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Physiological and growth responses of potato cultivars to heat stress

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Abstract

Climate warming is subjecting plants to heat stress, which can affect their physiological processes impacting their growth, development and productivity. Potato (*Solanum tuberosum*) is a staple food worldwide but potato crops are very sensitive to heat stress. We have studied the effects of heat stress on SPAD-measured leaf chlorophyll content, plant growth and tuber yield of 55 commercial potato cultivars in clonal tests under heat-stress (HS; 35 °C-day, 28 °C-night) and control non-stress (CK; 22 °C-day, 18 °C-night) conditions. The potato cultivars varied in their response to heat stress. Overall, heat stress reduced leaf size, increased the leaf chlorophyll SPAD values by up to 65% and plant height by 64%, but severely reduced the largest tuber weight by 93%. The HS-to-CK SPAD ratios were positively correlated with HS-to-CK plant height ratio, largest tuber weight under heat-stress, and the HS-to-CK largest tuber weight ratio. Potato cultivars displayed a correlated response to heat stress for their leaf chlorophyll content, plant height and tuber weight. We have identified the most heat-tolerant and heat-susceptible cultivars for these traits. Under heat-stress conditions, potato cultivars tend to have less reduction in tuber weight if their plants have higher increase in leaf chlorophyll content and plant height.

***Key Words: Solanum tuberosum* L., potato varieties, SPAD-measured chlorophyll content, plant height, tuber weight, heat stress, adaptive response**

Introduction

Global climate change is subjecting plants to abiotic stresses, such as heat stress, which can affect plants' physiological processes, consequently impacting their growth, development and productivity. Indeed warming temperatures from climate change cause more frequent heat stress to plants during the summer, and reduce crop yield and quality in many regions (Lafta and Lorenzen 1995). Heat stress affects growth, quality, and yield traits by impacting the structure and metabolic functions of cells and several physiological processes, such as structural alterations of protein complexes, changes in protein synthesis and enzyme activities, cellular structure and membrane functions, production of detrimental reactive oxygen species (ROS), decoupling of metabolic pathways, and damage to the photosynthetic apparatus (Taiz et al. 2015). In order to sustainably achieve global food security under climate change conditions, it is therefore essential to understand the response of crop plants to heat stress and its biological basis so that crop varieties tolerant to heat stress could be developed by selection, breeding and biotechnological approaches.

Potato (*Solanum tuberosum* L.) is a staple food word-wide, being the third most important crop after rice (*Oryza sativa*) and wheat (*Triticum* spp.). Its sustainable production is important for global food security. Potato is the key agricultural crop in Canada, especially in New Brunswick and Prince Edward Island, where it is the number one crop. However, potato crops are quite sensitive to heat stress, likely because potato originated from the highlands of the Andes. The ideal temperature for the growth of above-ground plant parts is 20-25 °C and the optimum temperature for tuber formation is 15- 20 °C (Rykaczewska 2013). The highest temperatures in most Canadian provinces were about 35 °C in July in recent years; for example, the highest monthly maximum temperature recorded in Ontario was 34.5 °C, 36.5 °C, 34.7 °C, respectively in 2015, 2016, and 2017 (http://climate.weather.gc.ca/prods_servs/cdn_climate_summary_e.html). Since potato is usually planted in late May or early June in Canada, the extreme high temperatures in summer definitely have an adverse influence on potato crops and subsequently harm potato production. In Ontario, as a result of extreme heat stress in summer, the potato yield decreased 17.2% in 2016 compared with the production in 2015

(Statistics Canada, 2016, https://www150.statcan.gc.ca/n1/daily-quotidien/161129/dq161129f-eng.htm).

High temperature can disturb the relationship balance between source and sink, delay the process of tuber formation and bulking, and finally result in tuber deformities and necrosis (Levy and Veilleux 2007). In fact, higher temperatures adversely affect tuber formation and tuber development in potato, and this inhibition of tuberization has been linked to the inhibition of tuberization signal *StSP6A* (an orthologue of Arabidopsis flowering *FT* locus (Navarro et al. 2011) at elevated temperatures (Ewing 1981; Hancock et al. 2014) and reduced accumulation of carbon into starch in the tuber at higher temperatures (Hancock et al. 2014). Also, an adverse effect on photosynthesis resulting from chlorophyll loss and reduced CO_2 fixation has been reported for tuber-forming *Solanum* species (Reynolds et al. 1990). A large number of differentially expressed genes involved in many biological processes and molecular functions as well as differential metabolite accumulation have been identified in response to mild to moderate heat stress in potato leaves and tubers (Hancock et al. 2014; Trapero-Mozos et al. 2018). Tolerance to elevated temperatures in potato is likely a polygenic trait and, thus expected to be substantially influenced by genotype-environment interactions. As such, potato cultivars may show a wide variety of variation in their response to heat stress. However, so far most studies on heat-stress response of potato have focused on some germplasm accessions (Reynolds and Ewing 1989) or only on a very few registered cultivars. In order to understand the biological basis of heat tolerance and select and develop potato varieties that are heat tolerant, it is critical to understand the variation in response of a large number of potato varieties/cultivars to heat stress. Indeed screening and breeding for heat-tolerant potato cultivars is urgently needed to stabilize potato productivity in the current and future warmer environment (Liu et al. 2006).

Potato is one of the key crops in Canada. For the potato production system to remain economically viable under the climate change conditions in Canada, it is critical to select and develop potato cultivars that are productive and tolerant to heat stress. In order to achieve this goal, first we need to understand the variation in physiological, growth and yield response of Canadian potato cultivars to heat stress, and identify productive and heat-tolerant cultivars. However, very little is known about these aspects. We have addressed this critical need in our study. The objective of our study was to examine variation among a large number of potato cultivars registered and used for production in Canada for their response to heat stress for chlorophyll content, plant growth and largest tuber weight traits. We examined the response of 55 potato cultivars (genotypes) to heat stress to determine whether there is a significant variation in their chlorophyll content, height growth and largest tuber weight traits in response to heat stress, whether these traits are inter-correlated, and whether there are cultivars that are heat tolerant and productive. Our working hypothesis is that there is significant genotypic variation among potato cultivars for their response to heat stress in chlorophyll content, plant growth and tuber formation, and relatively heat-tolerant cultivars could be identified based on these traits.

Materials and Methods

Plant material

Fifty-five potato varieties that are currently or previously registered in Canada were selected to investigate their response and tolerance to heat stress (Table 1). One-month-old *in-vitro* cultured plantlets of each of the 55 cultivars were obtained from the Plant Propagation Centre (New Brunswick, Canada). The plantlets were acclimated to the greenhouse environment in a half-strength modified Hoagland solution (Hammer et al., 1978) in a tray as previously described (Xie et al. 2018) for 12 days and then planted into potting mix (peat moss, Perlite, soil, vermiculite at 2:1:1:1, v:v).

The experiments were conducted in the greenhouse and growth chambers at the Fredericton Research and Development Centre, Agriculture and Agri-Food Canada, Fredericton, New Brunswick, Canada. The greenhouse experiment involved all 55 cultivars, whereas the growth chamber experiment included only two cultivars to further evaluate specific traits.

Greenhouse Experiment

Experimental design, heat treatment and growth conditions

We used a factorial experimental design. The greenhouse experiment had two temperature treatments. There were two replicates (blocks) within a treatment, and there were four plants of each cultivar within a replicate. Thus, the greenhouse experiment had 880 plants: cultivars × plants × replicates × treatments = 55×4×2×2 = 880 plants. The cloned plants of the same cultivar under two treatments were genetically identical.

The plants were tested under two conditions in two compartments of the greenhouse: control (CK) and heat stress (HS). The CK growth conditions used a photoperiod regime of 14h light and 10h dark, a day temperature of 22 ± 1°C, and a night temperature of 18 ± 1°C. The humidity was set to 70% but it was somewhat higher immediately after watering. All plants were first grown under these control non-stressed conditions for three weeks for acclimation to the greenhouse condition. After three weeks of growth in the potting mix under the control non-stressed conditions, half of the plantlets were transferred to another greenhouse compartment with a heat stress regime of 35 ± 1°C in the daytime and 28 ± 1°C at night; while the remaining plantlets were kept in the original greenhouse (22 °C/18 °C for day/night temperature) as the control. The heat treatment was started after three weeks of growth under the non-stress conditions because the potato crop is mainly planted in spring but experiences hot temperatures usually in the summer.

When plants were small, they were watered as needed and at the later stage twice a day to ensure there was no water deficiency. After two weeks of different temperature treatments, the plants were supported with bamboo sticks to keep them upright. The plants were fertilized once a week with N-P-K 20-20-20, also containing, various microelements (Plant Products Co. Ltd., https://www.plantprod.com, Brampton, Ontario, Canada). Plants were harvested 102 days after planting.

Chlorophyll content

The chlorophyll content of each plant was measured indirectly by using a hand-held Minolta SPAD-502 meter (2900 PDL, Spectrum Technologies, Inc). SPAD is an acronym for “Soil Plant Analysis Development. The SPAD values do not reflect the absolute chlorophyll content but are directly proportional to the actual chlorophyll content. The SPAD-502 meter can measure chlorophyll content indirectly by the absorbance in the 400-500 nm wavelength spectrum, using a near-infrared region as control. The SPAD values were measured on plants in both replicates of two treatments seven (SPADCK7D, SPADHS7D) and 16 days (SPADCK16D, SPADHS16D) after heat-stress treatment. The 4th opened leaf of each plant of the 55 cultivars was used to measure chlorophyll SPAD values. The 5th leaf was used if the 4th leaf was too small to measure. Each leaf was measured three times and the mean of the three readings was scored as the SPAD reading of that leaf. We ensured that the measurement was taken from the same area of the 4th or 5th leaf across the plants. We also measured the SPAD values after 32 days of treatment but only for one replicate in each of the two treatments (SPADCK32D, SPADHS32D). The 4th opened leaf measured for SPAD values 7 days after the start of heat treatment already emerged when the heat treatment started. For SPAD measurements on the 16 days and 32 days after the heat treatment, the 4th or 5th leaves emerged after starting the heat treatment. We calculated HS/CK SPAD ratios for each of the three temporal SPAD measurements: SPADR7D, SPADR16D, and SPADR32D.

Plant height and largest tuber weight

Plants were harvested 102 days after planting. We measured height of individual plants in both treatments (PHTCK, PHTHS) at the time of harvest and calculated HS/CK plant height ratio, PHTR. In addition, we measured the weight of the largest tuber for each plant in both CK and HS treatments at the time of harvest (LTWCK, LTWHS), and calculated HS/CK largest tuber weight ratio, LTWR. We measured the weight of the largest tuber because it can be unambiguously identified and measured. We think that the weight of the largest tuber is a good measure of the tuber size development and may serve as a surrogate indicator of yield. The total weight of tubers obviously is a good measure of the total yield. However, we had to arbitrarily decide whether tiny tubers should be counted in and from what size tubers should be counted in for measuring the total weight. Furthermore, we had to make sure that no tiny tubers accidently missed in the potting mix during harvesting. Therefore for unambiguity, we used the largest tuber weight as the measure of tuber size development and an indicator of yield.

Growth chamber experiment

We conducted two very small supplementary experiments with two cultivars (Russet Burbank and Kennebec) in the growth chamber set under the same control and heat-stress temperature conditions as described above for the greenhouse experiment. We conducted one experiment to measure leaf size in cultivar Russet Burbank to supplement the visual leaf morphological observations in the greenhouse experiment. Four cloned plants of Russet Burbank, two each in the CK and HS treatments, were used. The length and width of the 1st, 2nd, and 3rd opened leaves were measured after the heat-stress treatment of 0 h, 24 h, 2.5 days and 4 days. These leaves were quite small at the time of the start of the heat treatment. In the second growth chamber experiment, we evaluated the cloned plants of cultivar Kennebec in the CK and HS conditions for determining chlorophyll content in order to compare the results with the greenhouse experiment. SPAD values were measured for the 5th leaf of 19 plants in CK treatment and 22 plants in HS treatment 10 days after starting the heat treatment. The supplementary growth chamber experiments were a part of the separate experiment performed to undertake the transcriptomic work to identify differentially expressed genes in response to heat stress.

Data Analysis

Data was analyzed using univariate and multivariate statistical methods. Analysis of variance was performed to test for differences in the measured SPAD chlorophyll content, plant height and largest tuber weight as well as their ratios between each other due to temperature treatment, cultivars and replicates using the SAS Enterprise Guide (SAS Institute Inc., Cary, NC, Version 7.1). Means and standard errors or standard deviations for each trait for cultivars were calculated and the statistical significance of differences among cultivars for each trait was determined by Duncan's Multiple Range Test (DMRT) using the SAS Enterprise. Since the DMRT results for the measured traits were consistent to those for the trait HS/CK ratios, we have presented the results only for the ratios.

The correlations among different measured and derived traits were estimated by calculating Pearson correlation coefficients using the statistical tool in Excel 10. The mean trait value for each cultivar was used in the correlation analysis. We also performed regression analysis among the selected traits using the mean cultivar trait values. Pearson correlations among all traits and their significance levels were also calculated using the multivariate correlation option in SAS Enterprise (Version 7.1).

Multivariate analysis was performed using heat map and Principal Component Analysis (PCA) to examine the differentiation and grouping of the traits and cultivars. The heatmap.2 and gplots commands in R package (https://www.r-project.org/) were used to generate a heatmap using cultivar means of SPADCK7D, SPADHS7D, SPADR, PHTHS, PHTCK, LWTHS, LTWCK, PHTR, LTWR traits. In this analysis, first the Manhattan distances among cultivars and traits were calculated by the command "dist" and then the hierarchical cluster analysis was performed by the command "hclust" (row_distance, method = "ward.D2"). The PCA was performed using SAS (SAS Institute Inc., Cary, NC, version 9.4), based on the same nine traits. The ordination of the cultivars was determined by constructing a plot for Principal Component 1 (PC1) and Principal Component 2 (PC2).

Results

Chlorophyll content

Leaf chlorophyll SPAD values of plants in the heat-stress treatment were significantly ($P < 0.001$) higher than those of plants in the control treatment, consistently at three times of measurement: 7 days, 16 days and 32 days (Table 2; Table S1 and S2). A large genotypic variation among cultivars was observed for the SPAD values and their HS/CK ratios (Table 2; Tables S1 and S2). ANOVA showed that these differences in SPAD values and HS/CK SPAD ratios due to cultivars as well as temperature treatments were highly significant ($P < 0.01$).

The 7th day SPAD values for the plants in the control treatment ranged from 28.16 to 45.63, with an average of 37.22, whereas the SPAD values for the plants in the heat stress treatment ranged from 39.60 to 55.38, with an average of 45.31 (Table 2). The 7th day HS/CK SPAD ratios (SPADR7D) ranged from 1.06 for Adirondack Blue to 1.53 for Russet Norkotah, with an average of 1.22, suggesting an average 22% increase of SPAD values in HS-treated plants compared to control plants (Table 2). The cultivars that had a

SPADR7D greater than 1.35 (i.e., more than 35% increase in SPAD values) were Russet Norkotah (1.53), Chieftain (1.44), Eramosa (1.41), and Carlton (1.36) (Table 2).

A similar trend of increased SPAD values for heat-stressed plants as observed on the 7th day was detected at the 16th and 32nd day measurements (Tables S1 and S2); however the rank of certain cultivars based on SPAD values was changed (Tables 2 and S1). Indeed for certain cultivars the SPAD values decreased compared to the 7th day observation. The SPAD values 16 days after treatment ranged from 26.35to 42.93 in the CK treatment and from 29.04 to 59.69 in the HS treatment and, with an average value of 34.52 for plants under CK conditions and 49.08 for plants under HS conditions, with an average increase of 43% ($P < 0.05$, Table S2). The HS-to-CK ratio of SPAD values at the 16th day (SPADR16D) ranged from 0.98 in AC Chaleur to 1.77 in Carlton, with an average ratio of 1.43 (Table S1). The ratio of HS-to-CK SPAD value at the 32nd day after treatment further increased with a range from 1.17 (cultivar Elba) to 2.33 (cultivar Eramosa) and an average of 1.65 over 55 cultivars (Table S2). Thus the mean of the increase of SPAD values over the 55 cultivars was 65%. However, there were significant differences among cultivars for the extent of increase in the SPAD values (Table S2).

In a supplementary experiment in the growth chamber with the cultivar Kennebec, the mean SPAD value of the 5th leaf 10 days after starting the heat treatment was 46.61 in heat-stressed (HS) plants and 39.80 in CK plants ($P < 0.01$). Thus, the growth chamber experiment confirmed our observation of increased SPAD values in the heat-stressed potato plants.

The overall mean of SPAD values was significantly higher ($P < 0.05$ in ANOVA-Duncan test), in HS plants than in CK plants, regardless of the 7th day, 16th day, or the 32nd day measurement in the greenhouse experiment of the 55 cultivars (the HS/CK of SPAD value: 45.3/37.2, 49.1/34.5, and 55.5/34.2, for 7th day, 16th day, and 32nd day, respectively) or the 10th day measurement the supplementary growth room experiment of the cultivar Kennebec (HS/CK 46.6/39.8) (Fig. S1).

Plant leaf size and height

Under heat stress (HS) conditions, plants tended to grow taller (Table 3), with smaller, greener and thicker leaves than controls (CK). Four weeks after treatment, plants of most varieties were clearly taller than the non-stressed plants, such as in 'Yukon Gold' (Fig. 1A). However there was significant variation among the studied potato cultivars ($P < 0.01$) in their plant height response to heat stress (Table 3). The heat-stressed plants did not show any signs of obvious early senescence or dying, whereas most of the non-heat-stressed plants showed signs of yellowing in leaves at the time of harvest (Fig. 1B). The aboveground plant parts were larger in the heat-stressed plants (Fig. 1C) than the non-heat-stressed plants (Fig. 1B) at the time of harvest in most cultivars, as shown with plants of the cultivar 'Shepody' (Figs. 1A, 1B). The leaf length ratio (HS/CK) and the leaf width ratio (HS/CK) of the first, second and third leaves decreased 4 days after the start of heat treatment in Russet Burbank plants in the growth chamber experiment (Fig. 2).

The plant height ratio between HS plants and CK plants varied significantly among the cultivars ($P < 0.01$). It was greater than 1.0 in 45 out of 55 cultivars (Table 3), with an average of 1.64 among the 55 cultivars. This demonstrates that on average there was a 64% increase in plant height under the heat stress conditions compared to the non-stressed control conditions. The cultivars with a HS/CK plant height ratio larger than 2.5 were Eramosa (3.93), Caribe (3.25), Norland (2.98), AC Belmont (2.81), Chieftain (2.78), Yukon Gold (2.73), and Cherry Red (2.64) (Table 3). Ten cultivars showed a reduction of 2% to 53% in plant height under the heat-stress conditions as compared to control conditions, with an average of 10.4% (Table 3) and this was related to their high sensitivity to heat stress. The cultivar 'AC Chaleur' showed the largest reduction of 57% in plant height (Table 3). The correlation between the plant height ratio and the tuber weight ratio for these 10 cultivars was not significant (R = 0.56, P = 0.090).

Largest tuber weight

We observed significant variation in tuber formation and the largest tuber weight among the cultivars both under the control and heat-stress conditions (Table 4). Under the control non-stressed conditions, all cultivars formed tubers with the largest tuber weight ranging from 29.38 g to 115.65 g, with an average of 72.17 g (Table 4). Under the heat-stress conditions, the tuber formation and tuber weight were significantly inhibited. No tubers were formed in four cultivars (Table 4; All Blue, Butte, Ac Chaleur and White

Rose). In addition, 'Russet Burbank' produced only one small tuber of approximately 1.0 g in one of the 8 plants under heat stress. The largest tuber weight ranged from 0.12 g to 26.26 g, with an average of 4.76 g over the 51 cultivars (Table 4). Cultivars Eramosa, Chieftain, and AC Belmont were top cultivars for the largest tuber weight under HS conditions (Table 4). The ratio of the HS/CK largest tuber weight ranged from 0 to 0.30, with an average of 0.07 (Table 4), which means that the largest tuber weight was on average lower by 93.4% under the heat stress conditions. The differences among cultivars were highly significant ($P < 0.01$).

Correlation among traits

The Pearson correlation coefficient (R) (and their statistical significance levels) among all the measures and derived traits are presented in Table 5. SPAD values for three time intervals (7, 16, and 32 days) were positively and significantly correlated with each other for plants from each of the CK and HS treatments, showing a consistent temporal trend. Likewise, HS/CK SPAD ratios for 7-, 16- and 32-day measurements were positively and significantly correlated.

SPAD values for plants in CK treatment were negatively correlated with the LTW and LTWR of plants in the HS treatment. The results indicated that cultivars that had higher chlorophyll content in CK compared to the HS treatment were more susceptible to heat stress in terms of the largest tuber weight (Table 5). Only in one case, the CK SPAD values at day 32 were positively correlated with the height of the plants from the CK treatment (Fig. 3A). The 7th day SPAD values for plants in HS were negatively correlated with LTWHS and LTWR. The SPAD values measured for HS plants on the 16th day and 32nd day did not show any significant correlation with the harvest time LTWHS and LTWR (Table 5). However, the HS/CK SPAD ratios on each of the 7th, 16th, and 32nd day were all positively and significantly correlated with LTWHS and LTWR (Table 5; Fig. 3B).

The HS/CK plant height ratio and the largest tuber weight were positively and significantly correlated (Fig. 3C). There was also a positive correlation between the 32nd day HS/CK SPAD ratio and HS/CK plant height ratio (Fig. 3A) and largest tuber weight (Fig. 3B). In addition, the height of plants in HS treatment (PHTHS) was positively and significantly correlated with the 16th and 32nd day of SPAD values (SPADHS16D, SPADHS32D) for the HS treated plants (Table 5). This suggests that the heat stress tolerance in terms of plant height and the largest tuber weight was correlated with the increase in the chlorophyll content.

PHTR was positively correlated with SPADR7D, LTWHS, LTWR, SPADR16D, SPADR32D (Table 5), suggesting that cultivars tolerant to heat stress in terms of plant height are also tolerant to heat stress in terms of chlorophyll content and tuber size.

Heatmap

Heatmap analysis based on the traits SPADHS7D, SPADCK7D, PHTHS, PHTCK, LWTHS, LTWCK, SPADR, PHTR, and LTWR showed that SPADHS7D and SPADCK7D were in the same cluster. Interestingly PHTHS and PHTCK grouped with LTWCK in the same cluster. LTWR, SPADR7D, PHTR, and LTWHS were in the same cluster. Eramosa, Norqueen Russet, Norland DR, AC Belmont, Cherry Red, Caribe, and Chieftain were grouped together as a cluster. Since most members of this cluster had relatively large LTWHS, this cluster largely represents the cultivars more tolerant to heat stress than the other cultivars. All Blue, Butte and White Rose, which did not produce any tubers under heat stress, were grouped in the same cluster (Fig. 4).

Principal component analysis

The principal component analysis based on nine traits identified the first four principal components (PC) as significant with Eigen values >1.0 and accounted for a total of 82.7% of the total variation (Table S3). The first PC explained 41.6% and second PC 16.5% of the total variation. The first PC was dominated by the positive values for LTWHS, LTWR and PHTR, and the highest negative value for SPADCK7D (Table S4). The second PC was dominated by the positive values for SPADHS7D, SPADCK7D and PHTR, and the highest negative value for PHTCK (Table S4). The PC1 separated the cultivars Eramosa, Chieftain, AC Belmont, Caribe, and Cherry Red from the others (Fig. 5). These cultivars displayed higher heat tolerance (in terms of LTWR and PHTR) than others, and the results for the differentiation and grouping of these cultivars were consistent with those obtained from the heatmap (Fig. 4).

Discussion

Heat stress can adversely affect growth and yield in crop plants by altering several physiological processes and metabolic pathways. Here we have evaluated physiological, growth and tuber development response of 55 potato cultivars to heat stress by investigating chlorophyll content, plant growth and the largest tuber weight. This is the first large-scale screening of potato varieties registered in Canada for their heat-stress tolerance. We have demonstrated significant genotypic variation among potato cultivars for their response to heat stress in terms of chlorophyll content, plant height and the largest tuber weight and identified relatively heat-stress tolerant and susceptible potato cultivars. Despite significant inter-cultivar genotypic variation, our results establish that overall heat stress decreased leaf size, increased leaf chlorophyll content (SPAD values) and plant height growth but severely reduced the tuber formation and the largest tuber weight in the studied potato cultivars.

We measured leaf chlorophyll content based on SPAD index values, which are directly proportional (R^2=0.99) to the absolute chlorophyll content (Ling et al., 2011). Our results clearly demonstrate that leaf chlorophyll content as measured by SPAD index values increased under heat stress conditions in all cultivars. This was consistent at three different measurement times (7, 16 and 32 days) in the greenhouse experiment, with the exception of 'AC Chaleur' at 16^{th} day measurement when its SPAD value was reduced by 2% under heat stress. Our growth chamber experiment further confirmed increased SPAD values under heat stress conditions for the cultivar Kennebec. Thus, our results of heat-stress-induced increase in leaf chlorophyll content (SPAD values) are real and repeatable (Fig. S1). These results are further supported by our observation that potato plants' leaves in the heat-stress conditions were greener and darker than those in the non-stressed condition. Our results suggest that potato plants increase their leaf chlorophyll content as an acclimation response to heat stress.

Increased SPAD values may also be due to decreased leaf size and somewhat increased leaf thickness of potato plants that we observed under heat-stress conditions, which may have increased chlorophyll content per unit leaf area. Increased SPAD values may also be due to other cellular structural changes induced by heat stress. We cannot determine the exact cause underlying this observation in the present study, and further work is needed to investigate whether chlorophyll content in terms of leaf dry weight also increase under similar heat stress conditions in these potato cultivars. Nevertheless, SPAD values have been reported to increase with fresh and dry leaf thickness, specific leaf mass (specific leaf weight, SLW; leaf dry weight per unit leaf area), and leaf succulence, and decrease with specific leaf area (ratio of leaf area to leaf dry mass) and leaf water contents in several plant species (Yamamoto et al. 2002; Marenco et al. 2009).

Our observations of increased SPAD values in heat-stressed plants, although repeatable and consistent with leaf color and morphology of potato plants, are contrary to reduced SPAD index values in wheat (*Triticum aestivum*) and rice (*Oryza sativa*) plants under heat stress (Balouchi 2010; Liu et al. 2013; Tiwari et al. 2017). This may be due to the differences in species' response to heat stress and leaf structure. Potato leaf morphology and structure differs from that of wheat and rice, thus its SLW is likely different from that of wheat and rice. Yamamoto et al. (2002) have reported that the effect of SLW on SPAD index can vary between plant species. Our results of increased SPAD values under heat treatment are contrary to decreased chlorophyll content (based on extraction with acetone and spectrophotometric readings) under mild heat stress reported in potato cultivar Desiree (Hancock et al. 2014) and accession of the wild species *Solanum chacoense* (Reynolds et al. 1990). We do not know the reason for this inconsistency but it may be related to the differences in heat treatment conditions, measurement methods, and potato genotypes between our and Hancock et al. (2014) studies. Our experiment did not include the wild *Solanum chacoense*. However, our results for reduced chlorophyll content in cultivar AC Chaleur in response to heat stress at the 16^{th} day are consistent with Hancock et al. (2014). This exemplifies the genotypic effect on the response of potato cultivars to heat stress and suggests that more than one cultivar should be used in such studies. Also, a previous study (Demirel et al. 2017) in Turkey compared chlorophyll SPAD values in potato plants with harvest dates in June, and July for the normal growth period, and heat-stressed growth, respectively in uncontrolled conditions., The SPAD values were slightly lower for the plants harvested in July than for the plants harvested in June. It is unclear whether the slight reduction of SPAD values was due to the higher atmospheric temperature in July or due to some other weather-related factors, such as humidity or drought. In our current study, both CK and HS plants were treated under controlled environments and at the same time with no water stress. Further work is required to fully address the differences observed in chlorophyll content patterns in different studies.

Although, there was significant genotypic variation among cultivars, our study demonstrated that overall heat stress increases aboveground plant height in potato. This vigorous growth of aboveground part is most likely due to increased photosynthesis rate in leaves due to increased leaf chlorophyll content that we observed under increased temperature. This is supported by significant positive correlation of HS/CK plant height ratio with HS/CK SPAD index ratio at the 16th and 32nd day as well as visual observation of the potato plant leaves, which were much greener in the heat-stress treatment than in the non-stressed control treatment. The considerable increase of chlorophyll SPAD values in HS plants of most cultivars in the present study suggests that the photosynthesis rates increased under these HS conditions in these tested cultivars. Our results are consistent with an increase in net foliar photosynthesis of plants of cultivar Desirée under relatively mild heat stress (30 °C/20 °C, day/night) (Hancock et al. 2014), even though the reported chlorophyll content decreased in this cultivar. The increased plant height under heat-stress conditions in our study may also be due to higher shoot to tuber allocation of photosynthates. Our results of increased height growth under heat stress are in agreement with previous studies (Marinus and Bodlaender 1975; Paul et al. 2016).

Our study shows that heat stress severely reduces tuber formation and tuber weight, evident from no tuber formation in several potato cultivars and overall reduction of the largest tuber weight by 93.4% (Table 4). This was despite the fact that plants under heat stress remained largely green until harvest and their leaf chlorophyll SPAD values increased. It is generally known that heat stress adversely affects tuberization in potato, and our results are consistent with previous similar findings (Marinus and Bodlaender 1975; Paul et al. 2016; Demirel et al. 2017). It is known that heat stress inhibits tuber formation and tuber development by disrupting the tuberization signal (Ewing 1981; Hancock et al. 2014), an orthologue of Arabidopsis flowering *FT* locus (Navarro et al. 2011), changing the expression of the genes related to the circadian clock (Hancock et al. 2014), as well as reducing the accumulation of carbon into starch in the tuber (Hancock et al. 2014). The same or similar mechanisms may be responsible for the reduction in tuber formation and tuber development in our study.

Our study reveals that tuber yield in terms of the largest tuber weight was significantly and positively correlated with both HS/CK SPAD index and HS/CK plant height ratio. This suggests that cultivars that are relatively heat tolerant in terms of the largest tuber weight also have increased plant height and increased leaf chlorophyll content under heat stress conditions. This may represent a correlated response of potato cultivars for chlorophyll content, plant height and the largest tuber weight to heat stress. Demirel et al. (2017) also found correlation between SPAD values and tuber yield in potato. Significant positive correlations between leaf SPAD values and grain yield have also been reported for wheat (Barutçular et al. 2016).

From the trends that emerged from our data, it appears that increased leaf chlorophyll content, increased height and decreased tuber formation and the largest tuber weight may be the acclimation response of potato to heat stress. This response differed greatly among the 55 potato cultivars. The potato cultivars showed correlated response for chlorophyll content, plant height and tuber weight. Based on these multiple traits, relatively heat-stress tolerant and susceptible cultivars could be differentiated, as was evident from the heatmap and PCA results (Figs. 4 and 5). The HS/CK leaf chlorophyll, plant height and tuber weight ratios appear to be the most discriminatory traits. Since the tuber weight is one of the most important factors for potato agriculture, our study identifies relatively heat-stress tolerant and susceptible cultivars for this trait (Table 4). The PCA and heatmap separated a group of five cultivars (Eramosa, Chieftain, AC Belmont, Caribe, and Cherry Red), which could be considered as relatively most heat-stress tolerant, in terms of LTWR, among the 55 Canadian registered cultivars studied here. The cultivars Eramosa and Chieftain were found to be the most heat-stress tolerant when leaf chlorophyll content, plant height and the largest tuber weight were considered together. Russet Burbank, All Blue, Butte, AC Chaleur, and White Rose could be considered as the most susceptible potato cultivars to heat stress in terms of tuberization (crop yield). Russet Burbank was also found to be the most susceptible cultivar to heat in terms of tuber weight when 17 potato accessions/cultivars were tested in the field in Turkey (harvest in June for the normal growth period and July for heat stress treatments) (Demirel et al. 2017). This finding of Russet Burbank's susceptibility to heat under both controlled and uncontrolled environments may partly explain why the hot summer in Ontario caused severe reduction in potato production in 2016 because the cultivar 'Russet Burbank' was the primary planted cultivar. Indeed 'Russet Burbank' is the world's most important potato cultivar for production.

The heat stress tolerant and sensitive cultivars identified in our study can help in the selection and breeding of heat-stress tolerant potato cultivars, in identifying genes responsible for heat tolerance using genomic approaches, and creating new heat tolerant cultivars using genomics technologies, such as genome editing (e.g. Ma et al. (2017)).

Conclusion

We have for the first time performed a large-scale evaluation of potato varieties for their tolerance to heat-stress and identified relatively tolerant and susceptible cultivars. Significant genotypic variation among potato cultivars exists for their response to heat stress in terms of chlorophyll content, plant height and the largest tuber weight. Overall heat stress decreased leaf size, increased leaf chlorophyll content (in terms of chlorophyll SPAD values) and plant height but severely reduced the largest tuber weight, which may be considered as an acclimation response to heat stress. Potato cultivars display a correlated response to heat stress for their leaf chlorophyll content, plant height and tuber weight. Cultivars that are relatively heat tolerant in terms of the largest tuber weight also have increased plant height and increased leaf chlorophyll content under the heat stress conditions. Our study provides information of basic biological significance and is useful for potato farmers and breeders in selecting heat-stress tolerant cultivars, and for potato genomics studies and applications.

Author contributions

Study conception and design: XQL, RT and QY; Greenhouse and growth chamber work: RT, SN, GZ, GC, MH and XQL; Data analysis and manuscript direction: XQL and OPR; Manuscript writing and revision: OPR and XQL.

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Table 1 Potato cultivars and their parentage and origin.

Cultivar	Cultivar ID	Parentage	Origin (country)
AC Belmont	ACBe	Raritan x F51013	Canada
AC Blue Pride	ACBP	N713-16 x N889-78-3	Canada
Ac Chaleur	ACCh	Belleisle x N457	Canada
AC Ptarmigan	ACPt	ND699-13 x f59103	Canada
Adirondack Blue	Adri	Chieftain x Black Russian	USA
All Blue	Ablu	Unknown	USA or UK
Allegany	Alle	M297-17 x unknown	USA
Alturas	Alturas	A77182-1 x A75188-3	USA
Andover	Ando	Allegany x Atlantic	USA
Atlantic	Atl	Wauseon x B5141-6	USA
Belchip	Belc	Wauseon x USDA B5141-6	USA
Butte	Butt	A492-2 x Norgold Russet	USA
CalWhite	Calw	Pioneer x BC8370-4	USA
Caribe	Caribe	F55066 x USDA96-56	Canada
Carlton	Carlton	F47024 x F55069	Canada
Castile	Castile	Peconic x F107-30	USA
Centennial Russet	CRus	W12-3 x Nooksack	USA
Cherry red	CRed	ND4750-2R x LA1858	USA
Chieftain	Chieft	La1027-18 x La1354	USA
Chipeta	Chipeta	WNC612-13 x Wischip	USA
Defender	Def	Unknown	USA
Denali	Denali	B5141-6 x 1-62-90-64	USA
Desiree	Desi	Urgenta x Depesche	Netherlands
Elba	Elba	D29-10 x NY27	USA
Epicure	Epicure	Magnum Bonum x Early Regent	England
Eramosa	Eramosa	F52047 x F60019	Canada
Frontier Russet	FRus	A66102-16 x WN330-1	USA
Goldrush	Goldru	Lemihi Russet x ND450-3 Russ	USA
Innovator	Innov	Shepody x RZ-84-2580	Netherlands
Katahdin	Kata	USDA 40568 X USDA 24642	USA
Kennebec	Kenn	B127 x USDA 96-56	USA
Langlade	Langl	Kennebec x W631	USA
Mainechip	Main	AF186-2 x AF84-4	USA
Mazama	Mazama	ND1196-2R x Redsen	USA
Mirton Pearl	Mirton	Mira x F5318	Canada
Nipigon	Nipigon	F53026 x F51043	Canada
Niska	Niska	Wischip x B5141-6	USA

Norgold Russet	Norg	A119-1 x ND2475-8	USA
Norland DR	Norl	ND626 x Redkote	USA
Norqueen Russet	Norqu	WA 330 x ND9567-2 Russ	USA
Ranger Russet	Rang	Butte x A6595-3	USA
Raritan	Raritan	F4519 x 834C(29)	Canada
Red Cloud	RedC	NE 185.70-1 x Superior	USA
Red Lasoda	RedL	Mutation of La Soda	USA
Red Pontiac	RedP	Mutation of Pontic	USA
Russet Burbank	RB	A mutant of Burbank	USA
Russet Norkotah	RN	ND9526-4 Russ x ND9687-5 Russ	USA
Sebago	Sebago	Chippewa x Katahdin	USA
Shepody	SH	Bake King x F58050	Canada
Snowden	Snow	B5141-6 x Wischip	USA
Spunta	Spunta	Bea x USDA 96-56	Netherlands
Superior	Super	USDA96 x M59.44	USA
Viking	Viking	Redskin x Nordak	USA
White Rose	Whit	Seed ball from Jackson	USA
Yukon Gold	Yukon	W5279-4 x Norgleam	Canada

Note: the information on the potato cultivars is based on Barclay and Scott (Barclay and Scott 1997), except that the information of the cultivars Alturas, Cherry Red, and Innovator was according to Canadian Food Inspection Agency (http://www.inspection.gc.ca).

Table 2 The chlorophyll SPAD values of non-stressed (SPADCK7D) and heat-stressed (SPADHS7D) plants and their ratios (SPADR7D) after 7 days treatment of potato cultivars.

Cultivar	No. of HS plants	SPADCK7d	SPADHS7d	SPADR7d Mean ± SE
Russet Norkotah	8	29.45	44.58	1.53 ± 0.10 a
Chieftain	8	31.55	45.38	1.44 ± 0.06 ab
Eramosa	8	28.16	39.60	1.41 ± 0.06 abc
Carlton	8	34.19	46.43	1.36 ± 0.04 abcd
Shepody	8	30.63	40.90	1.34 ± 0.03 abcde
Mazama	8	36.40	48.54	1.33 ± 0.03 abcde
White Rose	8	31.86	42.13	1.32 ± 0.05 abcde
Russet Burbank	8	32.30	41.91	1.30 ± 0.04 bcdef
Caribe	8	33.45	43.25	1.30 ± 0.03 bcdef
Norgold Russet	8	33.75	43.69	1.29 ± 0.03 bcdefg
AC Blue Pride	8	33.58	43.36	1.29 ± 0.02 bcdefg
Mirton Pearl	8	35.39	45.76	1.29 ± 0.04 bcdefg
Cherry Red	8	34.10	44.18	1.29 ± 0.03 bcdefg
Red Pontiac	8	38.85	49.85	1.28 ± 0.01 fbcdeg
Red Lasoda	8	39.55	50.56	1.28 ± 0.01 bcdefg
Centennial Russet	8	38.18	48.69	1.27 ± 0.03 bcdefg
Raritan	8	37.48	47.65	1.27 ± 0.02 bcdefg
Viking	8	37.03	46.76	1.26 ± 0.03 bcdefg
Alturas	4	38.53	46.75	1.26 ± 0.04 bcdefgh
Niska	8	33.49	41.73	1.25 ± 0.01 bcdefgh
AC Ptarmigan	8	35.63	44.29	1.24 ± 0.02 bcdefgh
Yukon Gold	8	39.95	49.56	1.24 ± 0.03 bcdefgh
Desiree	8	38.14	47.40	1.24 ± 0.03 bcdefgh
Innovator	8	36.33	44.93	1.24 ± 0.01 bcdefgh
Superior	8	35.39	43.65	1.23 ± 0.04 bcdefgh
Nipigon	8	35.79	44.03	1.23 ± 0.04 bcdefgh
Atlantic	8	35.14	43.23	1.23 ± 0.03 bcdefgh
Spunta	8	39.98	48.38	1.22 ± 0.06 bcdefgh
Goldrush	8	34.99	42.48	1.22 ± 0.03 bcdefgh
Chipeta	8	35.85	43.43	1.21 ± 0.03 bcdefgh
AC Belmont	8	33.45	40.41	1.21 ± 0.03 cdefgh
Denali	8	37.09	44.80	1.21 ± 0.03 cdefgh
Butte	8	35.63	42.74	1.20 ± 0.05 cdefgh
Defender	8	43.88	52.58	1.20 ± 0.02 cdefgh
Red Cloud	8	42.49	50.40	1.19 ± 0.01 cdefgh
Sebago	8	37.63	44.58	1.18 ± 0.02 cdefgh

Epicure	8	39.70	46.54	1.18 ± 0.03 defgh
Castile	8	41.38	48.54	1.17 ± 0.03 defgh
Ranger Russet	8	36.05	41.88	1.16 ± 0.03 defgh
Belchip	8	36.38	42.26	1.16 ± 0.02 defgh
Andover	8	35.84	41.60	1.16 ± 0.02 defgh
CalWhite	8	43.86	50.85	1.16 ± 0.01 defgh
Norland DR	8	44.14	50.96	1.16 ± 0.02 defgh
Elba	4	37.23	41.38	1.16 ± 0.06 defgh
All Blue	8	39.86	45.68	1.15 ± 0.02 defgh
Ac Chaleur	4	45.63	55.38	1.15 ± 0.09 defgh
Mainechip	4	41.33	47.20	1.14 ± 0.05 defgh
Norqueen Russet	8	43.08	48.75	1.13 ± 0.02 defgh
Langlade	8	41.96	46.73	1.11 ± 0.04 efgh
Allegany	8	39.88	44.39	1.11 ± 0.01 efgh
Frontier Russet	8	38.24	41.46	1.08 ± 0.03 fgh
Katahdin	8	41.28	44.76	1.08 ± 0.02 fgh
Kennebec	4	38.19	41.45	1.08 ± 0.01 fgh
Snowden	8	36.74	39.40	1.07 ± 0.02 fgh
Adirondack Blue	6	41.38	44.07	1.06 ± 0.05 gh
Mean		**37.22**	**45.31**	**1.22**

Note: The ratio means followed by the same letter are not significantly different according to the Duncan's multiple range test at *P* <0.05. The ratio means were calculated directly from individual HS plants against the means value of 8 CK plants.

Table 3 Height of plants (PHT) in control (CK; 22° C) and heat-stressed (HS; 35° C) treatments and HS/CK plant height ratio (PHTR) at harvest.

Cultivar	No. of HS plants	22° C plant height (cm)	35° C plant height (cm)	HS/CK plant height ratio
Eramosa	8	25.14	98.88	3.93 ± 0.18 a
Caribe	8	28.80	93.50	3.25 ± 0.10 b
Norland DR	8	26.38	78.50	2.98 ± 0.22 bc
AC Belmont	8	27.81	78.25	2.81 ± 0.20 bcd
Chieftain	8	32.75	91.00	2.78 ± 0.24 bcd
Yukon Gold	8	41.25	112.5	2.73 ± 0.09 bcde
Cherry red	8	32.25	85.13	2.64 ± 0.21 bcdef
Innovator	8	41.50	102.25	2.46 ± 0.05 bcdefg
Norqueen Russet	8	40.25	99.00	2.46 ± 0.05 bcdefg
Russet Burbank	8	82.38	111.25	2.34 ± 0.03 cdefgh
AC Ptarmigan	8	31.63	69.88	2.21 ± 0.22 cdefghi
Norgold Russet	7	32.25	70.57	2.19 ± 0.05 cdefghij
Frontier Russet	8	50.13	106.50	2.12 ± 0.05 defghijk
Mazama	8	43.75	84.75	1.94 ± 0.17 efghijkl
Viking	8	55.38	105.13	1.90 ± 0.10 fghijklm
Carlton	8	60.25	111.75	1.85 ± 0.07 fghijklm
Centennial Russet	8	47.43	87.5	1.84 ± 0.13 fghijklm
Red cloud	8	42.81	78.38	1.83 ± 0.07 fghijklm
Langlade	8	52.13	94.25	1.81 ± 0.09 ghijklmn
Goldrush	8	60.38	103.13	1.71 ± 0.04 ghijklmno
Epicure	8	56.38	95.25	1.69 ± 0.06 ghijklmnop
Ranger Russet	8	60.13	99.38	1.65 ± 0.09 ghijklmnopq
Raritan	8	54.14	87.88	1.62 ± 0.07 hijklmnopqr
Spunta	8	61.38	98.13	1.60 ± 0.06 hijklmnopqrs
Superior	8	45.71	72.88	1.59 ± 0.08 hijklmnopqrs
Nipigon	8	67.63	106.13	1.57 ± 0.02 hijklmnopqrs
Mirton Pearl	8	63.63	94.88	1.49 ± 0.04 ijklmnopqrs
CalWhite	8	69.00	101.00	1.46 ± 0.06 ijklmnopqrs
Andover	8	61.63	89.63	1.45 ± 0.08 ijklmnopqrs
Katahdin	8	79.88	114.13	1.43 ± 0.03 ijklmnopqrs
Red Pontiac	8	64.38	88.50	1.37 ± 0.09 ijklmnopqrs
Russet Norkotah	8	47.50	113.00	1.37 ± 0.09 ijklmnopqrs
Niska	8	57.06	77.50	1.36 ± 0.04 jklmnopqrs
Denali	8	55.00	74.25	1.35 ± 0.11 jklmnopqrs
Snowden	8	46.00	61.38	1.33 ± 0.03 klmnopqrs

Kennebec	8	73.00	96.13	1.32 ± 0.02 klmnopqrs
Chipeta	8	75.50	96.75	1.28 ± 0.07 lmnopqrst
Castile	6	80.63	102.50	1.27 ± 0.11 lmnopqrst
Desiree	8	77.50	94.50	1.22 ± 0.07 lmnopqrst
Shepody	8	67.63	81.13	1.20 ± 0.05 lmnopqrst
Sebago	8	76.25	89.25	1.17 ± 0.04 lmnopqrst
White Rose	8	76.50	89.00	1.16 ± 0.08 lmnopqrst
AC Blue Pride	8	69.75	78.88	1.13 ± 0.11 lmnopqrst
Butte	8	58.13	63.75	1.10 ± 0.15 lmnopqrst
Atlantic	8	51.43	54.75	1.06 ± 0.07 mnopqrst
Mainechip	8	49.67	48.75	0.98 ± 0.29 nopqrst
Defender	8	84.25	81.75	0.97 ± 0.06 nopqrst
Belchip	8	94.75	89.38	0.94 ± 0.04 opqrst
Allegany	8	83.88	79.00	0.94 ± 0.06 opqrst
Red Lasoda	8	66.00	58.50	0.89 ± 0.02 opqrst
Alturas	8	89.63	76.75	0.86 ± 0.08 pqrst
Adirondack Blue	7	70.83	57.14	0.81 ± 0.22 qrst
All Blue	8	77.50	60.63	0.78 ± 0.03 rst
Elba	8	89.00	68.25	0.77 ± 0.23 st
Ac Chaleur	8	56.14	26.13	0.47 ± 0.18 t
Average		**58.40**	**85.98**	**1.64**

Note: The ratio means were calculated directly from individual HS plants against the means value of 8 CK plants. The ratio means followed by the same letter are not significantly different according to the Duncan's multiple range test at *P* <0.05.

Table 4. Weight of the largest tuber per plant (LTW) in control (CK; 22° C) and heat-stressed (HS; 35° C) treatments and HS/CK largest tuber weight ratio (LTWR) at harvest.

Cultivar	No. of HS plants	CK largest tuber weight (g)	HS largest tuber weight (g)	HS/CK largest tuber weight ratio
Eramosa	8	86.61	26.26	0.30 ± 0.04 a
Chieftain	8	62.75	17.86	0.28 ± 0.04 ab
AC Belmont	8	60.76	14.93	0.25 ± 0.04 abc
Superior	8	40.75	7.24	0.18 ± 0.06 abcd
Cherry Red	8	58.57	9.47	0.16 ± 0.05 bcde
Carlton	8	50.48	7.99	0.16 ± 0.08 bcdef
Denali	8	68.83	10.52	0.15 ± 0.06 bcdefg
Caribe	8	63.89	9.75	0.15 ± 0.05 bcdefg
Epicure	8	52.74	7.99	0.15 ± 0.05 bcdefg
Russet Norkotah	8	69.68	9.62	0.14 ± 0 bcdeg
AC Blue Pride	8	70.94	7.99	0.11 ± 0.04 cdefg
Innovator	8	77.41	7.57	0.10 ± 0.02 defg
Andover	8	52.48	4.87	0.09 ± 0.03 defg
Atlantic	8	59.69	5.53	0.09 ± 0.03 defg
Belchip	8	115.65	10.66	0.09 ± 0.02 defg
Norland Dark Red	8	77.02	7.04	0.09 ± 0.04 defg
Mazama	8	47.87	4.36	0.09 ± 0.03 edfg
Snowden	8	74.91	6.5	0.09 ± 0.04 edfg
Nipigon	8	84.95	7.29	0.09 ± 0.03 defg
Desiree	8	57.25	4.9	0.09 ± 0.02 defg
Mainechip	8	54.79	4.33	0.08 ± 0.04 defg
Viking	8	104.94	7.88	0.08 ± 0.03 defg
Shepody	8	91.54	6.51	0.07 ± 0.02 defg
Sebago	8	87.09	5.89	0.07 ± 0.02 defg
Raritan	8	80.92	4.69	0.06 ± 0.03 defg
Mirton Pearl	8	74.94	3.85	0.05 ± 0.02 defg
Niska	8	79.65	3.9	0.05 ± 0.02 defg
Goldrush	8	67.08	3.25	0.05 ± 0.02 defg
Katahdin	8	88.55	4.18	0.05 ± 0.02 defg
Defender	8	79.12	3.65	0.05 ± 0.02 defg
CalWhite	8	94.08	4.23	0.05 ± 0.01 defg

Red Cloud	8	93.26	3.85	0.04 ± 0.02 edfg
Red Pontiac	8	80.19	2.16	0.03 ± 0.02 edfg
AC Ptarmigan	8	37.38	0.95	0.03 ± 0.01 defg
Norqueen Russet	8	41.24	1	0.02 ± 0.02 defg
Castile	6	53.32	1.07	0.02 ± 0.01 defg
Langlade	8	87.85	1.89	0.02 ± 0.01 efg
Yukon Gold	8	90.78	1.63	0.02 ± 0.02 efg
Norgold Russet	7	71.34	1.21	0.02 ± 0.02 efg
Chipeta	8	94.59	1.42	0.02 ± 0.02 efg
Ranger Russet	8	71.18	1.06	0.01 ± 0.01 efg
Centennial Russet	8	66.41	0.97	0.01 ± 0.01 efg
Kennebec	8	88.91	1.01	0.01 ± 0.01 efg
Adirondack Blue	7	66.49	0.61	0.01 ± 0.01 efg
Red Lasoda	8	86.93	0.76	0.01 ± 0.01 efg
Frontier Russet	8	57.92	0.51	0.01 ± 0 efg
Spunta	8	78.32	0.32	0 ± 0 fg
Elba	8	98.76	0.35	0 ± 0 fg
Allegany	8	82.72	0.27	0 ± 0 fg
Alturas	8	88	0.27	0 ± 0 fg
Russet Burbank	8	67.24	0.12	0 g
All Blue	8	52.58	0	0 g
Butte	8	60.06	0	0 g
Ac Chaleur	8	88.34	0	0 g
White Rose	8	29.38	0	0 g
Average		72.17	4.76	0.07

Note: The ratio means were calculated directly from individual HS plants against the means value of 8 CK plants. The ratio means followed by the same letter are not significantly different according to the Duncan's multiple range test at $P < 0.05$.

Table 5. Pearson correlation coefficients among different measured and derived traits and statistical significance (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$)

	SPADCK7D	SPADHS7D	SPADR7D	LTWCK	LTWHS	LTWR	PHTCK	PHTHS	PHTR	SPADCK16D	SPADHS16D	SPADR16D	SPADCK32D	SPADHS32d	SPADR32D
SPADCKD	1	0.73 ***	-0.68 ***	0.17	-0.49 ***	-0.49 ***	0.16	-0.26 0.05	-0.33 *	0.75 ***	0.12	-0.58 ***	0.59 ***	0.48 ***	-0.28 *
SPADHS7D	0.73 ***	1	0.00	0.11	-0.29 *	-0.26 0.05	0.05	-0.14	-0.15	0.60 ***	0.41 **	-0.14	0.32 *	0.72 ***	0.14
SPADR7D	-0.68 ***	0.00	1	-0.13	0.45 **	0.46 ***	-0.19	0.24	0.33 *	-0.46 **	0.24	0.70 ***	-0.53 ***	0.07	0.57 ***
LTWCK	0.17	0.11	-0.13	1	0.05	-0.17	0.31 *	0.08	-0.15	0.24	0.02	-0.19	0.33 *	0.08	-0.23
LTWHS	-0.49 ***	-0.29 *	0.45 **	0.05	1	0.95 ***	-0.38 **	0.17	0.55 ***	-0.40 **	-0.01	0.40 **	-0.44 **	-0.06	0.44 **
LTWR	-0.49 ***	-0.26 0.05	0.46 ***	-0.17	0.95 ***	1	-0.44 **	0.12	0.54 ***	-0.42 **	0.00	0.42 **	-0.48 ***	-0.06	0.47 ***
PHTCK	0.16	0.05	-0.19	0.31 *	-0.38 **	-0.44 **	1	0.05	-0.81 ***	0.29 *	-0.05	-0.34 *	0.63 ***	0.01	-0.60 ***
PHTHS	-0.26 0.05	-0.14	0.24	0.08	0.17	0.12	0.05	1	0.44 **	0.02	0.44 **	0.42 **	-0.19	-0.17	0.09
PHTR	-0.33 *	-0.15	0.33 *	-0.15	0.55 ***	0.54 ***	-0.81 ***	0.44 **	1	-0.26 0.05	0.26 0.06	0.52 ***	-0.64 ***	-0.05	0.63 ***
SPADCK16D	0.75 ***	0.60 ***	-0.46 **	0.24	-0.40 **	-0.42 **	0.29 *	0.02	-0.26 0.05	1	0.49 ***	-0.43 **	0.61 ***	0.53 ***	-0.27 *
SPADHS16D	0.12	0.41 **	0.24	0.02	-0.01	0.00	-0.05	0.44 **	0.26 0.06	0.49 ***	1	0.57 ***	-0.02	0.45 **	0.30 *
SPADR16D	-0.58	-0.14	0.70	-0.19	0.40	0.42	-0.34	0.42	0.52	-0.43	0.57	1	-0.61	0.01	0.61

	***		***		**	**	*	**	***	**	***		***		***
SPADCK32D	0.59	0.32	-0.53	0.33	-0.44	-0.48	0.63	-0.19	-0.64	0.61	-0.02	-0.61	1	0.31	-0.75
	***	*	***	*	**	***	***		***	***		***		*	***
SPADHS32D	0.48	0.72	0.07	0.08	-0.06	-0.06	0.01	-0.17	-0.05	0.53	0.45	0.01	0.31	1	0.38
	***	***								***	**		*		**
SPADR32D	-0.28	0.14	0.57	-0.23	0.44	0.47	-0.60	0.09	0.63	-0.27	0.30	0.61	-0.75	0.38	1
	*		***		**	***	***		***	*	*	***	***	**	

Note:
CK: 22°C/18°C control conditions.
HS: 32C/28C heat treatment conditions.
SPADCK7D: The 7th day SPAD values for plants in CK.
SPADHS7D: The 7th day SPAD values for plants in HS.
SPADR7D: HS/CK SPAD ratios for the 7th day measurements.
LTWCK: The largest tuber weight of plants in CK.
LTWHS: The largest tuber weight of plants in HS.
LTWR: The HS/CK ratio of the largest tuber weight
PHTCK: The plant height at harvest for plants in CK.
PHTHS: The plant height at harvest for plants in HS.
PHTR: The plant height HS/CK ratio at harvest.
SPADCK16D: The 16th day SPAD values for plants in CK.
SPADHS16D: The 16th day SPAD values for plants in HS
SPADR16D: HS/CK SPAD ratios for the 16th day measurements
SPADCK32D: The 32nd day SPAD values for plants in CK.
SPADHS32D: The 32nd day SPAD values for plants in HS
SPADR32D: HS/CK SPAD ratios for the 32nd day measurements

FIGURE LEGENDS

Fig. 1. Leaf morphology and size in control (CK) and heat-stressed (HS) treatments. **A.** Plants of the cultivar Yukon Gold four weeks after the start of heat treatment in greenhouse experiment. **B:** CK Plants of the cultivar Shepody at harvest. **C.** HS Plants of the cultivar Shepody at harvest.

Fig. 2. Leaf length and width of plants in growth chambers. **CK:** Means of two plants under non-stress conditions. **HS.** Means of two plants with heat stress treatment. Cultivar: Russet Burbank.

Fig. 3. Correlation among plant height, largest tuber weight and SPAD chlorophyll values. analysis. A. SPADR32D vs PHR; B. SPADR32D vs. LTWP; C. PHR vs. LTWP. SPADR32D: chlorophyll SPAD HS/CK ratio. PHTR: plant height ratio between heat/control conditions. LTWR: largest tuber weight HS/CK ratio.

Fig. 4. Heatmap clustering of potato cultivars using multiple traits. SPADHS7D: chlorophyll SPAD of 35°C/28°C heat stressed plants. SPADCK7D: chlorophyll SPAD values of the plants under 22°C/18°C control conditions. PHTHS: plant height of the plants under 35°C/28°C heat stress. PHTCK: plant height of plants under 22°C/18°C control conditions. LWT35: largest tuber weight of plants under 35°C/28°C heat stress conditions. LTWCK: largest tuber weight of plants under 22C°/18°C control conditions. SPADR7D: chlorophyll SPAD HS/CK ratio. PHTR: plant height ratio between heat/control conditions. LTWR: largest tuber weight HS/CK ratio.

Fig. 5. Ordination of potato cultivars on the first two Principal Components. The cultivars in the encircled group are Eramosa, Chieftain, AC Belmont, Caribe, and Cherry Red (in the order from right to left).

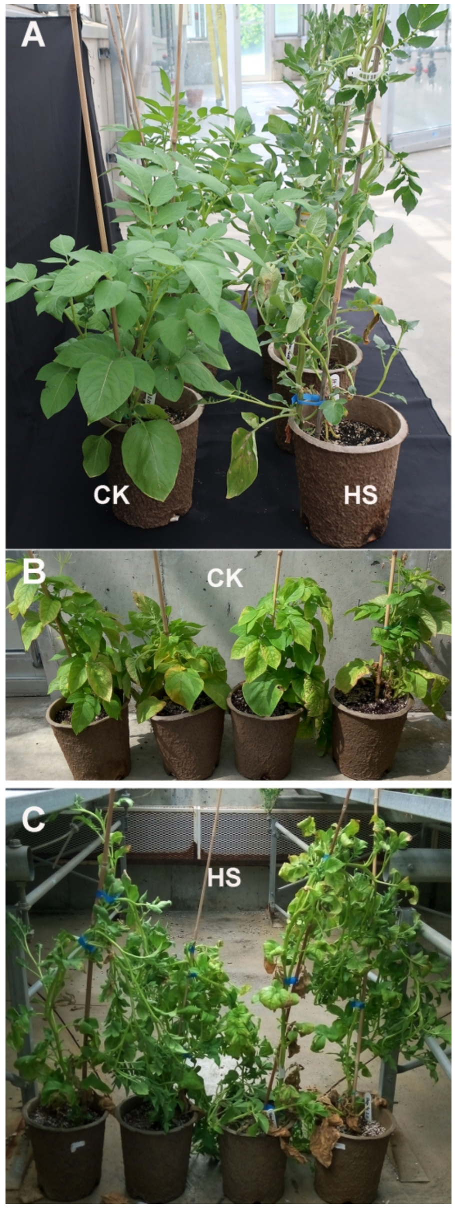


Fig. 1. Leaf morphology and size in control (CK) and heat-stressed (HS) treatments. A. Plants of the cultivar Yukon Gold four weeks after the start of heat treatment in greenhouse experiment. B: CK Plants of the cultivar Shepody at harvest. C. HS Plants of the cultivar Shepody at harvest.

78x209mm (300 x 300 DPI)

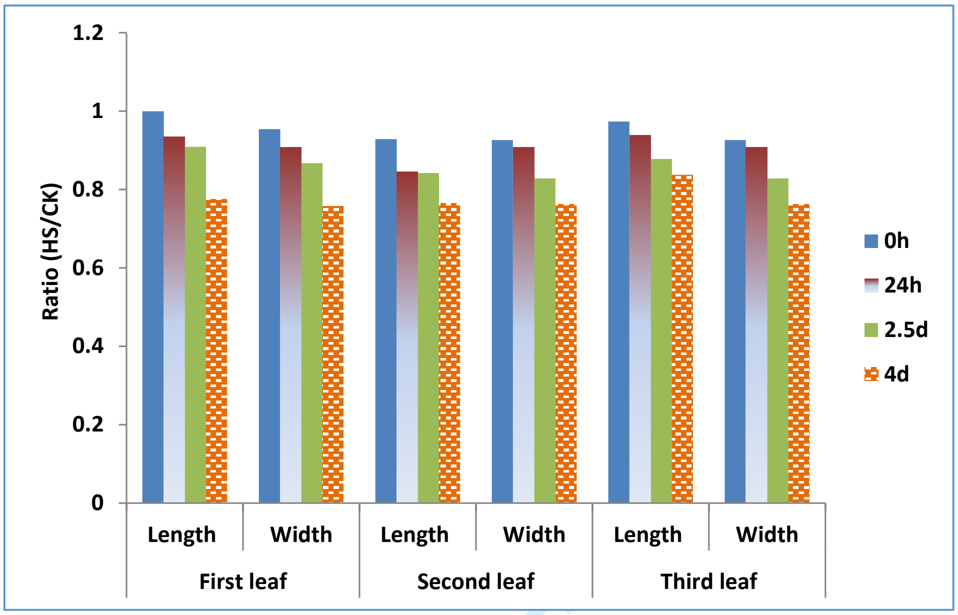
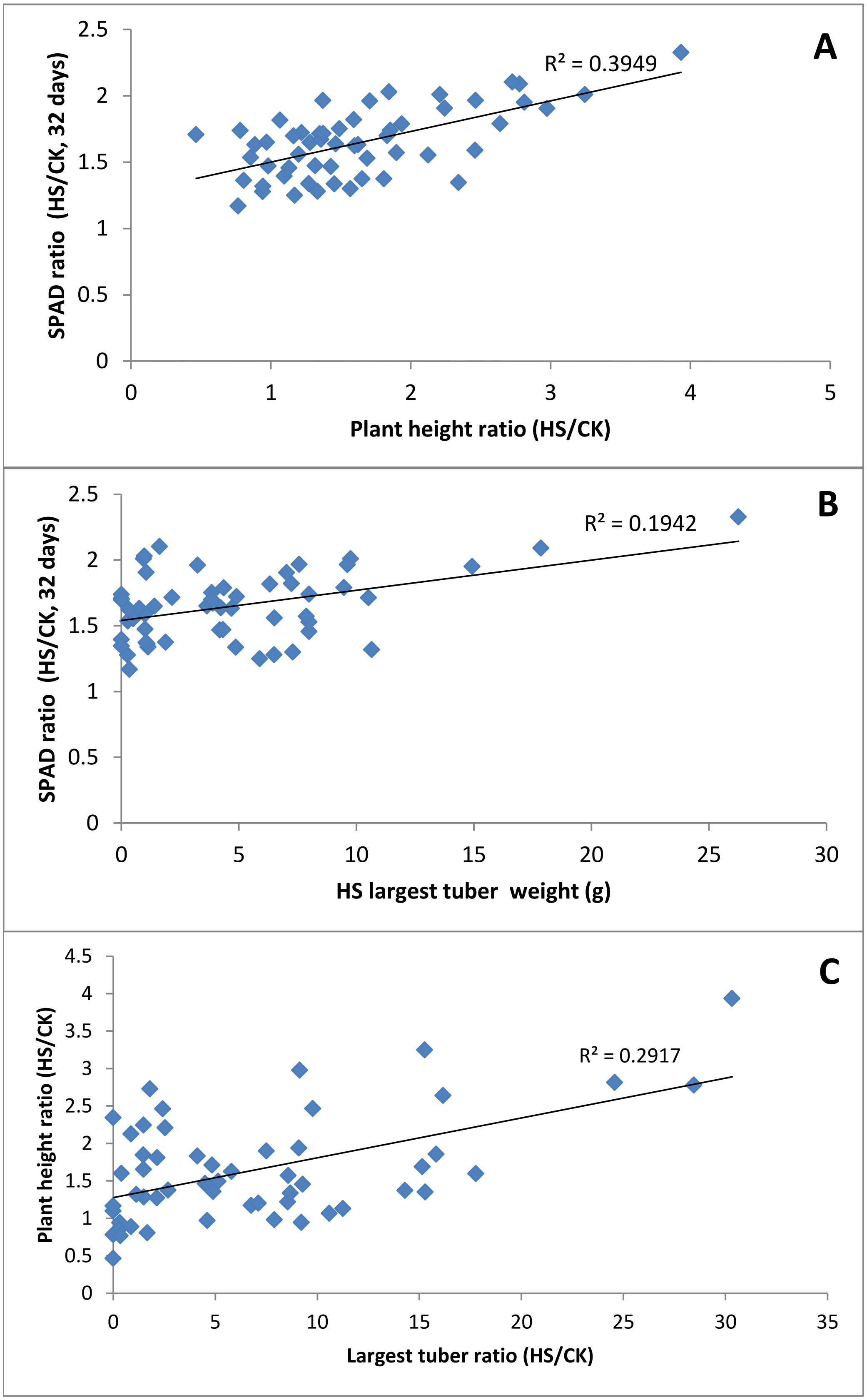
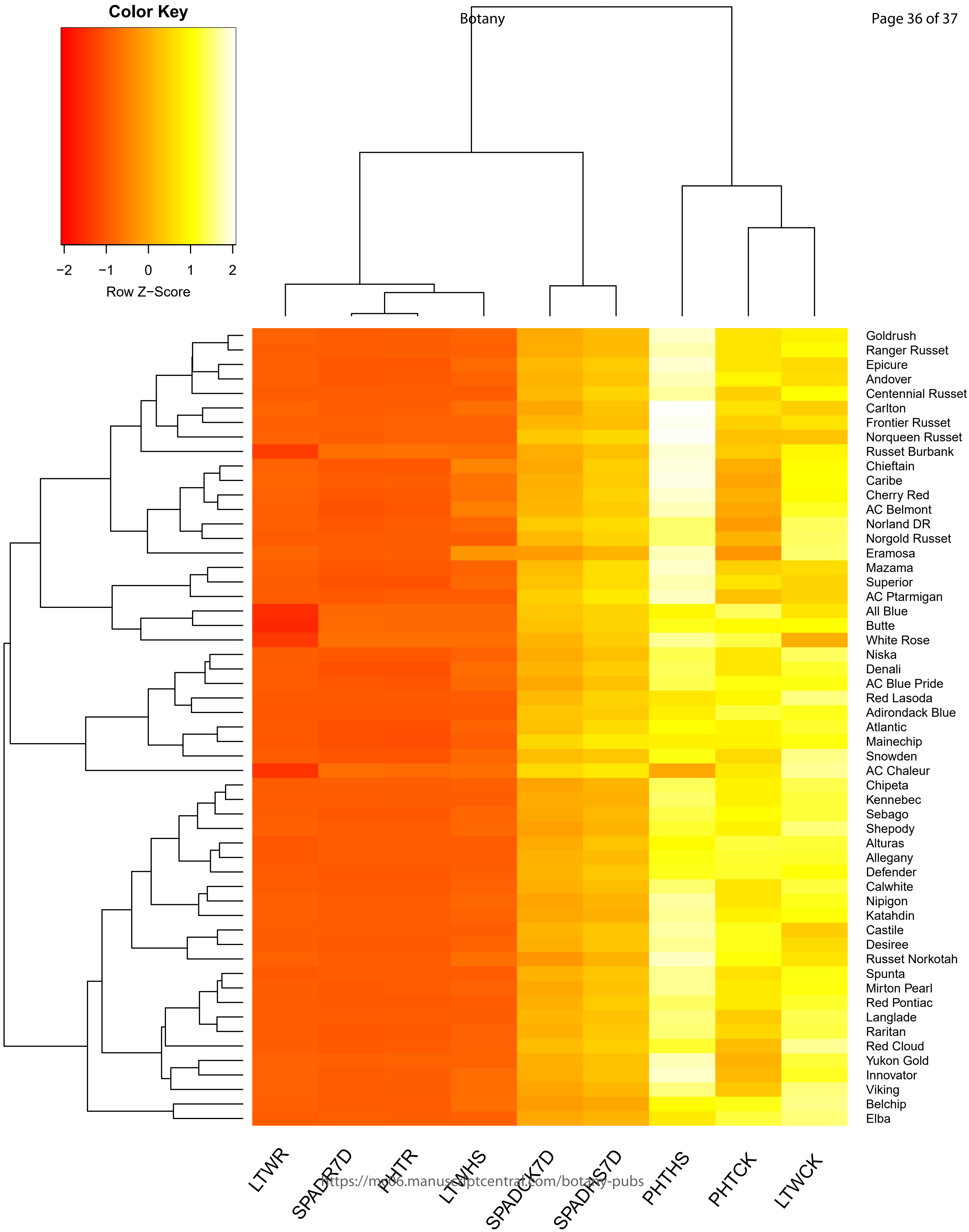
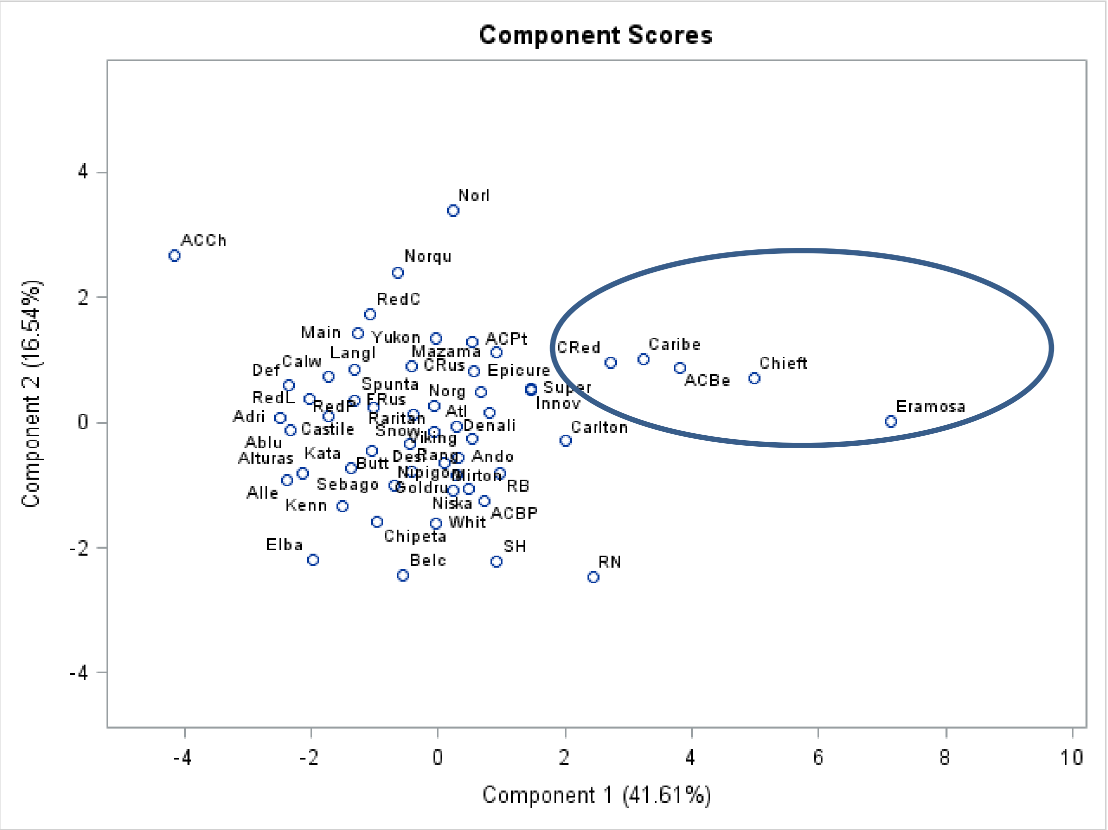

1.2
1
0.8
0.6
0.4
0.2
0
Ratio (HS/CK)
Length
Width
Length
Width
Length
Width
First leaf
Second leaf
Third leaf
0h
24h
2.5d
4d

Figure 2.


A
R² = 0.3949
SPAD ratio (HS/CK, 32 days)
Plant height ratio (HS/CK)
0
0.5
1
1.5
2
2.5
0
1
2
3
4
5
B
R² = 0.1942
SPAD ratio (HS/CK, 32 days)
HS largest tuber weight (g)
0
5
10
15
20
25
30
C
R² = 0.2917
Plant height ratio (HS/CK)
Largest tuber ratio (HS/CK)
3
3.5
4
4.5
35


Color Key
−2
−1
0
1
2
Row Z−Score
Goldrush
Ranger Russet
Epicure
Andover
Centennial Russet
Carlton
Frontier Russet
Norqueen Russet
Russet Burbank
Chieftain
Caribe
Cherry Red
AC Belmont
Norland DR
Norgold Russet
Eramosa
Mazama
Superior
AC Ptarmigan
All Blue
Butte
White Rose
Niska
Denali
AC Blue Pride
Red Lasoda
Adirondack Blue
Atlantic
Mainechip
Snowden
AC Chaleur
Chipeta
Kennebec
Sebago
Shepody
Alturas
Allegany
Defender
Calwhite
Nipigon
Katahdin
Castile
Desiree
Russet Norkotah
Spunta
Mirton Pearl
Red Pontiac
Langlade
Raritan
Red Cloud
Yukon Gold
Innovator
Viking
Belchip
Elba
LTWR
SPADR7D
PHTR
LTWHS
SPADCK7D
SPADHS7D
PHTHS
PHTCK
LTWCK


Component Scores
Norl
ACCh
Norqu
RedC
Main
Yukon
ACPt
Mazama
CRus
Epicure
Langl
Calw
Def
Spunta
Norg
Super
Innov
RedL
FRus
RedP
Adri
Raritan
Atl
Denali
Castile
Snow
Viking
Ablu
Kata
Alturas
Butt
Des
Rang
Ando
Nipigon
Mirton
Sebago
Goldru
RB
Alle
Kenn
Niska
ACBP
Whit
Chipeta
Elba
Belc
SH
RN
CRed
Caribe
ACBe
Chieft
Eramosa
Carlton
4
2
0
-2
-4
-4
-2
0
2
4
6
8
10
Component 2 (16.54%)
Component 1 (41.61%)