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A non-destructive method to predict polyphenol content in strawberry

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Abstract

Fruit total phenolic content (TPC) and total antioxidant capacity (TAC) are usually assessed by destructive wet chemistry. Yet, for precision agriculture and recent breeding program, more rapid and non-destructive methods are needed. A new portable device, Dualex, is used to access the epidermal polyphenol content of leaves in order to select seedlings with good fruit quality. A field experiment was conducted to investigate the potential of Dualex, applied to fruit breeding, on four selected strawberry genotypes ('Kent', 'Jewel', 'Saint-Pierre' and 'SJ8976-1') of known quality. Our results showed that Dualex readings either from the adaxial side (upper side, DUAD), abaxial side (lower side, DUAB), sum of DUAD and DUAB (Phen), soluble solids content (SSC), titratable acidity (TA), TPC and TAC of 'Jewel' and 'Kent' were significantly higher than those of 'SJ8976-1' and 'Saint-Pierre'. There were positive correlations between DUAD, DUAB and Phen vs. SSC, TA, TPC and TAC. The use of Dualex in strawberry breeding programs might be useful to estimate TA and SSC along with TPC and TAC to select for high fruit quality in a seedling population, which consequently reduces the time from crossing to naming and reduce field costs.

Key words: Breeding, Dualex, selection, strawberry, total antioxidant capacity, total phenolic content.

Introduction

Strawberries (*Fragaria* × *ananassa* Duch.) have long been regarded as having considerable health benefits due to their high levels of antioxidant compounds, which provide protection against harmful free radicals, and have been associated with lower incidence and mortality rates from heart diseases and cancers in addition to a number of other health benefits [1-5]. Previous studies have shown that the majority of the total antioxidant capacity (TAC) of strawberries may originate from their polyphenolic compounds and molecules having antioxidant properties which play important roles in several biological processes that sustain plant life and defense against external stresses [6, 7]. Polyphenols have been identified as ellagic acid, ellagic acid glycosides, ellagitannins, gallotannins, anthocyanins, flavonols, flavanols and coumaroyl glycosides [6, 8]. Moreover, the increasing interest in polyphenolic compounds is not only due to the appreciation of their broad pharmacological activity, but also to their effect on shelf life and disease resistance [9-11].

Strawberry breeding is a long cycle costing approximately a total of 10 years to name a variety. Each cycle starts with controlled crosses among selected parents and initial seedling evaluations based on plant vigor, yield, fruit size and important agronomic characteristics, and then approximately 20% of seedlings are retained for further evaluation in single plots. Selections will be tested and screened and a few will be tested in replicated trials in one site before being tested in several locations. Several methods have been used to shorten the breeding period including the use of chlorophyll fluorescence [12, 13] and chemical composition [7, 11] to select lines and further use of new method is an asset to reduce the time from crossing to naming.

Classical methods to measure total phenolic content (TPC) and TAC of strawberries are time consuming and costly. A new portable tool to quantify epidermis polyphenols on the leaf, based on chlorophyll fluorescence, Dualex (contraction of Dual + excitation) (Force-A, Paris, France), has been designed for a non-destructive *in situ* method which also allows for both a higher number of samples and repeated measurements during long-term experiments. Because Dualex emits UV radiations ($\lambda = 375$ nm) that are partly absorbed by the leaf epidermis (due to the presence of polyphenols whose absorbance peaks are in the UV-A and UV-B regions), the amount of light available for chlorophyll excitation is reduced. Meanwhile, a red reference light not absorbed by the epidermis is also emitted ($\lambda = 650$ nm). Epidermal transmittance is therefore calculated by the rate of fluorescence emission in response to chlorophyll excitation F(UV)/F(REF), where F(UV) is fluorescence detected in response to UV excitation, and F(REF) is fluorescence measured in response to excitation induced by the red light [14, 15].

Since Dualex is a new instrument, most reports have focused on its response to nitrogen fertility status in crops and monitoring of phenolic maturity of winegrapes [16-18]. To our knowledge, no work has been done on the relationship between Dualex readings vs. TPC and TAC in strawberries. Therefore, the aim of present work was to (i) evaluate soluble solids content (SSC), titratable acidity (TA), TPC and TAC on the fruits of selected strawberry lines, (ii) determine the changes of the epidermal polyphenols of

leaves using Dualex at three berry maturity stages (green, pink and red) and (iii) explore the correlation of Dualex readings on leaves vs. SSC, TA, TPC and TAC in order to establish the possibility of using Dualex to screen strawberry seedling rich in polyphenols during vegetative growth stage (seedlings). These results might open a new door for screening high nutritional value strawberry genotypes during seedling evaluation which will consequently shorten the period of field evaluation while reducing the time from crossing to naming.

Materials and Methods

Field experimental design: A complete randomized design with five replicates was used to evaluate three strawberry cultivars 'Kent', 'Jewel', 'Saint-Pierre' and one selection 'SJ8976-1', at Agriculture and Agri-Food Canada, L'Acadie Experimental Farm (longitude: 73.35 W; latitude 45.32 N), in L'Acadie, Quebec, Canada, during the growing season of 2009. Forty plants of each genotype were planted in a double row 30 cm x 30 cm apart in a 30 m long plot.

Dualex measurements: Dualex measurements were taken on the lamina of the uppermost fully expanded leaves, avoiding midribs, three times during berry maturity at green, pink and red stages. A minimum of 20 leaves with uniform appearance, in each plot, were selected. Dualex readings were obtained on the adaxial (upper side, named DUAD) and the abaxial (lower side, named DUAB) sides of leaves and values from both sides were summed up in order to estimate the total epidermal polyphenols of leaves (Phen) [19].

Sample preparation and extraction procedures: Thirty fruits at fully red stage were harvested from each plot and then rapidly put in a cooler and brought to the laboratory, where they were immediately cut in halves and frozen in liquid nitrogen. Samples of each genotype were stored at -80°C for further fruit quality analysis including SSC, TA, TPC and TAC.

Ten grams of fresh-frozen fruits of each strawberry genotype were ground in 50 mL of 50% methanol for 2 min at room temperature using a Polytron blender (Brinkmann Instruments, Westbury, NY, USA). The mixture was filtered through filter paper (Whatman No. 1) and then through a 0.45 µm Acrodisc syringe filter (Gelman Sciences, Ann Arbor, MI, USA). The final filtrate was then stored at -20°C before being analyzed. Extracts resulting from this procedure were used for TPC and TAC.

All chemicals were of analytical grade. Gallic acid, ferric chloride, sodium acetate, hydrochloric acid, sodium carbonate, 2,4,6-Tri(2-pyridyl)-1,3,5-triazine and the Folin-Ciocalteu reagent were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Determination of total phenolic content: TPC was determined according to the Folin-Ciocalteu (FC) method [20] with slight modifications. A standard or sample extract (0.02 mL) was mixed with 1.58 mL of water and 0.1 mL of FC reagent in a 10 mL vial. After 3 min, 0.3 mL of Na_2CO_3 (7.5%) was added, and the solution was allowed to stand for 30 min at 40°C. Absorption was measured at 765 nm with a LAMBDA L7110190 UV/visible spectrophotometer (PerkinElmer Ltd, Beaconsfield, BUCKS, United Kingdom). Gallic acid was used as a standard, and TPC was expressed as gallic acid equivalent (GAE) in µg g^{-1} of fresh-frozen weight. Concentrations beyond the highest point (500 µg mL^{-1}) of the linear range of the standard curve were diluted before final analysis.

Determination of total antioxidant capacity: TAC was determined by ferric reducing/antioxidant power (FRAP) according to the method of Benzie and Strain [21]. Briefly, 2.4 mL of freshly prepared FRAP reagent containing 10 mM of TPTZ in 40 mL of HCl, 20 mM of $FeCl_3 \cdot 6H_2O$ and 300 mM of acetate buffer, pH 3.6, in the ratio of 1:1:10 (v:v:v) was mixed with 80 µl of appropriately diluted sample. After 4 min the mixture was measured at 593 nm (LAMBDA L7110190 spectrophotometer, PerkinElmer Ltd, Beaconsfield, BUCKS, United Kingdom). The FRAP value of the samples was calculated on the basis of 500 µM Fe^{2+} ($FeSO_4 \cdot 7H_2O$). TAC was expressed as mg ascorbic acid equivalent (AAE) per gram fresh-frozen weight.

Determination of soluble solids content and titratable acidity: Five randomly selected strawberries were homogenized using a Supreme Juicerator (Acme Juicer Manufacturing Co., New Hartford, CT, USA), and SSC of the juice was determined at 20°C using a refractometer (Abbe Mark II, Reichert-Jung, Depew, NY, USA). TA was measured according to Khanizadeh *et al*. [11] by diluting 1 mL of strawberry juice with 9 mL of distilled water, adjusted to pH 8.1 using 0.1 N of NaOH (Accumet AB15 Basic pH meter, Fisher Scientific).

Statistical analysis: GLM and CORR procedure of SAS (SAS Institute, version 6, Cary, NC, USA) were used to analyze the data. Means were separated using the least significant difference (LSD) tested at the 0.05 level, when the F value was significant. Pearson's correlation coefficients were also calculated on a mean at the 0.05 level.

Results and Discussion

Soluble solids content and titratable acidity: SSC and TA are the most important quality indices of fruits ubiquitously used in standard quality controls. Strawberry flavor is derived from the interactive taste and aromas of many chemical constituents. SSC is mainly organic sugars, such as glucose, sucrose and fructose, which influence the taste, flavor and maturity of strawberries. In current study, the highest SSC was observed in 'Jewel' and 'Kent', followed by 'SJ8976-1' and 'Saint-Pierre' (Table 1). Significant differences were observed between the genotypes for TA. 'Jewel' and 'Kent' had the highest TA while 'SJ8976-1' and 'Saint-Pierre' had the lowest one. Our results showed that SSC and TA varied within genotypes. Although, not all strawberries with high SSC and TA will necessarily be of good quality, the absence of SSC and TA makes good quality unlikely [22, 23].

Total phenolic content and total antioxidant capacity: The phytochemical content of fruits is considered correlated with bioactive health benefits of diabetes mellitus, allergies, cancers, viral infections, headaches, stomach and duodenal ulcers as well as inflammatory diseases [1-5, 24]. The highest TPC and TAC were found in 'Jewel' and 'Kent' while 'SJ8976-1' and 'Saint-Pierre' showed the lowest (Table 1). Several previous studies also have shown that strawberries are a good source of natural antioxidants but vary with genotypes [10, 25]. Thus, screening strawberries rich in polyphenols is highly demanded on the market. Our study demonstrated that 'Jewel' and 'Kent' are good choices to be parents in new strawberry selections.

Table 1. Means of Dualex readings on leaves during three stages of berry maturity (green, pink and red) along with SSC, TA, TPC and TAC on fruits measured at red-berry stage of selected strawberry lines.

	Leaves									Fruits			
	Green-berry Stage			Pink-berry Stage			Red-berry Stage			Red-berry Stage			
	DUAD	DUAB	Phen	DUAD	DUAB	Phen	DUAD	DUAB	Phen	SSC (°Brix)	TA (%)	TPC [a] (μg GAE g^{-1})	TAC [b] (μg AAE g^{-1})
Kent	1.25±0.16	0.39±0.08	1.64±0.23	1.83±0.15	0.71±0.13	2.54±0.28	2.11±0.18	0.93±0.10	3.04±0.16	6.9±0.4	0.81±0.08	925.0±119.3	2006.4±147.3
Jewel	1.29±0.15	0.51±0.10	1.80±0.25	1.90±0.21	0.73±0.13	2.63±0.34	2.13±0.17	0.93±0.15	3.06±0.18	7.2±0.2	0.88±0.08	993.5±58.8	2149.3±159.1
Saint-Pierre	1.00±0.18	0.30±0.13	1.30±0.28	1.45±0.23	0.48±0.16	1.94±0.38	1.75±0.18	0.67±0.17	2.42±0.35	6.4±0.3	0.71±0.09	802.8±106.7	1681.2±97.6
SJ8976-1	1.02±0.16	0.35±0.15	1.38±0.30	1.60±0.11	0.51±0.12	2.11±0.20	1.84±0.14	0.73±0.15	2.57±0.29	6.5±0.3	0.70±0.12	805.7±68.2	1752.8±139.9
$LSD_{0.05}$	0.24	0.18	0.40	0.26	0.19	0.43	0.23	0.19	0.29	0.3	0.07	119.2	215.8

DUAD is Dualex readings from the adaxial side (upper side), DUAB is Dualex readings from the abaxial side and Phen is the sum of DUAD and DUAB.
All values are means of 5 replicates.
$LSD_{0.05}$: Least significant differences at 0.05 level.
[a] TPC expressed as mg gallic acid equivalent (GAE) per gram fresh-frozen weight.
[b] TAC expressed as mg ascorbic acid equivalent (AAE) per gram fresh-frozen weight.

Dualex readings on strawberry leaves: Our results concluded that there were significant changes in Dualex readings (DUAD, DUAB and Phen) on strawberry leaves during berry maturity and among genotypes (Table 1). Basically, the Dualex readings of 'Jewel' were higher, followed by 'Kent', 'SJ8976-1' and 'Saint-Pierre'. It is very interesting to see that the trend in Dualex readings on leaves was almost the same as for SSC, TA, TPC and TAC on fruits, especially at the optimum berry maturity (red stage). Furthermore, DUAD was higher than DUAB which is in agreement with others [14, 17, 26]. This might be due to the different optical properties of the adaxial and abaxial sides of leaves in respect to solar illumination and the strong relationship between the polyphenol accumulation and light intensity. Moreover, the Dualex readings on strawberry leaves were increasing at the three berry maturity stages (green, pink and red). This might be related to the maturity and/or senescence of the whole plant. Lenk *et al*. [27] presented similar results when significantly high flavonol concentrations and effective *in vivo* UV screening were detected in most exposed half-berries with berry ripening but not in non-exposed samples; they concluded that radiation-exposure conditions determine flavonol synthesis and berries need to achieve an advanced stage of ripeness before responding to radiation-exposure by synthesizing large amounts of UV-protecting flavonols.

Relationships between Dualex readings and SSC, TA, TPC and TAC: Significant positive correlations were found between Dualex readings on leaves vs. SSC, TA, TPC and TAC on fruits of selected strawberry genotypes at the pink and red-berry stages (Table 2). Higher correlations were observed at the red-berry stage with exception for TAC. Results indicated that there is a relationship between the polyphenols of leaf epidermis measured by Dualex and the classic fruit quality tests. Therefore, it is possible to use Dualex to select strawberry lines of good quality during vegetative growth, especially in a strawberry breeding program during seedling evaluation.

Since DUAD and DUAB are highly correlated, it would be possible to use measurements from one side of the leaf for estimation of chemical composition as suggested earlier by Tremblay *et al*. [17] and Cartelat *et al*. [19].

Conclusions

Development of new strawberry lines with specific levels of TAC stimulates greater interest in the nutraceutical and functional food industry. Based on our results, a positive correlation between leaf Dualex readings (vegetative growth) and fruit quality is observed. It appears that leaf epidermis Dualex readings can be used to predict fruit quality during seedling evaluations to accelerate the selection process and reduce overall costs associated with seedling field evaluation and worth being investigated further as a practical way of selecting seedling in a breeding program.

Table 2. Pearson's correlation coefficients (top right corner) and statistical significances (lower left corner) between Dualex readings during three stages of berry maturity (green, pink and red), SSC, TA, TPC and TAC on fruits at red-berry stage.

			Leaves									Fruits			
			Green-berry Stage			Pink-berry Stage			Red-berry Stage			Red-berry Stage			
			DUAD	DUAB	Phen	DUAD	DUAB	Phen	DUAD	DUAB	Phen	SSC	TA	TPC	TAC
Leaves	Green-	DUAD		0.84	0.97	0.77	0.76	0.78	0.79	0.56	0.76	0.30	0.64	0.44	0.58
	berry	DUAB	***		0.94	0.69	0.71	0.72	0.71	0.48	0.67	0.17	0.55	0.56	0.54
	Stage	Phen	***	***		0.77	0.77	0.79	0.79	0.55	0.75	0.26	0.63	0.51	0.59
	Pink-	DUAD	***	***	***		0.91	0.99	0.95	0.57	0.86	0.53	0.70	0.60	0.71
	berry	DUAB	***	***	***	***		0.97	0.94	0.61	0.88	0.48	0.69	0.57	0.64
	Stage	Phen	***	***	***	***	***		0.97	0.60	0.89	0.52	0.71	0.60	0.69
	Red-	DUAD	***	***	***	***	***	***		0.64	0.93	0.52	0.75	0.63	0.65
	berry	DUAB	*	*	*	**	**	**	**		0.88	0.61	0.74	0.46	0.47
	Stage	Phen	***	**	***	***	***	***	***	***		0.62	0.82	0.61	0.63
Fruits	Red-	SSC	NS	NS	NS	*	*	*	*	**	**		0.72	0.59	0.56
	berry	TA	**	*	**	***	***	***	***	***	***	***		0.54	0.58
	Stage	TPC	*	*	*	**	**	**	**	*	**	**	*		0.67
		TAC	**	*	**	***	**	***	**	*	**	**	**	**	

DUAD is Dualex readings from the adaxial side (upper side), DUAB is Dualex readings from the abaxial side and Phen is the sum of DUAD and DUAB.
*, **, *** indicate significant difference at 0.05, 0.01 and 0.001, respectively, and NS indicates no significant difference.

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