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Screening of Snap Bean (*Phaseolus vulgaris* L.) Genotypes for Phenolic Bioactive-Linked Anti-Hyperglycemic Properties Using *In Vitro* Models

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ABSTRACT

Phenolic bioactives from plant-based foods have significant antioxidative and associated medicinal and therapeutic properties and can be targeted against oxidative stress-linked chronic diseases such as type 2 diabetes (T2D). Legumes with phenolic antioxidant-linked human health function has both agro-ecological and public health benefits. Since phenolic bioactives vary among diverse genotypes, the major aim of this study was to screen and investigate the phenolic antioxidant-linked anti-hyperglycemic functionality of snap bean (*Phaseolus vulgaris*) genotypes as dietary and potential therapeutic target against early stages of T2D. The aqueous extracts of 5 genotypes of commonly consumed green snap bean pods grown separately under field and greenhouse conditions were investigated for total soluble phenolic content (TSP), phenolic acid profile, total antioxidant (TA) activity, α-amylase, and α-glucosidase enzyme inhibitory activities using *in vitro* assay models. Among genotypes, OR 5630 had moderate TSP content, moderate to high TA activity, and high α-amylase enzyme inhibitory activity, while moderate α-glucosidase enzyme inhibitory activity was observed in Ebro and Eagle genotypes both in the field and greenhouse grown pods. Therefore, the genotypes with moderate phenolic antioxidant-linked anti- hyperglycemic properties can be further investigated *in vivo* studies with animal models to integrate with healthy dietary intervention strategies or for potential therapeutic and medicinal applications against early stages of T2D and its associated complications.

INTRODUCTION

Diverse biological functions of secondary metabolites, such as phenolic bioactives in food crops provide multiple opportunities to utilize them for medicinally relevant human health applications including in dietary and potential

therapeutic strategies against non-communicable chronic diseases (NCDs). Although human cells cannot produce phenolic antioxidants themselves, dietary consumption of these phenolic bioactives or targeting them for therapeutic strategies from plant-based sources has diverse health benefits, especially against chronic oxidative stress-linked NCDs, such as T2D (Kahn et al., 2014; Pandey and Rizvi, 2009). Phenolic antioxidants of plant-based foods can either directly offset cellular damages caused by reactive oxygen species (ROS) (Rice-Evans et al., 1997) or can induce endogenous antioxidant enzyme responses when consumed as a part of the diet or targeted in therapeutic and medicinal applications (Shetty and Wahlqvist, 2004). Animal cells including human cells typically respond to these phenolic bioactives mainly through direct interactions with receptors or enzymes involved in metabolic processes of digestion, signal transduction, or through modifying gene expressions which may result from modification of the redox status of the cell induced from a series of redox- dependent reactions (Shalaby and Horwitz, 2015; Tsao and Rong, 2010). Therefore, when consumed or targeted in therapeutic and medicinally relevant strategies, phenolic bioactives can help in maintaining redox homeostasis by countering ROS-induced chronic oxidative stresses, which are commonly associated with hyperglycemia linked to T2D (Rochette et al., 2014). Beyond such specific bioactive role as a redox regulator, there are strong evidences of other important protective health relevant benefits of phenolic antioxidants of food crops modulating and inhibiting key disease pathways (Sarkar and Shetty, 2014; Shetty and Wahlqvist, 2004).

Numerous such protective functions of phenolic bioactives are associated with cardio-protection, anti-cancer, anti-aging, neuro-protection, and anti-diabetes benefits (Fernandez-Panchon et al., 2008; Pandey and Rizvi, 2009; Shahidi and Ambigaipalan, 2015). Phenolic bioactives and their metabolites also influence digestion, absorption, and metabolism of dietary carbohydrates (such as starch and sucrose), which has direct implications for T2D management (Hanhineva et al., 2010; McCue and Shetty, 2004). Specifically, phenolic bioactives from cereal grains, legumes, vegetables, and fruits potentially influence glucose metabolism in part by inhibiting α-amylase, and α-glucosidase enzyme activities, which are key digestive enzymes responsible for carbohydrate digestion and glucose absorption in the small intestine (Hanhineva et al., 2010; McCue and Shetty, 2004). Inhibition of these digestive enzymes could reduce the rate of glucose release and absorption in the small intestine and subsequently suppress postprandial hyperglycemia and therefore, dietary phenolics are excellent diet-based therapeutic targets to manage early stages of T2D (Hanhineva et al., 2010; Sarkar and Shetty, 2014). Previous *in vitro* studies have reported that phenolic bioactives of food crops, including legumes posses significant α-amylase and α-glucosidase enzyme inhibitory activities and can be targeted in dietary and therapeutic strategies to manage early stages of T2D (Hanhineva et al., 2010, Kwon et al., 2007; Ranilla et al., 2009; Singhal et al., 2014). Apart from this specific digestive role in improving glucose metabolism, legumes are also considered as an important antidote for preventing and managing T2D-linked cardiovascular diseases (Jukanti et al., 2017).

Most edible legumes also have a higher amount of resistant starch and amylose in the seeds which are resistant to rapid digestion in the small intestine and ultimately resulting in lower availability of glucose (Jukanti et al., 2017). The lower bioavailability coupled with slower entry of glucose in the bloodstream reduce the demand for insulin and thus lower the glycemic index and insulinemic postprandial response (Jukanti et al., 2017). In addition, legumes including common green beans and peas are also high in soluble and insoluble fiber, low in sodium and fat, and are an excellent source of complex carbohydrates, B vitamins,

folate, and minerals such as calcium, iron, magnesium and potassium (Roy et al., 2010). Fresh green beans including snap beans contain quercetin and kaempferol classes of phenolics and fresh pods have more complex phenolic profile compared to seeds (Escarpa and González, 2000). Higher anti- hyperglycemic properties and improvement in collagen accumulation was observed in *in vivo* animal model studies with green bean pod extracts (Pari and Venkateswaran, 2003; Roman-Ramos et al., 1995). Howvever, such human health relevant phenolic bioactive profiles of common snap beans vary significantly with cultivation and growing conditions, genotypes, and with other phenotypic traits (Kleintop et al., 2016). Although higher consumption of easy to use fresh snap bean has been encouraged due to diverse potential human health benefits, however a gap exists in biochemical rationale-based evidences for phenolic antioxidant-linked anti-hyperglycemic properties of specific snap bean genotypes and based on their different growing conditions that can be targeted for medicinally relevant health benefits. Therefore, the major aim of this study was to develop a strategy to screen different snap bean genotypes for their phenolic antioxidant-linked antihyperglycemic properties using rapid *in vitro* assay models and comparing between field and greenhouse grown snap bean pods as a biochemical rationale-based foundation for further long-term dietary and therapeutic applications as well as basis for further crop breeding strategies.

MATERIALS AND METHODS

Materials. In this study, green pods of 5 different genotypes of snap beans (*Phaseolus vulgaris* L.) (Bogota, Eagle, Ebro, Oregon 5630, and Tendergreen) were evaluated. Selection of these snap bean genotypes was based on their genetic diversity and common use of these genotypes in the market. Green pods of these snap bean genotypes were obtained from the Oregon State University, Corvallis, USA, in 2014 (from Bean CAP Snap Bean Diversity Panel) and frozen snap bean samples were analyzed at North Dakota State University, Fargo, ND, USA. All 5 snap bean genotypes evaluated in this study were planted and grown (crop year 2014) in field plots at the Oregon State University Vegetable Research Farm, Corvallis, Oregon. The soil type of the vegetable research farm is Chehails silty clay loam soil and located at latitude N44.571209, longitude W123.243261 at 77 m.a.s.l. The site is continuously planted with snap bean over the years and randomized complete block design was used. All standard field practices were used to plant, grow, and harvest snap beans (Kleintop et al., 2016). After harvest snap beans pods were immediately frozen and transported to North Dakota State University for biochemical analyses.

In a separate experiment, snap been seeds collected from Oregon State University, Corvallis, USA were grown in the greenhouse (AES Research Complex Greenhouse, North Dakota State University, Fargo, ND, USA) in 2015. Snap bean plants were grown in 15 cm diameter pots using professional grade potting mix. The greenhouse temperature was kept constant between 25 and 26°C. A photoperiod of 14 h light and 10 h dark was used, and beneficial nematodes (weekly application) were used to minimize thrips and other insect infestation in the greenhouse. Each genotype was planted in eight pots and used as biological replicates. After harvest, pods were transferred to the laboratory and were frozen immediately before extraction. Prior to cold-water extraction, all pods were washed with distilled water and excess water was soaked and blotted out using paper towel. The chemical reagents used for biochemical analysis were porcine pancreatic α-amylase, starch, equivalent of intestinal α-glucosidase from yeast (*Saccharomyces cerevisiae*), p-nitrophenyl-α-D-glucopyranoside (*pNPG*) and $ABTS^+$ radical cation-decolorization agent. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Sample Extraction. Pod samples were cut into small pieces and weighed. Green snap bean pods of each genotype were extracted separately using distilled water at a ratio of 20 g of the sample (pod) per 50 mL of distilled water. All samples were homogenized for 10 minutes using a Waring blender. The samples were later centrifuged at 10,015 g for two times with 20 min each. The supernatant was collected and stored at 2°C in refrigerator during the study period. Only aqueous extracts were targeted, as these are the most relevant for physiological and metabolic function in terms of digestion and anti-hyperglycemic functional properties.

Total Soluble Phenolic Content. Total soluble phenolic content of snap bean aqueous extract was determined by a method described by Shetty et al. (Shetty et al., 1995). In brief, 0.5 mL of the sample was transferred to a test tube and mixed with 0.5 mL of water, 1 mL of 95% ethanol and 5 mL of distilled water. To each sample, 0.5 mL of 50% (v / v) Folin-Ciocalteau reagent was added and mixed. After 5 min, 1 mL of 5% Na_2CO_3 was added and mixed and then kept in the dark for 60 min incubation. After 60 min incubation the absorbance of the sample was read at 725 nm using UV/VIS spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The absorbance values were converted into total soluble phenolic content and expressed in micrograms per gram of fresh weight (FW). Standard curves were established using various concentrations of gallic acid in 95% ethanol.

Antioxidant Activity by ABTS [2, 2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)]Free Radical Scavenging Assay. Total antioxidant activity of aqueous extract of snap bean pods was determined by the ABTS + free radical cation scavenging assay (Re et al., 1999). ABTS (Sigma Chemical Co. St. Louis, MO) reagent was dissolved in water to a concentration of 7 mM. Then ABTS stock solution was prepared using 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulfate. The mixture was allowed to stand in the dark at room temperature for 12-16 h prior to use in the assay. Before being added to the sample, the stock solution was diluted with ethanol (95%) in the ratio 1:88 to obtain an absorbance of 0.70 ± 0.02 at 734 nm and was equilibrated at 30°C. One milliliter of the ABTS solution was then added to glass tubes containing 50 µL of sample extract and then mixed using vortex for 30 sec. After 2.5 min of incubation, the absorbance of the mixture was read at 734 nm using UV/VIS spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The readings were compared with controls, which contained 50 µL of 95% ethanol instead of aqueous extract of snap bean green pods. The Trolox reference standard for relative antioxidant activities was prepared with 5 mM stock solution of Trolox in ethanol. Percent inhibition was calculated by:

$$ABTS\ Inhibition\ (\%) = \frac{(Abs\ control - Abs\ sample)}{Abs\ control} \times 100$$

α-Amylase Inhibitory Activity Assay. Porcine pancreatic α-amylase was purchased from Sigma Chemical Co (St. Louis, MO, USA). A volume of 500 µL of supernatant and 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α-amylase enzyme solution (0.5 $mg.mL^{-1}$) was incubated at 25ºC for 10 min. After pre-incubation, 500 µL of a 1% starch solution in sodium phosphate buffer 0.02 M (pH 6.9 with 0.006 M NaCl) was added to each test tube at timed intervals (Kwon et al., 2007). The reaction mixtures were then incubated at 25ºC for 10 min. The reaction was stopped by adding 1.0 mL of 3, 5-dinitro salicylic acid (DNS) color reagent. The test tubes were then incubated in a water bath with boiling water (90-100 ºC) for 10 min and later cooled at room temperature. The reaction mixture was then diluted upon addition of 10 mL of distilled water and the absorbance of the sample was measured at 540 nm using UV/VIS spectrophotometer (Thermo Fisher

Scientific, Waltham, MA, USA). Percent inhibition was calculated as follows:

$$\alpha - Amylase\ Inhibition\ (\%) = (\frac{Abs\ control - (Abs\ sample - Abs\ Sample\ Blank)}{Abs\ Control} \times 100$$

α-Glucosidase Enzyme Inhibitory Activity Assay. α-Glucosidase assay was performed using 50 µL of the supernatant and 100 µL of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1.0 $U.mL^{-1}$) and incubated in 96-well plates at 25°C for 10 min (Kwon et al., 2007). After pre-incubation, 50 µL of a solution of 5 mM p-nitrophenyl-α-D-glucopyranoside in 0.1 M phosphate buffer (pH 6.9) was added to each well at time intervals. The reaction mixtures were then incubated at 25°C for 5 min. Before and after incubation, the absorbance readings were recorded at 405 nm by a microplate reader (Thermomax, Molecular Device Co., Virginia, USA) and compared with a control, which contained 50 µL of buffer instead of aqueous extract of pods. The inhibitory activity of α- glucosidase was expressed as % inhibition and calculated as follows:

$$\alpha - Glucosidase\ Inhibition\ (\%) = \frac{(\Delta\ Abs\ control - \Delta\ Abs\ sample)}{\Delta\ Abs\ control} \times 100$$

Phenolic Acid Profile Determination Using High Performance Liquid Chromatography (HPLC). Green pod sample extracts of snap beans were micro- centrifuged for 10 min at 13,000 rpm, from which a small volume (5 µL) of the sample was subjected to chromatographic analysis using reverse phase HPLC (Agilent 1260 Infinity Series equipped with DAD 1100 diode array detector; Agilent Technologies, Palo Alto, CA). A gradient elution method, involving 10 mM phosphoric acid (pH 2.5; Solvent A) and 100% methanol (Solvent B), was used. Sample extracts were eluted on a C-18 analytical column (Agilent Supelco SB-C18 250 × 4.6 mm internal diameter) with a packing material particle size of 5 µm, at a flow rate of 0.7 mL/min at ambient temperature, with a total run time of 25 min. Pure standards of gallic acid, catechin, chlorogenic acid, ferullic acid, quercetin, p-coumaric acid, and kaemferol in 100% methanol were used to calibrate retention times on the standard curve. The chromatograms so obtained were analyzed using the Agilent Chemstation integration software.

Statistical Analysis. The entire laboratory based biochemical (*in vitro*) and bioactive analysis was repeated four times from same snap bean sample obtained from Oregon State University from crop year 2014 and also from the greenhouse experiment at North Dakota State University in 2015. The analysis of each experiment were performed in triplicates. The means and standard deviations were calculated from the replicates of the experiments and analysis were performed using Microsoft Excel XP. Data were subjected to analysis of variance (ANOVA) to determine the significant differences between treatments. Significant differences were analyzed using Tukey test ($P<0.05$) by the R program (www.r-project.org).

RESULTS

Total Soluble Phenolic (TSP) Content and Total Antioxidant (TA) Activity. Total soluble phenolic (TSP) content and total antioxidant (TA) activity of snap bean genotypes from field and greenhouse were determined. Statistically significant variations in TSP content and TA activity was observed between snap bean genotypes ($p<0.05$) (Figure 1 A, B & 2 A, B).

The TSP content of aqueous extracts of snap bean pods ranged from 197.9 to 330.5 (µgGAE. g^{-1} FW) in field grown pods (Figure 1A) and 127.8 to 322.4 (µgGAE. g^{-1} FW) in greenhouse grown pods. In the field grown pods, highest TSP content was found in Tendergreen followed by OR 5630 (Figure 1A). Interestingly, when grown under greenhouse condition

Tendergreen had lowest TSP content, while OR 5630 had higher TSP content (Figure 1B). The variation of TSP content in Tendergreen might be due to the different growing condition (between field and greenhouse) or due to some other environmental factors.

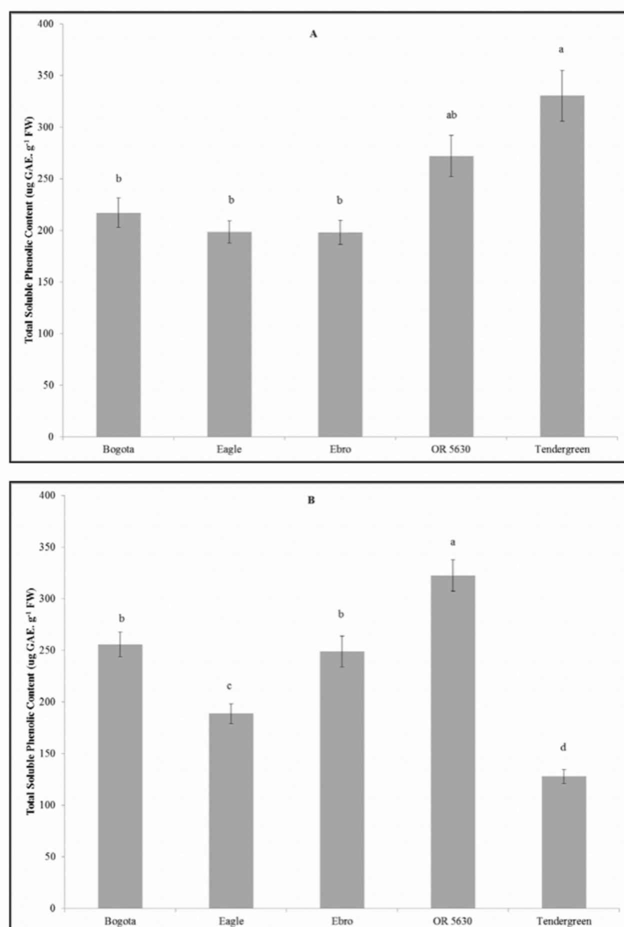

Figure 1. Total soluble phenolic (TSP) content (µg GAE. g^{-1} FW) of aqueous pod extracts of 5 different snap bean genotypes grown under field (A) and greenhouse (B) conditions. Different lowercase letters represent significant differences in TSP content among snap bean genotypes at $p<0.05$ probability level.

The TA activity of 5 snap bean genotypes varied significantly (p<0.05) and ranged from 32.6 to 79.3% (ABTS % inhibition) in pods grown under field condition (Figure 2A) while 43.0 to 89.6 % (ABTS % inhibition) in snap beans grown under greenhouse condition (Figure 2B). Similar to the TSP content, Tendergreen snap bean genotype had significantly higher (p<0.05) TA activity followed by Ebro, OR 5630, Eagle, and Bogota from field experiment (Figure 2A). In the greenhouse experiment higher TA activity was observed in Ebro snap bean genotype followed by OR 5630, Bogota, and Eagle (Figure 2B). Like TSP content, lower TA activity was observed in Tendergreen snap bean genotype grown under greenhouse conditions (Figure 2B).

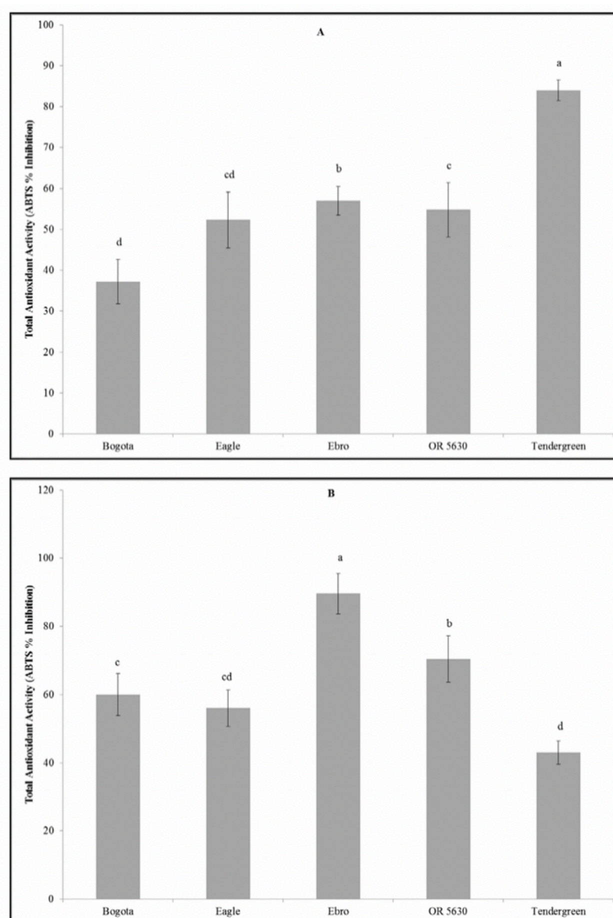

Figure 2. Total antioxidant (TA) activity (ABTS % inhibition) of aqueous pod extracts of 5 different snap bean genotypes grown under field (A) and greenhouse (B) conditions. Different lowercase letters represent significant differences in TA activity among snap bean genotypes at $p<0.05$ probability level.

α-Amylase and α-Glucosidase Inhibitory Activity. In this study, significant differences in α-amylase inhibitory activity was observed among 5 snap bean genotypes (p<0.05) (Figure 3A, B). Alpha-amylase enzyme inhibitory activity of aqueous pod extracts of snap bean genotypes grown under field condition was ranged from 43.9 to 83.6 (% inhibition) (Figure 3A), while it was ranged between 26 to 80 (% inhibition) in snap bean

pods grown under greenhouse condition (Figure 3B). Among snap bean genotypes OR 5630 and Bogota had higher α- amylase enzyme inhibitory activity when compared to Eagle and Ebro. Similar to TSP content and TA activity, α-amylase inhibitory activity of Tendergreen snap bean genotype varied significantly between field and greenhouse experiments.

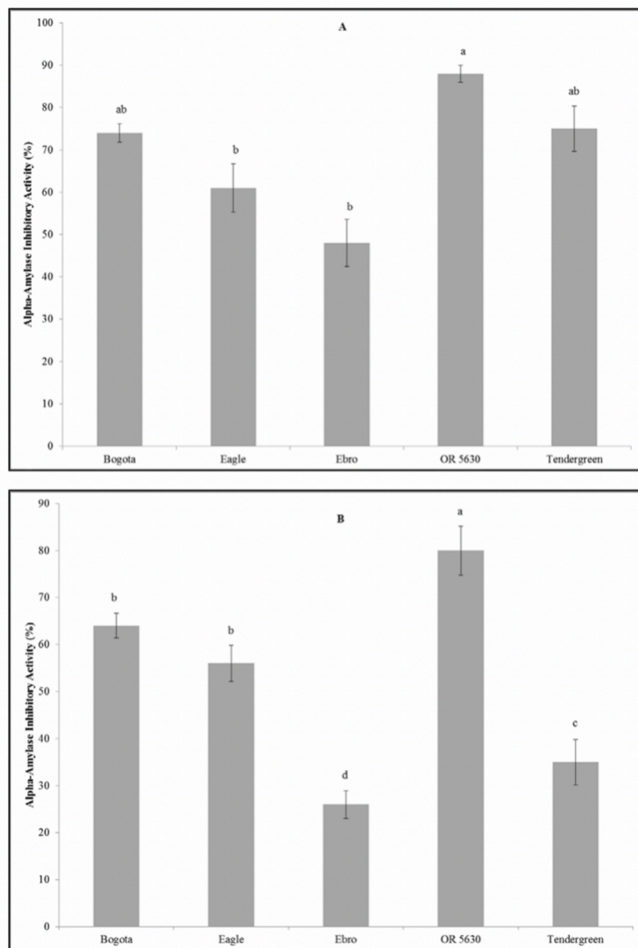

Figure 3. α-Amylase enzyme inhibitory activity (% inhibition) of aqueous pod extracts of 5 different snap bean genotypes grown under field (A) and greenhouse (B) conditions. Different lowercase letters represent significant differences in α- amylase inhibitory activity among snap bean genotypes at $p<0.05$ probability level.

Inhibitory potentials of snap bean genotypes against another key digestive enzyme, α-glucosidase was also evaluated using *in vitro* assay model. Significant dose dependent response (undiluted, half-diluted, and one-fifth diluted) in α- glucosidase inhibitory activity was observed in all 5 snap bean genotypes in both field and greenhouse grown pods (Table 1). In undiluted sample, α-glucosidase enzyme inhibitory activity of snap bean genotypes from field experiment ranged from 24.9 to 50.5 (% inhibition), while it ranged between 26.4 to 52.6 (% inhibition) in greenhouse grown pods. Among snap bean genotypes Eagle and Ebro had higher α-glucosidase enzyme inhibitory activity at all doses when compared with other 3 snap bean genotypes and under both greenhouse and field conditions (Table 1). Overall low to moderate α-glucosidase enzyme inhibitory activity was observed in this study.

Table 1. Dose dependent response in α-glucosidase enzyme inhibitory activity (% inhibition) of aqueous green pod extracts of 5 snap bean genotypes from field and greenhouse.

	α-glucosidase (%) inhibition					
Genotypes	Undiluted (0.4 mg/mL)		½ Diluted (0.2 mg/mL)		1/5 Diluted (0.08 mg/mL)	
	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse
Bogota	24.8 b*	26.4 c	14.1 b	15.2 b	8.3 a	12.1 a
Eagle	47.9 a	52.6 a	33.5 a	26.1 a	21.6 a	18.6 a
Ebro	50.5 a	52.4 a	27.8 ab	28.3 a	21.5 a	17.4 a
OR 5630	38.4 ab	38.6 b	27.5 ab	25.4 a	14.5 a	13.2 a
Tendergreen	34.4 ab	28.9 c	20.4 ab	16.3 b	13.5 a	9.8 a

*Different lowercase letters (vertically) in different columns represent significant differences between different snap bean genotypes under each dilution at $P<0.05$ probability level.

Phenolic Acids Profile. Individual phenolic acids of 5 snap bean genotypes were determined using HPLC method. Major phenolic acids detected in this study were quercetin, catechin, and gallic acid (Table 2). Previous studies have detected quercetin, kaempferol, rutin, catechin and epicatechin as major phenolic acids in green pods of green bean cultivars (Abu-Reidah et al. 2013; Escarpa and González, 2000). Use of different solvent, extraction types, and HPLC protocol used in this study may have contributed for detecting only 3 phenolic acids in snap bean genotypes. Similar to TSP content higher concentrations of individual phenolic acid, especially quercetin was also observed in Tendergreen snap bean

genotype grown under field condition when compared to others (Table 2).

DISCUSSION

Since secondary metabolites, such as phenolic bioactives of food crops have relevance in cellular redox-regulation it can also translate into therapeutic and medicinal properties and human health benefits for managing oxidation breakdown-linked NCDs, such as T2D. Therefore, targeting food crops with higher phenolic antioxidant profile for therapeutic applications against oxidative-stress linked T2D has sound rationale. However, phenolic bioactive profiles and associated human health benefits of food crops including snap beans targeted in this study vary significantly among genotypes, different growing conditions (environment), and between genotype × phenotype × environment interactions (Kleintop et al., 2016).

Table 2. Major phenolic acid content identified by high performance liquid chromatography (HPLC) of 5 snap bean genotypes grown in the field and greenhouse condition.

	Phenolic Acids ($\mu g.g^{-1}$ FW)					
Genotypes	Gallic Acid		Catechin		Quercetin	
	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse
Bogota	7.8* a	8.9 a	13.2 a	16.2 a	45.67 b	40.6 b
Eagle	8.3 a	9.2 a	11.2 a	14.4 a	19.24 c	32.4 bc
Ebro	7.8 a	10.1 a	9.8 a	18.6 a	20.62 c	28.6 c
OR 5630	8.2 a	12.4 a	10.2 a	13.2 a	42.42 b	52.1 a
Tendergreen	8.5 a	6.8 a	15.2 a	12.4 a	55.56 a	32.6 bc

*Different lowercase letters (vertically) in different columns represent significant differences in individual phenolic acid concentration between different snap bean genotypes at $P<0.05$ probability level.

Therefore, rapid *in vitro* screening of different genotypes of snap beans for human health relevant phenolic antioxidant profile and associated functionalities targeting chronic diseases has significant merit for further development of superior varieties and wider use for medicinal and therapeutic applications. Based on this context and biochemical rationale, 5 different snap bean genotypes both from field (one year) and greenhouse experiments were evaluated and screened for phenolic antioxidant-linked anti- hyperglycemic functionality targeting early stages of T2D using rapid *in vitro* assay models. This provides the biochemical-based rationale and foundation for further development of targeted varieties for wider health targeted applications.

Overall, moderate level (127.8 to 330 μgGAE. g^{-1} FW) of TSP content was found in all 5 snap bean genotypes targeted in this study and grown under field and greenhouse conditions, which has relevance for diverse human health benefits of phenolic bioactives including their antioxidant-linked anti-hyperglycemic functionality that was evaluated subsequently in this study. Baardseth et al. (Baardseth et al., 2010) previously reported similar range of TSP content in other green bean genotypes (221 μgGAE. g^{-1} FW). Although Kleintop et al. (2016) also reported significantly higher TSP content (1.01 mgGAE. g^{-1} FW) in Tendergreen, the level of TSP content of OR 5630 reported by the same study was similar to the finding in this study. The higher level of TSP content in Tendergreen in earlier study might be due to the use of different solvents (acetone based extract), and extraction protocols, or due to the differences in crop year (crop year 2010), as growing conditions and environment could have significantly contributed to the higher level of TSP in Tendergreen (Kleintop et al., 2016). The level of TA activity of snap bean genotypes observed in this study was significantly higher than what another study reported previously (Gomes et al., 2009). Overall, moderate TSP content and moderate to high TA activity of snap bean genotype (OR5630) grown under both field and greenhouse conditions found in this study could potentially have significant relevance for potential therapeutic applications against chronic oxidative stress associated with T2D and other NCDs.

Previously, the anti-hyperlipidemic and antioxidant activity in aqueous extract of dried green beans was reported (Venkateswaran and

Pari, 2002). The anti- hyperlipidemic effect of this previous study was targeted for reduction in blood glucose level, triglycerides, free fatty acids, phospholipids and total cholesterol when bean extracts were administered in diabetes-induced rats (Venkateswaran and Pari, 2002). The pathogenesis of T2D typically involves increase of free radicals in the body coupled with the deficiency of antioxidant protection and further chronic oxidative stress from free radicals that are formed disproportionately by higher glucose oxidation, glycation of non-enzymatic proteins and subsequent oxidative degradation of glycosylated proteins (Ceriello, 2000; Maritim et al., 2003). However, food crops such as snap beans with moderate level of phenolic bioactive-linked antioxidant capacity have potential to provide protection against such chronic oxidative stresses and therefore, can be targeted for the medicinally relevant dietary and therapeutic applications against oxidation-linked chronic inflammation commonly associated with early stages T2D. Furthermore, inhibition of digestive enzymes, such as α-amylase and α- glucosidase with non-synthetic and natural plant based sources is a safe therapeutic strategy to prevent and manage chronic hyperglycemia linked to early stages T2D (Hanhineva et al., 2010; Kwon et al., 2007). In addition, a 45-75% α-amylase inhibitory activity was reported in beans previously (Melzig and Funke, 2007). The role of α- amylase in glucose metabolism is related to its ability for catalyzing the hydrolysis of α-1,4 glycosidic bond of starch, glycogen, and other oligosaccharides which results in the rapid release of simple sugars in the intestine (Ali et al, 2006; Pereira et al., 2010). However, the inhibition of this enzyme in the human digestive tract helps to manage postprandial hyperglycemia by slowing down the breakdown of starch and other complex carbohydrates (Negri and Negri, 2005). Overall, moderate to high α-amylase enzyme inhibitory activity was observed in all 5 snap bean genotypes in this study, and therefore these genotypes can be targeted in medicinally relevant therapeutic strategies to manage hyperglycemia-linked to T2D.

Managing postprandial hyperglycemia is critical for overall management of T2D (Baron, 1998). Therefore, controlling postprandial glucose level in the blood is of utmost importance in the treatment of T2D and its associated macro and micro-vascular complications (Ali et al., 2006; Subramanian et al., 2008). Thus, several synthetic and non-synthetic natural sources that have potential to inhibit α- amylase and α-glucosidase were evaluated and then targeted to design pharmaceutical drugs for T2D treatments (Kobayashi et al., 2000). However, synthetic inhibitors can cause significant side effects, some of which may increase the incidence of renal tumors, can cause liver damage, and acute hepatitis (Carascosa et al., 1997; Charpentier et al., 2000). Therefore, finding non-synthetic natural sources with moderate to high α-amylase and α-glucosidase has significant relevance for long-term and safe management of T2D using therapeutic strategies. In this context snap bean genotypes such as OR5630, Ebro, and Eagle can be targeted and integrated in medicinally relevant diets or in therapeutic strategies to control hyperglycemia-linked to early stages of T2D.

The health relevant benefits of food crops are not only just associated with total soluble phenolic content but also based on the composition of phenolic bioactives. Among detected phenolic acids, higher concentrations of quercetin was observed in all snap bean genotypes both under field and greenhouse conditions. Quercetin is most abundant flavonol widely distributed in different plant species and has significant human health benefits (Larson et al., 2012). Reduction of serum cholesterol and phospholipid levels was observed in rat with addition of 1% quercetin in diet (Odbayar et al., 2006). Similarly, reduction of blood pressure was also found with quercetin supplementation in hypertensive subjects (Edwards et al., 2007). Therefore, higher level of quercetin in snap bean pods may have significant relevance in

managing macro and micro-vascular risks such as hypertension and dyslipidemia, which are commonly associated with T2D. Quercetin, gallic acid, and catechin are also known as natural antioxidants and have significant human health benefits including anti-inflammatory properties (Saibabu et al., 2015). Anti-hyperglycemic and anti-lipidperoxidative properties of gallic acid was observed in diabetic rats (Punithavathi et al., 2011). Similarly, consumption of catechin rich beverage has also shown improvement in blood glucose control in T2D patients (Nagao et al., 2009). Therefore, the major phenolic acids found in snap bean genotypes in this study have potential to contribute to the evaluated antioxidant and anti-hyperglycemic functions and can be targeted as functional biomarkers in therapeutic strategies for management of early stages of T2D and its associated complications.

CONCLUSION

Legumes such as snap beans are considered as healthy food choices due to their diverse bioactive profiles including phenolic metabolites. However, these phenolic bioactives and associated health benefits of snap bean vary widely among different genotypes and due to growing conditions. Therefore, prior to the targeted utilization of snap beans in medicinally relevant therapeutic strategies, it is essential to screen and evaluate different genotypes and from different growing conditions for their optimum phenolic bioactive-linked anti-hyperglycemic functionality using rapid *in vitro* assay models. Overall, moderately high and relevant TSP content, hig h TA acti vit y and high α-amylase inhibitory activity were found in Oregon5630 snap bean genotypes, while high α-glucosidase inhibitory activity was observed in Ebro and Eagle genotypes in this study. However, significant variations in phenolic-linked antioxidant and anti- hyperglycemic functionalities were observed in Tendergreen genotype when compared between field and greenhouse growing conditions and therefore, needs further evaluation. However, snap bean genotypes with moderate phenolic-linked anti-hyperglycemic functionality can be targeted in therapeutic strategies against chronic hyperglycemia and chronic oxidative stress, commonly associated with early stages T2D. Further clinical studies with animal models and wider screening of other snap bean genotypes for medicinally relevant targeted properties are required to validate the findings of this *in vitro* study and further to advance the use of snap bean in medicinally relevant therapeutic intervention strategies targeting early stages of T2D and associated risks.

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