDT sensors (Sun and Young, 2001) were placed vertically into Non-Acclimated and Non-Stressed (NA), Drought Acclimated and Stressed (DAS) and Non-Acclimated and Stressed (NAS) to read soil moisture level. Twenty seven plants (9 plants from each of the three genotypes Fv, V and RB) were used.

Drought acclimation, drought stress and recovery cycles

When plants were about six weeks old, the first Drought Acclimation cycle (1st DA) was imposed in Fv-DAS, V-DAS and RB-DAS by withholding water until 10% soil moisture content was achieved, then re-water to 35-40% soil water content. In NA and NAS treatments, plants were watered to maintain 35-40% soil water content and fertilized as usual. A second drought acclimation cycle (2nd DA) was applied in the same way. At this stage, all DAS and NAS treatments were put into the first drought stress cycle (1st DS). NA treated plants were watered as usual. When the soil water content in the DAS and NAS pots dropped to 0%, plants were not watered until they showed visible wilting (after 5-7 days with 75% leaves wilted). All plants were then re-watered to raise the soil water content to 35-40% and were allowed to recover (1st R) for 5-7 days in the same greenhouse conditions until there were no wilted leaves. After recovery, a second drought stress cycle (2nd DS) was applied in the same way as the first and a second recovery cycle (2nd R) followed.

Leaf cuticle platelets – area

At the end of the 1st DS, leaf imprints of one lateral leaflet of the youngest fully expanded leaf of all three plants of each treatment (NA, DAS and NAS) and two genotypes Fv and RB (total 18 plants) were taken using Suzuki's Universal Micro-Printing (SUMP) method. SUMP solution was painted on the SUMP disc and the disc was pressed against the adaxial surface of the leaf for one minute to get the leaf imprint. Imprints were observed at 400 magnification with a Leica light microscope. The light level of the microscope and the distance between the imprint and the microscope lens were always the same. All images had the same scale and size. The original microscope image can have a scar bar (Figure 1). Five images from each imprint were selected; four large leaf cuticle platelets were randomly selected from one image and manually digitized using ESRI ArcGIS (Figure 2). Polygon areas were calculated in square micrometers (μm^2). In genotype RB, both DAS and NAS treatments resulted in smaller ($p < 0.05$) areas of leaf cuticle platelets compared to NA. We digitized 208 polygons in 52 images. The statistical analysis of these polygons is discussed in the next section.

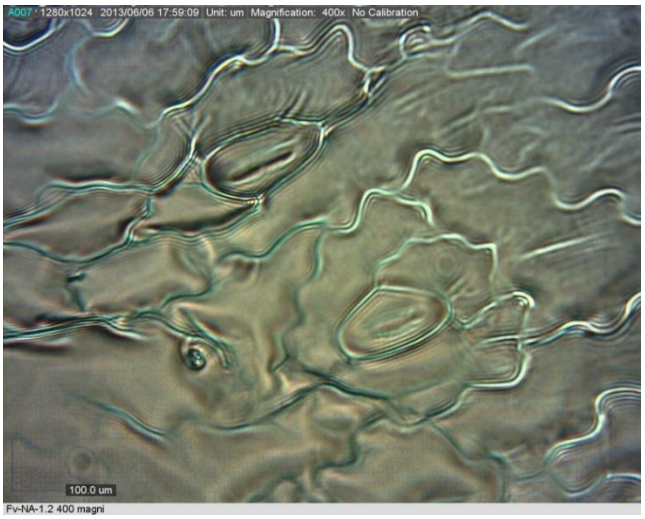

Figure 1. Sample image with scale bar

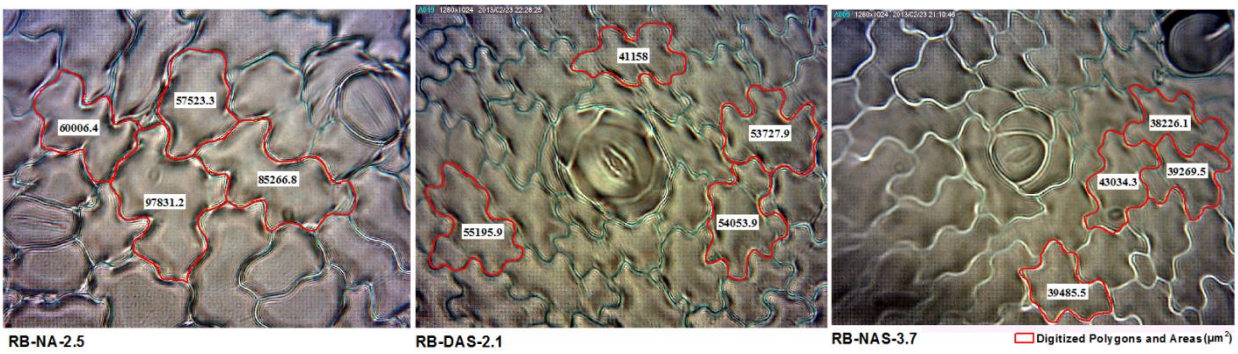

Figure 2. Digitized polygons with areas from three treatments (NA, DAS and NAS) of the potato genotype Russet Burbank

Leaf cuticle platelets –thickness

The original images were two dimensional and did not have thickness or elevation information. In the Microsoft Paint environment, all images had similar brightness and the top part was brighter than the bottom part of the leaf cuticle platelet (Figure 3). As it took only a few seconds to take the image, the brightness of the images was assumed to be unaffected. Thus the calculated thickness is the relative thickness between treatments and not the absolute thickness. The relative cuticle layer thickness was calculated with the procedure outlined below.

1. Images were added into ESRI ArcMap. Because the colour palette was stretched over a greater range of values in ArcMap than Windows Paint, the color of images displayed in ArcMap was slightly different from the colour in Windows Paint; should not affect the values of the raster for analysis purposes.
2. Fishnets of the images were created and georeferenced.
3. The same boundary was created for all the images; top and bottom parts of the image that might have scale and text information were excluded.
4. The Reclassify (Spatial Analyst) tool was used to reclassify 256 values of an image into 9 classes with the Natural Breaks classification method, and the processing extent was set the same as the boundary of the mask boundary. Natural Breaks classes were selected instead of other classification methods (e.g., Equal Interval, Quantile, etc.) because Natural Breaks identifies class breaks of similar values to maximize the differences between classes.
5. An area for each of the 9 classes (cell size × cell size × count of cells) was calculated.

Figure 3, shows that classes 1 to 4 in the legend were at the bottom part of the platelet and classes 5 to 9 were at the top part of leaf cuticle platelets. We calculated the

thickness for 183 images. The statistical analysis of these 183 images is discussed in the next section. Figure 4 shows the problems encountered during 3D confocal microscopy and hand sectioning as stated in Table 1 before.

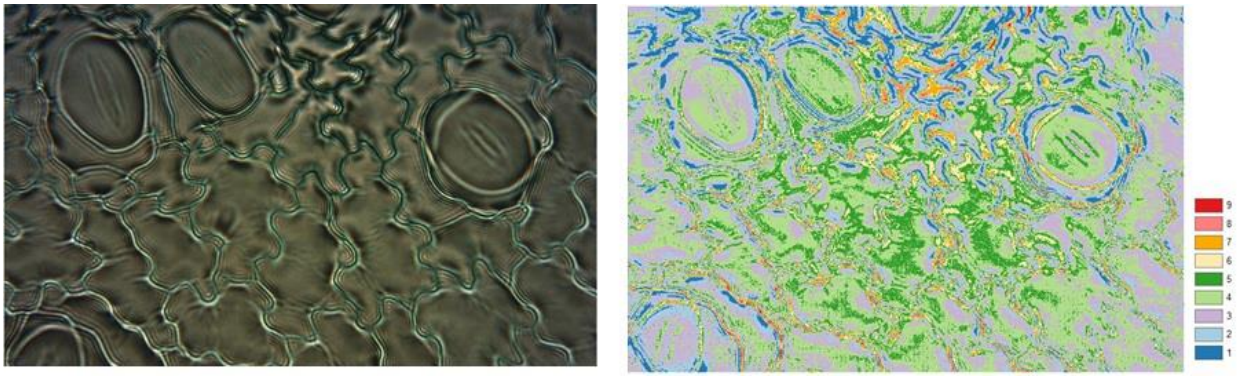

Figure 3. Original image of the leaf imprint (left) and thickness (right) of the leaf cuticle layer for 9 classes of the potato genotype Vigor.

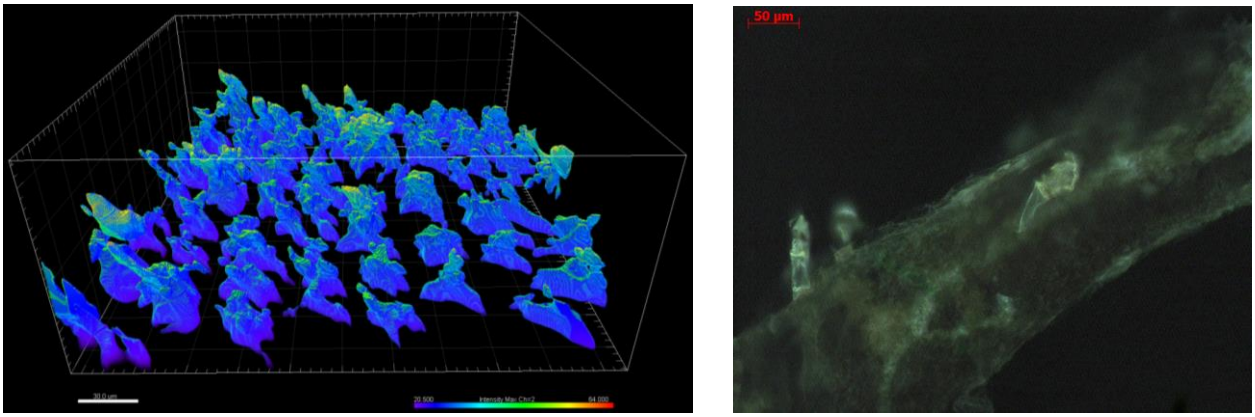

Figure 4. Three dimensional view of leaf cuticle platelets from the same leaf imprint used in Figure 3 using 3D Confocal Microscopy (left). Image was taken at a different position of the leaf imprint. Hand section from fresh leaf sample of 'Vigor' (right).

Statistical analysis

Data were analyzed with statistical software SPSS version 20.0. The overall difference among groups was determined using general linear model at a 95% confidence interval. Pairwise comparison was determined with the Tukey test. A p value < 0.05 suggest the results are significant.

Results

Leaf cuticle platelets – area

Areas of the 208 polygons in 52 images were compared with SPSS. In Fv and RB, both DAS and NAS treatments resulted in smaller (p<0.05) leaf cuticle platelets compared to

NA (data not shown). Therefore, drought stress induced smaller cuticular platelets in both Fv and RB genotypes.

Leaf cuticle platelets – thickness

We used the SPSS to analyze the thickness of the leaf cuticle platelets in 183 images. There was no genotype-dependant effect in leaf cuticle layer thickness as Fv and RB had equivalent thickness. DAS treatment resulted in the highest thickness. Therefore, drought acclimation induced the thickest cuticular layer (Table 2).

Table 2. Thickness of the leaf cuticle layer in three different treatments (NA, DAS, and NAS) in two potato genotypes (Fv and RB) at the end of the first drought stress (1st DS) cycle. Group not having same letter is significantly different ($p<0.05$) than the others.

		Mean thickness (μm) ± Standard Error
Genotype	Fv	233.94 ± 60.49 a
	RB	157.65 ± 59.81 a
Treatment	NA	120.96 ± 78.58 a
	DAS	298.06 ± 61.93 b
	NAS	168.36 ± 61.63 a

Conclusions

This study examined the effects of drought acclimation on drought stress resistance in three potato genotypes [Fv12246-6 (Fv), 'Vigor' (V) and 'Russet Burbank' (RB)] in a greenhouse environment. Two important aspects (area of leaf cuticle layer platelets and thickness of cuticle layer) of the drought stress resistance mechanism in potato leaves were examined using ESRI ArcGIS. The characteristics measured here were both found to be genotype and treatment dependent. The use of ESRI ArcGIS to measure leaf cuticle layer area and thickness was an effective way to determine resistance mechanism adopted by potato plants under intense drought stress conditions.

Acknowledgements

The authors are thankful to SAGES for funding this project, Dr. Benoit Bizimungu for supplying potato tubers and Shanna Bennman for the confocal microscopy work.

References

Arve, L.E., Torre, S., Olsen, J.E. & Tanino, K.K. (2011). Stomatal responses to drought stress and air humidity. In Arun Shankar (Editor), *Abiotic Stress/ Book 1*. INTECH Open Access Publisher. ISBN 978-953-307-394-1.

Bargel, H., Koch, K., Cerman, Z. & Neinhuis, C. (2006). Structure-functions relationships of the plant cuticle and cuticular waxes – a smart material? *Functional Plant Biology*, *33*, 893-

910.
Boyer, J.S., Wong, S.C. & Farquhar, G.D. (1997). CO_2 and water vapor exchange across leaf cuticle (epidermis) at various water potentials. *Plant Physiology, 114,* 185-191.
Sun, Z. J., and G. D. Young. 2001. A cost effective soil moisture instrument based on time-domain transmission measurement. *Int. Symp. and Worksh. on Time Domain Reflectometry for Innovative Geotechnical Applications, 2nd, Evanston, IL. 2001.*
Tanaka, Y., Sano, T., Tamaoki, M., Nakajima, N., Kondo, N. & Hasejawa, S. (2005). Ethylene inhibits Abscisic Acid-Induced Stomatal Closure in Arabidopsis. *Plant Physiology, 138,* 2337-2343.
van Loon, C.D. (1981). The effect of water stress on potato growth, development, and yield. *American Potato Journal, 58*, 51-69.
Yordanov, I., Velikova, V. and Tsonev, T. 2000. Plant responses to drought, acclimation and stress tolerance. Photosynthetica, 38(1): 171-186.