Genotype and Environment Effects on Prebiotic Carbohydrate Concentrations in Kabuli Chickpea Cultivars and Breeding Lines Grown in the U.S. Pacific Northwest

George Vandemark1*, Samadhi Thavarajah2,3, Niroshan Siva2 and Dil Thavarajah2

Prebiotic carbohydrates are compounds that include simple sugars, sugar alcohols, and raffinose family oligosaccharides, which are fermented by gut bacteria and can influence the species profile of the gut microbiome to reduce obesity and weight gain. Prebiotic carbohydrates are also associated with several health benefits including reduced insulin dependence and incidence of colorectal cancer. Although pulse crops such as chickpea have been important sources of nutrition for human diets for thousands of years, relatively little is known about the profiles of prebiotic carbohydrates in pulse crops. The objectives of this study were to characterize the type and concentration of seed prebiotic carbohydrates in 18 kabuli chickpea genotypes grown in 2017 and 2018 in Idaho and Washington, and partition variance components conditioning these nutritional quality traits in chickpea. Genotype effects were significant for fructose, sucrose, raffinose, and kestose. Environment effects were also significant for several carbohydrates. However, year effects were the greatest sources of variance for all carbohydrates. Concentrations of most carbohydrates were significantly greater in 2017, when there was less precipitation during the growing season coupled with greater heat stress during grain filling than in 2018. This may reflect the role of many of these carbohydrates as osmoprotectants produced in response to heat and water stress. Overall, our results suggest that a survey of more genetically diverse plant materials, such as a chickpea ‘mini-core’ collection, may reveal genotypes that produce significantly greater concentrations of selected prebiotic carbohydrates and could be used to introduce desirable nutritional traits into adapted chickpea cultivars.

Keywords: biofortification, breeding, chickpea, gut microbiome, nutrition
INTRODUCTION

Chickpea (Cicer arietinum L.) was one of eight ‘founder crops’ domesticated 9,000–11,000 years ago by Neolithic communities in riparian zones along the Tigris and Euphrates rivers in what is now Turkey and Syria (Lev-Yadun et al., 2000). Currently chickpea is the third most important pulse crop in terms of global production, after dry bean (Phaseolus vulgaris L.) and dry pea (Pisum sativum L.), with over 14.7 million Mt produced in 2017 (FAOSTAT, 2019). India is responsible for more than 80% of annual global production with Myanmar, Ethiopia, Turkey, and Pakistan being other major producers (FAOSTAT, 2019).

Chickpeas can be divided into two major classes, ‘kabuli’ and ‘desi’, based on seed characteristics. Desi chickpeas have a ‘teardrop’ shape and tend to be smaller in size and have thicker and darker seed coats than kabuli chickpeas, which have a rounder shape and tend to be larger and lighter in color (Toker, 2009). Desi chickpeas are typically dehulled to remove seed coats and then split and cooked to produce dhal, or are ground to make flour, whereas kabuli chickpeas are usually cooked whole without removing seed coats and then used for salads, canned, or for making edible spreads such as hummus (Yadav et al., 2007).

The first chickpeas grown commercially in the U.S. were large, light colored kabuli chickpeas known as ‘Spanish White’, which were grown in the San Joaquin Valley of southern California (Muehlbauer et al., 1982). Chickpea production began to expand in the 1980s to areas of Idaho and Washington where the predominate cropping system was dryland wheat and barley grown in rotation with lentil and pea. Commercial chickpea production in the U.S. consists almost entirely of kabuli chickpeas (Vandemark et al., 2014a). Currently chickpea is an important component of dryland production systems throughout the U.S. Pacific Northwest and Northern Plains. In 2017 more than 240,000 ha of chickpeas were harvested in the U.S. with a production value greater than $200 million (NASS, 2019). In 2017, Washington and Idaho together accounted for approximately 51% of total U.S. chickpea production, while Montana and North Dakota together accounted for approximately 43% of total production (NASS, 2019).

Biofortification, a process by which crop plants have higher concentrations of nutritional factors such as proteins, carbohydrates, or minerals, has been proposed as a way of improving human and animal nutrition (White and Broadley, 2005). Biofortification may be accomplished through management practices, the development of new cultivars with improved nutritional qualities through plant breeding, or a combination of management and genetic approaches (de Benoist et al., 2008). At least three billion people globally suffer from malnutrition caused from dietary deficiencies in iron (Fe) or zinc (Zn) (de Benoist et al., 2008; Wessels and Brown, 2012). Nutritional characterization of chickpea has largely been limited to determining seed concentrations of minerals (Bueckert et al., 2011; Ray et al., 2014; Vandemark et al., 2018) and dietary fiber (Chen et al., 2016). Non-genetic sources of variance including environment, year and their interactions have been found to have greater magnitudes of effect than genetic variance for several important minerals of global concern, including Fe, Mg, and Zn (Ray et al., 2014; Vandemark et al., 2018).

In contrast to health consequences associated with dietary deficiencies, excesses in food consumption, coupled with genetic and environmental factors, have resulted in increases in the global incidence of obesity, coronary artery disease (CAD), and diabetes. Prebiotic carbohydrates are compounds found in many food sources that have been associated with diverse health benefits (Carlson et al., 2018). The definition of ‘prebiotic’ in the scientific community has evolved over more than 20 years of discussion and research and is most currently ‘A nondigestible compound that, through its metabolism by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus conferring a beneficial physiologic effect on the host’ (Bindels et al., 2015). Prebiotic carbohydrates include the simple sugars glucose and sucrose, several sugar alcohols (SA) including sorbitol and mannitol, fructooligosaccharides (FOS) such as kestose and nystose, and raffinose family oligosaccharides (RFOs), which include raffinose, stachyose, and verbascose (Peterbauer and Richter, 2001). Prebiotic carbohydrates are fermented by gut bacteria and can influence the species profile of the gut microbiome, including increasing concentration of Bifidobacteria sp. that are associated with reduced obesity and weight gain (Schwertz et al., 2010). Fermentation of prebiotic carbohydrates produces short chain fatty acids (SCFA) that are associated with several health benefits including reduced obesity and insulin dependence (Gao et al., 2009) and protection against development of colorectal cancer (Keku et al., 2015).

Significant genotype, location, and year effects have been detected for seed concentrations of several prebiotic carbohydrates in lentil (Lens culinaris L.), including sorbitol, mannitol, and verbascose (Johnson et al., 2013). However, the effects of genetic and non-genetic sources of variance on seed prebiotic carbohydrate concentrations have not been estimated for chickpea. Understanding these effects is essential for developing new chickpea cultivars that produce seed with higher concentrations of selected prebiotic carbohydrates across different environments. The objectives of this study were to characterize concentrations of seed prebiotic carbohydrates in 18 kabuli chickpea genotypes grown in Washington and Idaho and partition variance components conditioning these nutritional quality traits in chickpea.

MATERIALS AND METHODS

Plant Materials and Field Trials

This study examined 18 café kabuli chickpea entries (Table 1), which included five cultivars, Billy Beans, CDC Frontier, CDC Orion, Royal, and Sierra, and 12 breeding lines. All entries were planted at two locations: Genesee, ID, (46.55° N, 116.92° W), and
Pullman, WA (46.73° N, 117.18° W) in both 2017 and 2018. All seeds were treated before planting with fludioxonil (0.56 g kg⁻¹, Syngenta, Greensboro, NC, USA), mfenoxam (0.38 g kg⁻¹, Syngenta) and thiabendazole (1.87 g kg⁻¹, Syngenta) to control fungal diseases, thiamethoxam (0.66 ml kg⁻¹, Syngenta) for insect control, and molybdenum (0.35 g kg⁻¹). Approximately 0.5 g *Mesorhizobium ciceri* inoculant (1 × 10⁸ CFU g⁻¹; Exceed, Cambridge, MA, USA) was applied to each seed packet one day before planting. Chickpeas were planted at a density of 43 seeds m⁻² in a 1.5 m × 6.1 m block (~430,000 seeds ha⁻¹). All yield trials used a randomized complete block design with three replications. Weeds were controlled by a single post-plant/pre-emergence application of metribuzin (0.42 kg ha⁻¹, Bayer Crop Science, Raleigh, NC, USA) and linuron (1.34 kg ha⁻¹, NovaSource, Phoenix, AZ, USA). All plots were exclusively rainfed and no supplemental irrigation was applied. Plots at Pullman were evaluated during the growing season for field traits including resistance to *Resistant Starch*.

### TABLE 1 | Mean⁵ yield, hundred seed weight (HSW) and days to mature for chickpea cultivars and breeding lines grown at Pullman, WA and Genesee, ID in both 2017 and 2018.

| Entry                  | Pedigree                        | Pullman Yield (kg/ha) | Genesee Yield (kg/ha) | HSW (g)¹ | Days to Mature ¹   |
|------------------------|---------------------------------|-----------------------|-----------------------|----------|-------------------|
|                        |                                 | 2017                  | 2018                  | 2017     | 2018              | 2017     | 2018              |
| CDC Frontier           | FLIP 91-22C/ICC 14912           | 1163 AB               | 2302 AB               | 1960 A   | 3553 A            | 36.0 H   | 104 AB            |
| CDC Orion              | FLIP 95-48C/93-120-63K          | 1052 AB               | 2423 A                | 752 BC   | 3750 A            | 41.7 G   | 98 AB             |
| Royal                  | HB-19/CA9783142                 | 644 B                 | 2175 AB               | 591 C    | 2516 A            | 54.8 A   | 106 AB            |
| Sierra                 | CA188359/CA188608               | 809 AB                | 1796 C                | 853 BC   | 2757 A            | 49.6 BCD | 103 AB            |
| Billy Beans Landrace   |                                 | 1205 AB               | 2264 AB               | 1299 ABC | 3181 A            | 29.5 I   | 97 B              |
| CA0790B0429C           | HB-14/CA9783142                 | 1141 AB               | 2002 AB               | 1126 BC  | 2712 A            | 48.3 BCDE| 106 AB            |
| CA0790B0647C           | Masella 2/CA9783153C            | 1350 AB               | 2344 AB               | 1039 BC  | 2995 A            | 47.8 CDEF| 101 AB            |
| CA0990A0239C           | CA9990B1887C/CA9990233W         | 791 AB                | 2175 AB               | 1267 ABC | 3481 A            | 52.1 ABC | 106 AB            |
| CA13900002C            | PIS59061/Goji                 | 1000 AB               | 2299 AB               | 854 BC   | 3067 A            | 45.0 EFG  | 101 AB            |
| CA13900023C            | CA9990B383C/Sierra             | 1070 AB               | 2023 AB               | 952 BC   | 3143 A            | 51.3 ABC  | 102 AB            |
| CA13900046C            | CA9990187S1/CA0569C091         | 986 AB                | 2030 AB               | 1074 BC  | 2792 A            | 35.7 H    | 102 AB            |
| CA13900119C            | CA469C020C/CA9890233W           | 897 AB                | 2151 AB               | 1309 ABC | 2533 A            | 48.5 BCDE | 105 AB            |
| CA13900129C            | CA469C020C/CA99901604C         | 1435 A                | 2368 A                | 860 BC   | 3750 A            | 49.0 BCDE | 110 A             |
| CA13900139C            | CA469C020C/CA9990187S1         | 1134 AB               | 2409 A                | 1168 BC  | 3769 A            | 45.7 DEF  | 105 AB            |
| CA13900147C            | CA469C020C/Dwletty              | 871 A                 | 1937 AB               | 1517 AB  | 3105 A            | 51.4 ABC  | 105 AB            |
| CA13900149C            | CA469C020C/Dwletty              | 647 B                 | 1789 C                | 884 BC   | 2537 A            | 52.3 AB   | 108 AB            |
| CA13900151C            | CA469C020C/Dwletty              | 1360 A                | 2316 AB               | 1463 BC  | 3322 A            | 43.7 FG   | 102 AB            |
| CA13900162C            | CA469C020C/Sierra              | 1060 AB               | 2293 AB               | 1966 A   | 2756 A            | 48.9 BCDE | 106 AB            |
| Grand Mean             |                                 | 1034                  | 2167                 | 1161     | 3087 A            | 48.2      | 104               |

⁵Means within a column followed by the same letter are not significantly different (Tukey’s HSD, α = 0.05).

Mean yield trials conducted at Pullman, WA in 2017 and 2018.

**Prebiotic Carbohydrates**

Ground seed samples (500 mg) were placed in 15-ml polypropylene conical tubes and 10 ml ddH₂O was added to each tube, which were incubated for 1 h at 80°C (Muir et al., 2009). Samples were centrifuged at 3,000g for 10 min. An aliquot (1 ml) of the supernatant was diluted with 9 ml ddH₂O, and the diluted supernatant was filtered through a 13 mm × 0.45 μm nylon syringe filter (Fisher Scientific, Waltham, MA, USA) prior to analysis. Prebiotic carbohydrate concentrations (SA, RFO, and FOS) were measured using high performance anion exchange chromatography (HPAE) (Dionex, ICS-5000, Sunnyvale, CA, USA) as previously described (Feinberg et al., 2009; Johnson et al., 2013). SA (sorbitol and mannitol), RFO (raffinose, stachyose, and verbascose), and FOS (kestose) were identified and quantified using pure standards (> 99%), and concentrations were detected within a linear range of 3 to 1,000 μg g⁻¹ with a minimum detection limit of 0.2 μg g⁻¹. A lab reference (CDC Redberry lentil) was used to ensure the accuracy and reproducibility of detection. The peak areas of the external reference, glucose (100 ppm), SA (3–1,000 ppm), RFO (3–1,000 ppm), and FOS (3–1,000 ppm) were routinely analyzed for method consistency and detector sensitivity, with an error of less than 5%.

**Resistant Starch**

RS concentrations were determined as previously described (McCleary and Monaghan, 2002) using a commercial assay (Megazyme, 2012). Ground samples (500 mg) were incubated with 4 ml of 100 mM sodium malate (pH 6) containing α-amylase (10 mg ml⁻¹) and amyloglucosidase (3 U ml⁻¹) for 16 h in a water bath (37°C) with 200 strokes/min vertical shaking (Orbit shaker bath, Lab Line Instruments Inc., Melrose Park, IL, USA). After incubation, 4 ml of 95% ethanol were added, and the samples were centrifuged at 1,500g for 10 min at room temperature. The pellets were re-suspended with 6 ml of ethanol (50% v/v), centrifuged, and decanted. The resuspension and centrifugation processes were done twice.
Supernatants from the three centrifugations were pooled and brought to a volume of 100 ml in ddH₂O. The pellets were dissolved in 2 ml of potassium hydroxide (2 M) in an ice bath (-0°C) while stirring with a magnetic stirrer for 20 min. The suspensions were diluted with 8 ml of sodium acetate buffer (1.2 M, pH 3.8), with 0.1 ml of 3,300 U ml⁻¹ amylglucosidase then immediately added followed by incubation at 50°C for 30 min. The suspension was then centrifuged at 1,500×g for 10 min at room temperature. Aliquots (0.1 ml) of both the supernatant containing the RS fractions and the diluted washings containing the soluble starch (SS) fractions were transferred separately to 10-ml glass tubes. A reagent blank was prepared using 0.1 ml sodium acetate buffer (pH 4.5). An aliquot (3 ml) of GOPOD reagent was added to each tube, which were incubated in a water bath at 50°C for 20 min. Absorption was measured using a spectrophotometer (Genesys 20, Thermo Scientific, NC, USA) at 510 nm. Starch fractions were calculated as follows:

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RS = \frac{X \times (\text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{glucose}} \times W_{\text{sample}})},
\]

\[
SS = \frac{Y \times (\text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{glucose}} \times W_{\text{sample}})},
\]

where \(\text{Abs}_{\text{sample}}\) and \(\text{Abs}_{\text{glucose}}\) are the absorbance value of sample and glucose corrected against reagent blank, respectively; \(W_{\text{sample}}\) is the moisture corrected weight of sample; and \(X\) and \(Y\) are the dilutions factors for RS and SS, respectively. Regular corn starch (RS concentration 1.0 ± 0.1% (w/w)) was used to verify the data, and batches were checked regularly to ensure an analytical error of less than 10%.

**Chemicals**

Solvents and standards used for high performance anion exchange chromatography (HPAE) and enzymatic assays were purchased from Fisher Scientific (Asheville, NC, USA), Sigma-Aldrich (St. Louis, MO, USA), and VWR International (Satellite Blvd, Suwanee, GA, USA). Distilled and deionized water (ddH₂O; NANO-pure Diamond, Barnstead, IA, USA) was used in these analyses.

**Statistical Analysis**

Entries (genotypes) were considered fixed factors and locations (environments), replications (blocks) within locations, and years were considered random factors. Combined ANOVA was conducted across both locations and years to detect effects of genotypes, environments, and their interactions. Entry means were compared between all pairs using Tukey's HSD test (\(\alpha = 0.05\)). Pairwise correlations were determined between seed carbohydrate concentrations and yield from data combined across both locations and years, and correlations were also determined between carbohydrate concentrations, HSW and days to mature for data obtained at Pullman, WA in 2017 and 2018. All statistical analyses were performed with JMP software (SAS, Cary, NC, USA).

**RESULTS**

**Chickpea Seed Carbohydrate Concentrations**

Mean squares of combined analysis of variance for chickpea seed carbohydrate concentrations are presented in Table 2. Genotype effects were significant for fructose, sucrose, rafstose, and kestose. Genotype effects were greatest for the simple sugars fructose and sucrose. Environment effects were also significant for several carbohydrates including sorbitol, glucose, fructose, kestose, and soluble starch. Environment effects were greatest for fructose, soluble starch, and glucose. Year effects were significant for all carbohydrates. Year effects were the greatest sources of variance for all carbohydrates. A significant genotype × environment effect was only observed for fructose. Significant genotype × year effects were observed for fructose and rafstose, however, the magnitudes of these effects were minor in comparison with year effects.

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**TABLE 2** | Mean squares of combined ANOVA, and coefficient of variation (CV) for concentrations of prebiotic carbohydrates in chickpea cultivars and breeding lines grown in Idaho and Washington*.

| Prebiotic Carbohydrate | Genotype (G) | Environment (E) | Year (Y) | G x E | G x Y | E x Y | G x E x Y | CV (%) |
|------------------------|--------------|-----------------|----------|-------|-------|-------|-----------|-------|
| Sorbitol               | 26,215       | 102,540*        | 11,511,773*** | 17,334 | 18,696 | 280,608*** | 17,710 | 18.6    |
| Mannitol               | 146          | 843*            | 27,467*** | 171   | 177   | 733*   | 181      | 71.1    |
| Glucose                | 52           | 2,295***        | 7,587***  | 103   | 62    | 1,820*** | 88       | 34.2    |
| Fructose               | 25***        | 471***          | 354***    | 12*** | 19*** | 191***  | 14***    | 78.8    |
| Sucrose                | 543,676***   | 43,134          | 24,978,312*** | 118,945 | 75,914 | 215,543 | 108,939  | 17.5    |
| Stachyose              | 49,531       | 100,736         | 27,014,050*** | 72,468 | 53,655 | 1,864,656*** | 76,584  | 18.2    |
| Raffinose              | 22,926**     | 5,091           | 873,905*** | 9,544 | 15,955* | 329,788*** | 8,169    | 28.6    |
| Verbascone             | 12,403       | 8,481           | 17,172,870*** | 8,888 | 10,780 | 15,715  | 8,169    | 28.6    |
| Kestose                | 258*         | 1,328*          | 17,612***  | 171   | 177   | 733*   | 181      | 43.9    |
| Res. starch            | 1.3          | 15.8*           | 2.5       | 74    | 55    | 421    | 63       | 19.2    |
| Sol. starch            | 92           | 763***          | 2,014***  | 74    | 55    | 421    | 63       | 19.2    |

* Study included 18 kabuli genotypes evaluated at two environments (Pullman, WA and Genesee, ID) in 2017 and 2018.

**Resistant starch concentrations were only determined for samples harvested at Pullman and Genesee in 2017.

*** Significant at \(P < 0.0001\).
Environment × year effects were significant for all carbohydrates except sucrose, verbascose, and soluble starch. The greatest interaction effect for all carbohydrates was the environment × year effect. A significant genotype × environment × year effect was only observed for fructose.

The most abundant carbohydrate in chickpea seed was sucrose, which on average constituted greater than 1.6% of total seed weight, followed by stachyose and sorbitol (Table 3). Sucrose represented greater than 95% of total simple sugars (sucrose + fructose + glucose). Stachyose represented greater than 50% of total RFO (stachyose + rafnosose + verbascose), which was the most abundant class of prebiotic carbohydrates. The least abundant carbohydrates in chickpea seed were fructose and mannitol. Concentrations of glucose and kestose were similar in chickpea seeds. Significant differences between means of chickpea entries were detected only for seed concentrations of sucrose. CA13900023C had a significantly higher sucrose concentration than CA13900046C, but no other significant differences were detected. Soluble starch on average constituted 41% of total seed weight and was approximately 10× more abundant than resistant starch.

Mean concentrations of carbohydrates across locations and years are presented in Table 4. For the majority of carbohydrates, including sorbitol, mannitol, glucose, sucrose, stachyose, rafnosose, verbascose, and kestose, mean concentrations at both locations in 2017 were significantly greater than both locations in 2018. Significant differences in mean concentrations of carbohydrates between Pullman-2017 and Genesee-2017 were only observed for mannitol and rafnosose. Significant differences in mean concentrations between Pullman-2018 and Genesee-2018 were observed for several carbohydrates including sorbitol, glucose, fructose, rafnosose, kestose, and soluble starch.

### Correlations Between Carbohydrate Concentrations, Yield, HSW, and Days to Mature

Significant correlations (P < 0.05) between carbohydrate concentrations were observed for the majority of pairwise

#### TABLE 3 | Mean<sup>a</sup> concentrations of prebiotic carbohydrates for chickpea cultivars and breeding lines grown at Pullman, WA and Genesee, ID in both 2017 and 2018.

| Entry                      | Sorbitol mg/100 g | Mannitol mg/100 g | Glucose mg/100 g | Fructose mg/100 g | Sucrose mg/100 g | Stachyose mg/100 g | Rafnosose mg/100 g | Verbacose mg/100 g | Kestose mg/100 g | Soluble Starch g/100 g |
|---------------------------|-------------------|-------------------|------------------|------------------|-----------------|-------------------|-------------------|-------------------|-----------------|----------------------|
| Billy Beans               | 708 A             | 11.0 A            | 28.0 A           | 0.82 A           | 1,378 AB        | 1,235 A           | 406 A             | 340 A             | 25.2 A          | 38.9 A               |
| CA0790B0043C             | 710 A             | 12.2 A            | 28.6 A           | 4.82 A           | 1,911 AB        | 1,228 A           | 514 A             | 355 A             | 30.3 A          | 41.5 A               |
| CA0790B0547C             | 606 A             | 15.3 A            | 27.7 A           | 3.36 A           | 1,921 AB        | 1,175 A           | 534 A             | 310 A             | 24.9 A          | 39.8 A               |
| CDC Frontier              | 670 A             | 12.9 A            | 27.3 A           | 4.53 A           | 1,758 AB        | 1,108 A           | 408 A             | 246 A             | 17.2 A          | 39.1 A               |
| CDC Orion                | 670 A             | 13.4 A            | 31.5 A           | 2.98 A           | 1,610 AB        | 1,239 A           | 442 A             | 340 A             | 20.9 A          | 41.3 A               |
| Royal                     | 757 A             | 12.0 A            | 30.1 A           | 0.91 A           | 1,337 B         | 1,241 A           | 474 A             | 333 A             | 27.0 A          | 41.4 A               |
| Sierra                    | 649 A             | 19.5 A            | 27.0 A           | 4.29 A           | 1,777 AB        | 1,312 A           | 495 A             | 376 A             | 27.4 A          | 40.6 A               |
| Grand Mean                | 698 A             | 13.3 A            | 29.6 A           | 2.31 A           | 1696            | 1,228 A           | 455 A             | 332 A             | 26.5 A          | 41.0 A               |

<sup>a</sup> Means within a column followed by the same letter are not significantly different (Tukey’s HSD, α = 0.05).

#### TABLE 4 | Mean<sup>a</sup> concentrations by location and year of prebiotic carbohydrates for chickpea cultivars and breeding lines grown at Pullman, WA and Genesee, ID in both 2017 and 2018.

| Location-Year       | Sorbitol mg/100 g | Mannitol mg/100 g | Glucose mg/100 g | Fructose mg/100 g | Sucrose mg/100 g | Stachyose mg/100 g | Rafnosose mg/100 g | Verbacose mg/100 g | Kestose mg/100 g | Soluble Starch g/100 g |
|---------------------|-------------------|-------------------|------------------|------------------|-----------------|-------------------|-------------------|-------------------|-----------------|----------------------|
| Pullman 2017        | 917 A             | 20.7 B            | 35.1 A           | 1.26 B           | 2,057 A         | 1,659 A           | 554 A             | 635 A             | 36.0 A          | 43.5 A               |
| Pullman 2018        | 945 A             | 28.6 A            | 35.9 A           | 1.55 B           | 2,016 A         | 1,509 A           | 484 B             | 603 A             | 34.6 A          | 44.5 A               |
| Genesee 2017        | 523 B             | 1.82 C            | 17.3 C           | 1.29 B           | 1,306 B         | 1,308 B           | 335 D             | 46.0 B            | 21.5 B          | 34.7 B               |
| Genesee 2018        | 406 C             | 1.77 C            | 29.8 B           | 6.02 A           | 1,393 B         | 1,393 B           | 435 C             | 50.5 B            | 12.8 C          | 41.3 A               |

<sup>a</sup> Means within a column followed by the same letter are not significantly different (Tukey’s HSD, α = 0.05).
combinations and only correlations with \( r \geq 0.80 \) will be noted. The highest positive correlations between carbohydrate concentrations were observed between verbascose and sorbitol \((r = 0.93)\), verbascose and stachyose \((r = 0.92)\), stachyose and sorbitol \((r = 0.88)\), stachyose and sucrose \((r = 0.85)\), and verbascose and sucrose \((r = 0.82)\).

Correlations between seed carbohydrate concentrations and agronomic traits tended to be less than those observed between different carbohydrate concentrations. Correlations between carbohydrate concentrations and HSW or days to flower had relatively low magnitude \((r < 0.40)\) or not significant. Correlations between carbohydrate concentrations and days to mature tended to positive for most carbohydrates and were highest for sorbitol \((r = 0.67)\) and verbascose \((r = 0.65)\). However, significant negative correlations of appreciable magnitude were observed between several carbohydrate concentrations and plot yield. The highest negative correlations with yield were observed for the RFOs verbascose \((r = -0.80)\) and stachyose \((r = -0.77)\), followed by simple sugars sorbitol \((r = -0.66)\) and mannitol \((r = -0.65)\).

**DISCUSSION**

Significant genotype effects were detected for several prebiotic carbohydrates (Table 2). However, non-genetic sources of variance including year effects and environment × year interaction effects were the greatest sources of variance for all carbohydrates (Table 2). These results suggest that only limited gains may be made in these traits using adapted parental materials. Minor genotype effects, or in many cases a lack of significant genotype effects are likely due in part to the relatively narrow genetic base present in the examined chickpea cultivars and breeding lines (Table 1). Three breeding lines are full-sibs derived from CA0469C020C/Dweley and seven breeding lines share as a parent CA0469C020C, which has resistance to Ascochyta blight and is a full-sib line to CA0469C025C, a germplasm with improved parent CA0469C020C/Dweley and seven breeding lines share as a

Year effects were the greatest source of variance for all carbohydrate concentrations (Table 2). For the majority of carbohydrates, mean concentrations in 2017 were significantly greater than in 2018 (Table 4). Monthly average temperatures and total monthly precipitation are presented in Table 5 for Pullman, WA and Genesee, ID during 2017 and 2018. Average temperatures early in the growing season (April and May) were warmer in 2018 than 2017 at Pullman and Genesee. However, average temperatures later in the growing season (July and August) were cooler in 2018 than 2017 at both locations. Both locations received more precipitation early in the growing season (April and May) in 2018 than 2017. These data suggest that the higher concentrations of many carbohydrates observed in 2017 may be the result of lower precipitation during the growing season coupled with greater heat stress later in the season (July and August) during grain filling. This may reflect the role of many of these compounds as osmoprotectants produced in response to heat and water stress.

Total RFO content in chickpea seed averaged 2.0% of dry weight, which is consistent with reports for other seeds ranging from 2 to 10% (Peterbauer and Richter, 2001). The most abundant RFO in chickpea seed was stachyose (Table 2). This is consistent with previous reports for other legume seeds, including dry bean (P. vulgaris L.) (McPhee et al., 2002) and soybean (Glycine max L.) (Kumar et al., 2010) for which stachyose was more abundant than raffinose.

A positive correlation with \( r > 0.80 \) was observed between seed concentrations of verbascose and stachyose. This likely reflects their shared RFO biosynthetic pathway in seeds, in which galactosylation of raffinose leads to production of stachyose, to which an additional galactosyl residue is transferred to produce verbascose (Peterbauer and Richter, 2001). Similarly high correlations were also observed between these two RFOs, sucrose, and sorbitol. The high correlations between sucrose, stachyose, and verbascose can also be explained by the role of sucrose as the first galactosyl residue acceptor in the RFO biosynthetic pathway. High correlations

**TABLE 5** | Average monthly temperature and precipitation during growing season in Pullman\(^a\), WA and Genesee\(^b\), ID in 2017 and 2018.

| Average Temperature (°C) | Total Precipitation (mm) |
|---------------------------|--------------------------|
|                           | Pullman 2017 | Pullman 2018 | Genesee 2017 | Genesee 2018 | Pullman 2017 | Pullman 2018 | Genesee 2017 | Genesee 2018 |
| April                     | 7.4           | 7.9           | 6.6           | 6.9           | 36.6           | 45.5           | 76.7           | 97.0           |
| May                       | 12.2          | 14.7          | 11.7          | 14.2          | 39.6          | 47.0          | 57.2          | 67.3          |
| June                      | 16.1          | 15.1          | 15.6          | 14.4          | 20.8          | 22.9          | 38.6          | 40.1          |
| July                      | 20.7          | 19.7          | 20.5          | 19.4          | 0.5           | 0.3           | 1.0           | 18.0          |
| August                    | 20.4          | 19.1          | 20.4          | 19.1          | 1.0           | 6.9           | 3.0           | 18.0          |

\(^a\) Data from Washington State University AgWeatherNet (https://weather.wsu.edu).
\(^b\) Data from U.S. National Center for Climate Information (https://www.ncdc.noaa.gov).
between sorbitol, stachyose and verbascose likely reflect that along with sucrose, SA such as sorbitol are primary products of photosynthesis and a major source of translocated carbohydrate to seed (Slewinski and Braun, 2010).

Only minor or non-significant correlations were observed between seed carbohydrate concentrations and seed size (HSW). However, high negative correlations were observed between yield and concentrations of RFOs verbascose and stachyose, and between yield and SAs sorbitol and mannitol. Although RFOs primarily function to store carbon in seeds, they are also known to accumulate in response to abiotic stress factors including heat (Pankulangara et al., 2004) and drought (Downie et al., 2003). Sorbitol has been shown to accumulate in several plant species in response to various abiotic factors including osmotic (Pommerrenig et al., 2007) and drought stress (Li et al., 2012).

Similarly, accumulation of mannitol has been shown to increase (Pommerrenig et al., 2007) and drought stress (Li et al., 2012). Sorbitol has been shown to accumulate in several plant species in

**REFERENCES**

Abebe, T., Guenzi, A., Martin, B., and Cushman, J. (2003). Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. *Plant Physiol.* 131, 1748–1755. doi: 10.1104/pp.102.003616

Bindels, L. B., Delzenne, N. M., Cani, P. D., and Walter, J. (2015). Towards a more comprehensive concept for prebiotics. *Nat. Rev. Gastroenterol. Hepatol.* 12, 303–310. doi: 10.1038/nrgastro.2015.47

Bueckert, R. A., Thavarajah, P., and Pritchard, J. (2011). Phytic acid and mineral micronutrients in field-grown chickpea (*Cicer arietinum L.*) cultivars from western Canada. *Eur. Food Res. Technol.* 233, 203–212. doi: 10.1007/s00217-011-1495-8

Carlson, J. L., Erickson, J. E., Lloyd, B. B., and Slavin, J. L. (2018). Health effects and sources of prebiotic dietary fiber. *Curr. Dev. Nutr.* 2, nyz005. doi: 10.1093/cdn/nyz005

Chen, Y., McGee, R., Vandemark, G., Brick, M., and Thompson, H. J. (2016). Dietary fiber analysis of four pulses using AOAC 2011.25: Implications for human health. *Nutrients* 8, 829. doi: 10.3390/nu8120829

de Benoist, B., McLean, E., Egli, I., and Cogswell, M. (2008). “Worldwide prevalence of anaemia 1993-2005,” in *WHO global database on anaemia* (Geneva, Switzerland: WHO Press), 2008.

Downie, B., Gurusunghe, S., Dahal, P., Thacker, R., Snyder, J. C., Nonogaki, H., et al. (2003). Expression of a galactinol synthase gene in tomato seed is up-regulated before maturation desiccation and again after imbibition whenever high concentrations of selected prebiotic carbohydrates and could be used to introduce desirable nutritional traits into adapted chickpea cultivars.

**DATA AVAILABILITY STATEMENT**

The datasets generated for this study are available on request to the corresponding author.

**AUTHOR CONTRIBUTIONS**

GV and DT conceived this work. GV planned and carried out field experiments including data collection, harvesting and cleaning seed samples. DT directed laboratory work to determine prebiotic carbohydrate profiles, maintained equipment for high performance anion exchange chromatography (HPAEC), and analyzed data. ST and NS performed laboratory work and collected data. GV performed statistical analysis. GV and DT drafted the manuscript. All authors read and approved the manuscript.

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drought stress. *Plant Mol. Biol. Rep.* 30, 123–130. doi: 10.1007/s11105-011-0323-4

Mcburney, B. V., and Monaghan, D. A. (2002). Measurement of resistant starch. *J. AOAC Int.* 85, 665–675.

McPhee, K. E., Zemetra, R. S., Brown, J., and Myers, J. R. (2002). Genetic analysis of the raffinose family oligosaccharides in common bean. *J. Amer. Soc Hortic. Sci.* 127, 376–382. doi: 10.21273/JASHS.127.3.376

Megazyme (2012). Resistant starch assay procedure. RSTAR 11/02. Megazyme Intl.

Muehlbauer, F. J., Kaiser, W. J., Bezdicek, D. F., Morrison, K. J., and Swan, D. G. (2012). Measurement of short-chain carbohydrates in common Australian vegetables and fruits by high-performance liquid chromatography (HPLC). *J. Agr. Food Sci.* 1112 (1982). Description and culture of chickpeas. Washington State University Ext. Bull.

Muir, J. G., Rose, R., Rosella, O., Liels, K., Barrett, J. S., Shepherd, S. J., et al. (2009). Measurement of resistant starch. *Plant Mol. Biol. Rep.*

Muehlbauer, F. J., Kaiser, W. J., Bezdicek, D. F., Morrison, K. J., and Swan, D. G. (2012). Measurement of short-chain carbohydrates in common Australian vegetables and fruits by high-performance liquid chromatography (HPLC). *J. Agr. Food Chem.* 57, 554–565. doi: 10.1021/jf0802700

NASS (National Agricultural Statistics Service) (2019). https://www.nass.usda.gov/Statistics_by_Subject/result.php?3E8E3F30-6430-3270-982E-4B9E18640793&sector=CROPS&group=FIELD%20CROPS&comm=BEANS. (Accessed 19 April 2019).

Panikulangara, T., Eggers-Shumacher, G., Wunderlich, M., Stransky, H., and NASS (National Agricultural Statistics Service) (2019). https://www.nass.usda.gov/Statistics_by_Subject/result.php?3E8E3F30-6430-3270-982E-4B9E18640793&sector=CROPS&group=FIELD%20CROPS&comm=BEANS. (Accessed 19 April 2019).

Peterbauer, T., and Richter, A. (2001). Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. *Seed Sci. Res.* 11, 185–197. doi: 10.1079/SSR200175

Pommerrenig, B., Papini-Terzi, F., and Sauer, N. (2007). Differential regulation of sorbitol and sucrose loading in the phloem of *Platycodon grandiflorum* in response to salt stress. *Plant Physiol.* 144, 1029–1038. doi: 10.1104/pp.106.089151

Ray, H., Bett, K., Tar’an, B., Vandenberg, A., Thavarajah, D., and Warkentin, T. (2014). Mineral micronutrient content of cultivars of field pea, chickpea, common bean, and lentil grown in Saskatchewan, Canada. *Crop Sci.* 54, 1698–1708. doi: 10.2135/cropsci2013.11.0744

Schwiertz, A., Taras, D., Schafer, K., Beijer, S., Bos, N. A., Donus, C., et al. (2010). Microbiota and SCFA in lean and overweight healthy subjects. *Obesity* 18, 190–195. doi: 10.1038/oby.2009.167

Slewinski, T. L., and Braun, D. M. (2010). Current perspectives on the regulation of whole-plant carbohydrate partitioning. *Plant Sci.* 178, 341–349. doi: 10.1016/j.plantsci.2010.01.010

Tokber, C. (2009). A note on the evolution of kabuli chickpeas as shown by induced mutations in *Cicer reticulatum* Ladizinsky. *Genet. Resour. Crop Evol.* 56, 7–12. doi: 10.1007/s10722-008-9336-8

Upadhya, H. D., and Ortiz, R. (2001). A mini core subset for capturing diversity and promoting utilization of chickpea genetic resources in crop improvement. *Theor. Appl. Genet.* 102, 1292–1298. doi: 10.1007/s00122-001-0556-y

Vandemark, G., Brick, M., Osorno, J., Kelly, J., and Urrea, C. (2014a). Edible grain legumes. Eds. S. Smith, B. Diers, J. Specht and B. Carver (Madison, WI), Yield Gains Major U.S. Field Crops, American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc.), 87–124. doi: 10.2135/cssaspecpub33.c5

Vandemark, G., Muehlbauer, F. J., Mihov, M., Chen, W., McPhee, K., and Chen, C. (2014b). Registration of ‘CA0469C025C’ chickpea. *J. Plant Regist.* 8, 303–307. doi: 10.3198/jpr2013.09.0057crg

Vandemark, G. J., Grusak, M. A., and McGee, R. J. (2018). Mineral concentrations of chickpea and lentil cultivars and breeding lines grown in the U.S. Pacific Northwest. *Crop J.* 6, 253–262. doi: 10.1016/j.cj.2017.12.003

Wessells, K. R., and Brown, K. H. (2012). Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. *PloS One* 7, e50568. doi: 10.1371/journal.pone.0050568

White, P. J., and Broadley, M. R. (2005). Biofortifying crops with essential mineral elements. *Trends Plant Sci.* 10, 586–593. doi: 10.1016/j.tplants.2005.10.001

Yadav, S. S., Longnecker, N., Dusunceli, F., and Bejiga, G. (2007). “Uses, consumption and utilization,” in Chickpea breeding and management yield gains major U.S. field crops. Eds. S. S. Yadav, R. Redden, W. Chen and B. Sharma (Oxfordshire, UK, 2007: CABI Intl.), 72–100. doi: 10.2139/ssm.1735326

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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