Enhancing In-crop Diversity in Common Bean by Planting Cultivar Mixtures and Its Effect on Productivity

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Common bean (*Phaseolus vulgaris* L.) is the most important food legume crop worldwide. Canadian beans, especially large seeded cultivars of Andean origin, have relatively narrow genetic diversities. Establishing crops with mixtures of cultivars instead of pure lines is a simple, cost effective way to increase genetic diversity in the field. A number of studies have demonstrated the benefits of mixture cropping over monocropping in controlling disease, increasing water use efficiency, and increasing yield stability. The objective of this study was to determine the effects of increasing in-field diversity, by using mixtures of bean cultivars instead of monocultures, on productivity. The feasibility of growing bean cultivar mixtures in southern Ontario environments was confirmed with a small pilot study that was conducted with four bean cultivars and restricted number of mixtures at two locations in 2017. Mixture performance experiments were performed with seven diverse bean genotypes at two Ontario locations [Woodstock and Elora (two planting dates) research stations] as pure stands and all possible binary mixtures (planted in alternate rows or as completely random mixtures) in 2018. Conventional plot-based above ground crop data were collected. Mixing efficiencies were calculated from the yield data using a relative yield of the mixture (RYM) index. Diallel analysis was used to identify general mixing ability of cultivars and specific mixing abilities of mixtures. Significant differences among seven bean cultivars and their mixtures were identified in all three environments for all analyzed traits. The results indicated multiple benefits of planting mixtures compared to monocultures A number of mixtures overyielded component cultivars grown in pure stands; they had higher yields, RYM index values >1 and positive specific mixing abilities (for yield) in both types of biblends. The research has the potential to provide a theoretical basis for the use of precision agriculture tools to plant fields with mixtures instead of monocultures. It could lead to greater in-field diversity in the crop and in the above and below ground ecosystems that might provide greater buffering capacity and resiliency to the cropping system as well as increased ecosystem services.

Keywords: common bean, cultivar mixtures, diversity, yield, mixing efficiency

INTRODUCTION

Coupled with effective inputs and improved management practices, plant cultivars that were improved during the green revolution doubled world grain production in 40 years (between 1960 and 2000) and averted predicted mass starvation and world order changes during a period of rapid global population growth (Khush, 2001; Tilman et al., 2002). Similar concerns exist about feeding a projected population of over nine billion people by year 2050 (Cardinale et al., 2012). However, there is increasing public interest in addressing food security concerns with crop production practices that increase diversity at all levels, including the soil microbiome, the crop cultivars, and the production systems that are utilized as well as reducing the use of inputs such as fertilizers and agrochemicals (Østergård et al., 2009; Bommarco et al., 2013; Gaba et al., 2014; Schipanski et al., 2016). A number of studies have demonstrated that mixed cropping systems have benefits over monocultures in reducing environmental impacts and in increasing above and below ground biodiversity, field productivity and yield stability, soil quality, weed/disease/pest control, energy use efficiency and profitability (Jolliffe and Wanjau, 1999; Malézieux et al., 2009).

Intraspecific mixtures (intracropping), which is simultaneous cultivation of two or more cultivars of the same species, can increase the in-field genetic diversity of a crop (Tooker and Frank, 2012; Vidala et al., 2017; Reiss and Drinkwater, 2018). The efficiency of these mixtures may be enhanced by blending high-yielding cultivars varying in their reactions to environmental stressors (Kiær et al., 2012). A meta-analysis of grain yields in cereals based on 26 published studies (documenting 246 instances of wheat and barley cultivar mixtures located in temperate regions) confirmed that yields in mixtures are higher than yields in pure stands. Mixing small grain cultivars is practiced in a number of regions in the world and management associated with these mixtures (mixing seeds, harvesting, and marketing) does not interfere with production (Mundt, 2002). In addition, the results obtained by Horner et al. (2019) from field pea mixtures, suggested that cultivar diversification increased yields and altered root bacterial and fungal communities and promoted their interactions.

Various spatio-temporal arrangements of crops can be utilized to perform intercropping, including planting mechanical mixtures of cultivars, or planting alternate rows, or strips in a field or planting them in relay. Each intercropping system is characterized by specific interactions between mixture components, with the highest frequency occurring in fields established with mixed crops. The selection of the mixing system depends largely on socioeconomic conditions and access to input, machinery and labor (Fageria, 1992).

Data from intercropping can be evaluated in a number of ways, including univariate and multivariate analyses of observed variables and usually, more than one method of analysis is necessary (Mead and Riley, 1981; Wijesinha et al., 1982; Mead, 1990; Federer, 1993). Diallel analysis was used to test for mixing ability analogous to genetic analysis of combining ability and select the best performing components for various intercrops. For example, Gallandt et al. (2001) used diallel analysis to evaluate pure stands and 15 biblends of six winter wheat cultivars grown in 33 environments in eastern Washington (USA). They identified a 1.5% yield advantage for mixtures compared to the average of their components grown in pure stands. In addition, significant correlations between yields of the mixtures and their components in pure stands suggested that mixture performance can be predicted from information from pure stands.

In addition to the raw data, numerous indices (which evaluate intercropping systems from agronomic, economic, ecological, and nutritional viewpoints) have been developed to assess yield potential, plant competition, and economic feasibility of various mixtures. Weigelt and Jolliffe (2003) reviewed over 50 indices commonly used in evaluating intercropping efficiencies. Indices such the relative yield of the mixture (RYM) calculates mixing efficiency by dividing yield response of the entire mixture by the average value of the mixture components evaluated in pure stands (Trenbath, 1974; Wilson, 1988). In mixtures with RYM = 1.0, there is no mixing (intercropping) effect; value of RYM <1.0 indicates mixing disadvantage (some type of competition among mixture components); and, mixtures having RYM >1.0 are more efficient than the monocultures (facilitation, complementarity, or avoidance of competition between the mixture components) (Williams and McCarthy, 2001).

Since intercropping performance can be affected by numerous factors, including crop selection (number and species), plant density, and competition among mixing components, the selection of the right index is critical in evaluating and interpreting the potential interactions and advantages of intercropping (Weigelt and Jolliffe, 2003; Bedoussac and Justes, 2011). Considering that there is no perfect index (Williams and McCarthy, 2001), the use of several indices, especially when more than one aspect of competition is being studied, could be beneficial.

The common (dry) bean (*Phaseolus vulgaris* L.) is the most important legume crop consumed directly by humans. Similar to other major crops, domestication and migration reduced the genetic diversity in common bean, particularly in the Andean compared to the Mesoamerican gene pool (Bitocchi et al., 2013). Similarly, a pedigree-based estimate of genetic diversity of Canadian bean cultivars indicated that the large seeded beans of Andean origin (such as kidney and cranberry) were most limited in their genetic diversity (Navabi et al., 2014).

A cost-effective way to increase in crop genetic diversity is to grow mixtures of cultivars, which is a common practice in many regions of Africa and South America. For example, in Eastern Africa, small holder farmers are planting common bean cultivar mixtures composed of two to 30 components varying in seed color, shape and size (Smithson and Lenne, 1996). Similarly, farmers in many regions of Brazil, are sowing mixtures of several bean lines (multilines) to ensure greater yield stability (Botelho et al., 2008). It was shown that the mixtures of bean cultivars can reduce the development and spreading of anthracnose (Ntahimpera et al., 1996; Prasad et al., 2016) and bean fly infestations (Ssekandi et al., 2016) in fields. Federer et al. (1982) compared the yields of 28 dry bean biblends in equal proportion to evaluate the mixing effect of the cultivars. Some cultivars had higher specific mixing effects than others,

but the results were not consistent over environments. Similarly, Riley et al. (1993) identified inconsistent responses for seed yield among common bean biblends across five different mixing ratios. This might be partially due to a narrow genetic diversity resulting in combinations of components from the same gene pool.

Southern Ontario is a major bean growing region in Canada (http://www.pulsecanada.com/producers-industry/about-pulses/growing-regions/) but there is no information on the performance of bean cultivar mixtures for this area. Therefore, the main objective of this study was to determine the agronomic (including crop yield) effects of increasing crop diversity in Ontario production systems by using common bean cultivars bred for this region in mixtures instead of monocultures. The work also tested the effects of the mixture type, namely, random mixing, or planting components in alternating rows on the performance of the mixture. In addition, the potential of predicting mixture performance from information measured from its components evaluated in pure stands, was tested. To meet these objectives, a number of diverse, high yielding common bean genotypes were evaluated for their performance in pure stands and binary mixtures, using a replacement design at a single density, in replicated trials in two Ontario locations in 2017 and 2018.

MATERIALS AND METHODS

Genotype Selection and Mixture Formation

The 2017 mixture study was initiated with four diverse, high yielding genotypes from the University of Guelph bean breeding program, including small seeded (Mesoamerican) white bean cultivars Lighthouse (Khanal et al., 2017) and Rexeter (Smith et al., 2012a), a black bean breeding line ACUG 15-B4 (registered as OAC Vortex in 2019), and a large seeded (Andean) light red kidney bean OAC Inferno (Smith et al., 2012b). In 2018, three additional cultivars were added, including a navy bean cultivar Bolt (Khanal et al., 2016b), a dark red kidney bean cultivar Dynasty (Khanal et al., 2016a), and a cranberry bean cultivar Red Rider (Park et al., 2009). Therefore, seven diverse genotypes [four Mesoamerican (small seeded: Lighthouse, Rexeter, Bolt and OAC Vortex) and three Andean (large seeded: OAC Inferno, Dynasty and Red Rider) gene pool] were evaluated in this study in 2018. The genotypes varied in growth habit, seed size, maturity, and resistance to three major common bean diseases {CBB [common bacterial blight caused by gram-negative bacteria *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*)], Ant [Anthracnose caused by seed-borne fungus *Colletotrichum lindemuthianum*(Sacc. & Magnus) Brosi & Cav.] and BCMV (*Bean common mosaic virus*, a member of the genus *Potyvirus*) an aphid-transmitted seed borne viral disease}. These genotypes (except new cultivar OAC Vortex) are checks in the trials performed by the Ontario Pulse Crop Committee (**Table 1**).

The genotypes were evaluated in pure stands and two types of binary mixtures [alternate row mixture (ARM) and same row (completely random) mixture (SRM), as described in Essah and Stoskopf, 2002]. An equal proportion replacement (substitutive) design at a single density was used as a mixing technique (Jolliffe, 2000). The aim of the work in 2017 was to test the feasibility

of mixing bean cultivars in Ontario environments and only a limited number of mixtures were formed. In all genotypic mixtures, the white bean cultivar Lighthouse was mixed with one of the other three genotypes [Rexeter, OAC Vortex (ACUG 15-B4) or OAC Inferno] in a 1:1 seed number ratio. In 2018, binary mixtures were formed for all possible combinations of seven cultivars (Lighthouse, Rexeter, OAC Vortex, OAC Inferno, Bolt, Dynasty, and Red Rider). The mixtures and pure stands had the same density (n) and each mixture component had a n/2 density (**Figure 1**).

Mixture names are composed of three letters: the first letter indicates cultivar component 1 [L (Lighthouse), R (Rexeter), B (Bolt), A (OAC Vortex, work initiated with the test name ACUG 15-B4), I (OAC Inferno), D (Dynasty) and RR (Red Rider)], the second letter indicates cultivar component 2 and the third letter represents mixture type [M stands for the same row mixture (SRM) and R is for the alternate row mixture (ARM)].

Field Evaluation

Field experiments were conducted at University of Guelph research stations at Elora [ERS; 2,680 CHU (Crop Heat Unit; Brown, 1993), London loam soil] and Woodstock (WRS; 2,890 CHU, Guelph loam soil; Voisey, 1971), Ontario in 2017 and 2018. In both years, the previous crop was winter wheat at the WRS. At the ERS in both years the first planting followed alfalfa and the second (2018) planting followed barley.

In 2017, the trials with 10 entries (four pure stands, three SRMs, and three ARMs) were carried out using a randomized complete block design (RCBD) with four replications. The rows were 6 m long and between rows spaces were 0.36 m. Each entry was planted in two adjacent 4-row plots (88 seeds row^{-1}) with a distance of 0.50 m between plots. Experiments were machine planted on June 6, 2017 at the WRS and on June 7, 2017 at the ERS. At both locations, fertilizer [200 kg ha^{-1} of 5-20-20 (10 kg ha^{-1} of N, 40 kg ha^{-1} of P and 40 kg ha^{-1} of K)] and herbicides [ppi tank mix of Pursuit® (BASF-Canada) at 200 ml ha^{-1} (active ingredient Imazethapyr at 48 g ha^{-1}) and 1.75 l ha^{-1} of Dual II Magnum® (active ingredient S-Metolachlor Group 2 at 1.6 kg ha^{-1}, Syngenta-Canada)] were applied before planting. No other chemicals were applied to control anthracnose, white mold and/or bacterial blights in the fields. Plots were combine harvested on September 19, 2017 at WRS and on September 29, 2017 at ERS.

In 2018, the trials with 49 entries (seven pure stands, 21 SRMs, and 21 ARMs) were planted as a square (7 × 7) lattice design with four replications. The experiments were machine planted on June 5, 2018 at the WRS and on June 7, 2018 for the first planting and on June 12, 2018 for the second planting at the ERS. The row spacing, seed density, fertilizer, and herbicide applications were same as in the 2017 experiments. The plots were combine harvested on September 18, 2018 at WRS and on October 10, 2018 planting 1 and October 23, 2018 planting 2 at ERS, respectively.

Data Collection

In all mixtures, both genotypic components were harvested and analyzed together. Plot-based crop data were collected for the

TABLE 1 | Characteristics of seven common bean genotypes selected for the mixture study conducted at two Ontario locations in 2017 and 2018.

Characteristics		Common bean genotypes						
		Lighthouse (L)	Rexeter (R)	Bolt (B)	OAC Vortex (A)	OAC Inferno (I)	Dynasty (D)	Red Rider (RR)
Test name		OAC 09-4	OAC 07-2	ACUG 10-1	ACUG 15-B4	OAC 07-L1	OAC 07-6D1	H4099-23481
Pedigree		spscbbr136/ PI207262//ICB-17 10/Vax4///OAC Speedvale/Avanti// OAC 99-1/OAC Rex	OAC Rex/AC Kippen	OAC Rex/A98083//AC Compass/B98143/// RESW2138/ B981045//B98213/ Kippen	HR199-4587/Zorro	HR85-1885/ Montcalm// USWA-39/AC Litekid///Foxfire/AC Elk//Sacramento/ AC Calmont	double-cross between HR85-1885 and Montcalm and USWA-39 and AC Litekid	SVM Taylor Horticulture and Dolly
Registration		2012	2011	2013	2019	2011	2012	2008
Gene pool[a]		MA	MA	MA	MA	A	A	A
Market class		Navy	Navy	Navy	Black	Light red kidney	Dark red kidney	Cranberry
Type		Indeterminate, upright	Indeterminate, upright	Indeterminate, upright	Indeterminate, upright	Determinate	Determinate	Determinate
Maturity		Full-season	Full-season	Early-season	Full-season	Mid to full season	Mid to late season	Mid to late season
Yield potential		High	High	High	High	Good	Excellent	High
Cooking quality		Good	Acceptable	Acceptable	Good	Acceptable	Good	Acceptable
Disease resistance[b]		CBB; Ant race 23; BCMV races 1 and 15	CBB; BCMV race 1	Ant race 73; BCMV races 1 and 15	CBB	Ant races 17 and 23; BCMV race 1	Ant races 17 and 73; BCMV race 1	BCMV race 15
References		Khanal et al. (2017)	Smith et al. (2012a)	Khanal et al. (2016b)	NA[c]	Smith et al. (2012b)	Khanal et al. (2016a)	Park et al. (2009)
2016 OPCC data[d]	YD	2,753	2,999	2,579	4,005	2,236	2,442	2,488
	SW	22.8	21.3	24.4	23.4	66.8	61.7	60.8
	DM	100	101	94	95	98	91	91
	HR	2.5	2.4	2.1	2.6	3.3	2.3	2.3
	CBB	3.0	3.0	4.0	3.3	5.0	4.5	5.0
	Ant	4	8	0	8	0	0	0

[a]Gene pool: MA, Mesoamerican; A, Andean.

[b]Disease resistance: CBB, common bacterial blight; Ant, Anthracnose; BCMV, bean common mosaic virus.
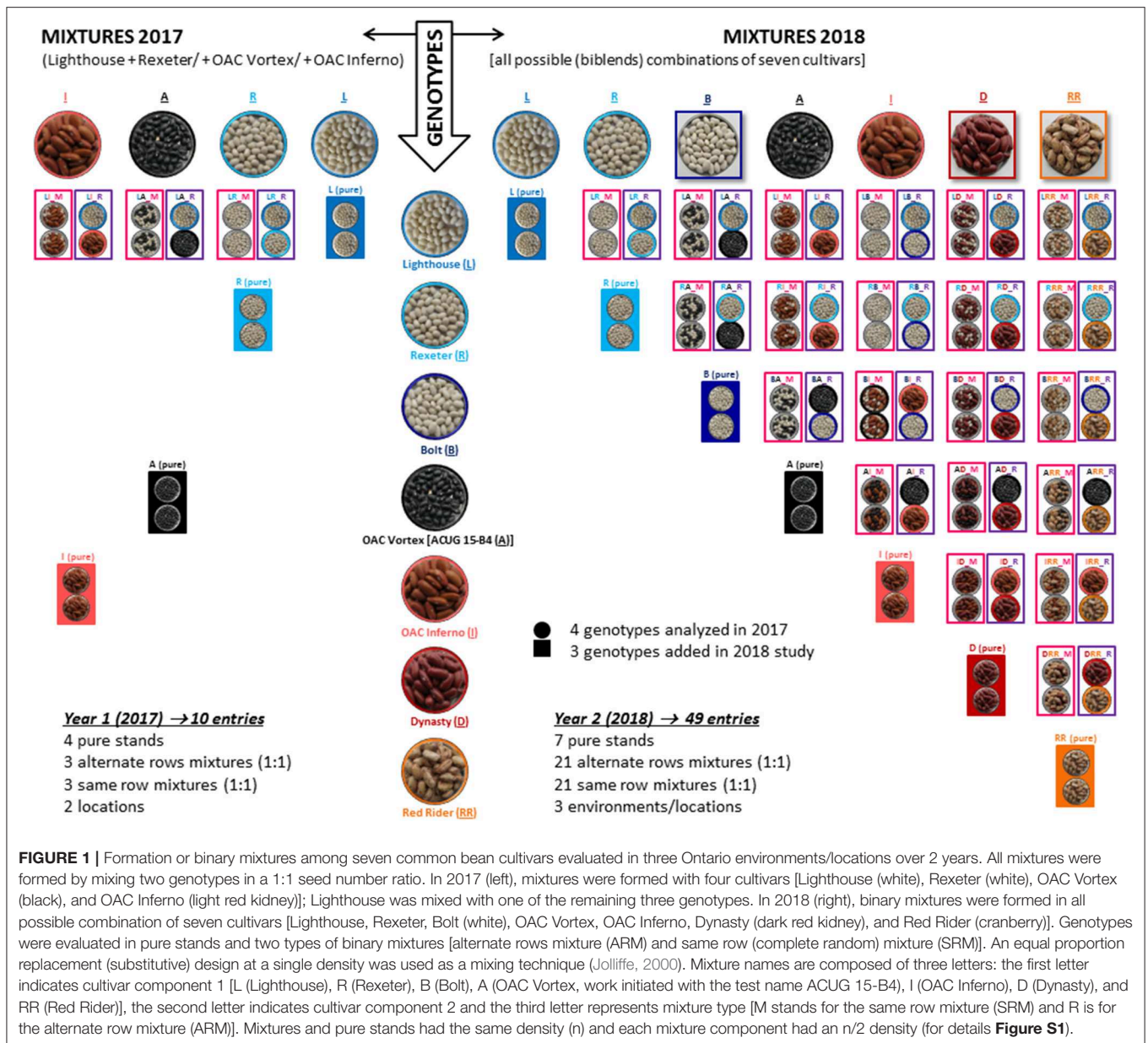
[c]NA, not available reference.

[d]Beans were evaluated as a part of the OPCC (Ontario Pulse Crop Committee, details are available at: www.gobeans.ca) performance and registration trials conducted over several Ontario locations in 2016; cultivars OAC Inferno and Dynasty were used as regional checks in 2016 trials.

Traits: YD, yield (kg ha−1); SW, seed weight (g); DM, maturity (days); HR, harvestability (visual rating of the stem's uprightness and the distance from pods to the ground using a scale 1–5, where 1 = excellent, 5 = poor); CBB, common bacterial blight severity was rated (CBB nursery at the AAFC Harrow) based on a leaf area infection with a 0–5 scale where 0 = no symptoms, 1= <5%, 2 = 5–10%, 3 =10–25%, 4 = 25–50%, and 5 = 50–100%; Ant, anthracnose severity was rated (Anthracnose nursery at the University of Guelph Elora research station) separately on leaves and pods using a scale from 0 to 9 (where for leaf veins 0 = no symptoms; 5 = some leaf vein darkening on some leaves; 9 = nearly 100% leaf veins darkened on most leaves; and for pods 0 = no symptoms; 5 = several small lesions per pod; 9 = most pods covered with coalescing lesions); ratings of 0–3 are considered resistant, while ratings over 3 can are reported as susceptible.

conventional above ground traits. Flowering was determined as the number of days from planting to 50% of plants in a plot with at least one fully opened flower. Maturity was reported as the number of days from planting to 95% of the pods in a plot being dry and ready to harvest. Plant height (cm) was measured from the soil surface to the tip of a main stem at the mid-pod filling stage. Seed weight (g) was computed as a weight of 100 seeds adjusted to 18% moisture content. Yield was a measure of a plot seed weight, expressed in kg ha^{-1}, and adjusted to 18% moisture content. Harvestability was determined at maturity using a scale of 1 (erect, suitable for direct combining harvest) to 5 (prostrate, unsuitable for machine harvesting). Relative leaf chlorophyll content was quantified as greenness of the plants using a portable non-destructive SPAD (Soil Plant

Analysis Development) chlorophyll meter (SPAD-502, Minolta, Japan) on eight, fully expanded, young leaves in each plot, and expressed as SPAD values. In 2017 the SPAD readings were done at a mid-pod filling stage [63 days after planting (DAP) on four randomly selected plants (two leaves/one reading)] (Monje and Bugbee, 1992). In 2018, the SPAD readings were collected at full flowering [43/44 DAP on eight randomly selected plants (one reading, one leaf, one plant row^{-1})]. Anthracnose disease reaction was evaluated under natural field infection using a 0–9 scale (Pastor-Corrales et al., 1995), where entries with ratings of 0–3 were considered resistant and ratings 4–9 were marked as susceptible. No anthracnose symptoms were identified in 2018.

Mixing efficiency was calculated from the yield data using indices applicable for the mixtures in which separate crop

FIGURE 1 | Formation or binary mixtures among seven common bean cultivars evaluated in three Ontario environments/locations over 2 years. All mixtures were formed by mixing two genotypes in a 1:1 seed number ratio. In 2017 (left), mixtures were formed with four cultivars [Lighthouse (white), Rexeter (white), OAC Vortex (black), and OAC Inferno (light red kidney)]; Lighthouse was mixed with one of the remaining three genotypes. In 2018 (right), binary mixtures were formed in all possible combination of seven cultivars [Lighthouse, Rexeter, Bolt (white), OAC Vortex, OAC Inferno, Dynasty (dark red kidney), and Red Rider (cranberry)]. Genotypes were evaluated in pure stands and two types of binary mixtures [alternate rows mixture (ARM) and same row (complete random) mixture (SRM)]. An equal proportion replacement (substitutive) design at a single density was used as a mixing technique (Jolliffe, 2000). Mixture names are composed of three letters: the first letter indicates cultivar component 1 [L (Lighthouse), R (Rexeter), B (Bolt), A (OAC Vortex, work initiated with the test name ACUG 15-B4), I (OAC Inferno), D (Dynasty), and RR (Red Rider)], the second letter indicates cultivar component 2 and the third letter represents mixture type [M stands for the same row mixture (SRM) and R is for the alternate row mixture (ARM)]. Mixtures and pure stands had the same density (n) and each mixture component had an n/2 density (for details **Figure S1**).

responses are not available (**Figure S1**). A relative yield of the mixture (RYM) index was calculated with the formula RYM $= Y_M/[(Y_{P1}+Y_{P2})/2]$ (Trenbath, 1974; Wilson, 1988). In the formula, Y_{P1} and Y_{P2} are yields of pure stands of cultivar component 1 (P1) and component 2 (P2), respectively; Y_M is a yield of the cultivar mixture (combined response of two mixture cultivar components). RYM measures weather the yield of the mixture is greater than the mean of the two monocultures (considers the proportion at which the species were grown). The values of RYM >1.0 indicate greater yield of the mixture compared to the mean of the two monocultures.

Additionally, mixtures were compared to the average of the two cultivar components (mid-parent) as well as a better performing cultivar component (better parent) when evaluated

in a pure stands. Mid-component (parent) Superiority (MS, or relative measure of mixing effect) was calculated using the formula MS $= \{[Y_M - (Y_{P1} + Y_{P2})/2]/(Y_{P1} + Y_{P2})/2\} \times 100$ (Kiær et al., 2012; Wang et al., 2016). The same results can be obtained from the RYM values using the formula: $Y_I = $ (RYM - 1) $\times$ 100 (Williams and McCarthy, 2001), as the percentages of the overall yield increase or decrease (Y_I). Better-component (parent) Superiority (BS, or maximal mixing effect or overyield) was calculated using the formula BS $= [(Y_M - Y_{BP})/Y_{BP}] \times$ 100 (Trenbath, 1974; Kiær et al., 2012; Wang et al., 2016). In the formula, BP indicates a better performing (higher yielding) cultivar component evaluated in a pure stand. The advantage of mixing bean cultivars was also calculated by comparing yield (kg ha^{-1}) of the mixture with the difference between the

mixture yield and yield of the cultivar component evaluated as monocrops (midcomponent, better yielding component, and lower yielding component).

Data Analysis

Crop data were analyzed as RCBD (2017) and lattice design (2018) using the generalized linear mixed models (Glimmix) procedure in SAS (Statistical Analysis System) v.9.4 software (SAS Institute, 2013). Treatments [cropping systems (genotypes)], locations, and interactions were considered as fixed effects. Replications (block and iblock) were evaluated as random effects using "random" and "covtest" statements. When necessary, the "nobound" option was used to remove boundary constraints on covariances (0), which allows their estimates to be negative. A few outliers were removed, and data were re-analyzed. If significant treatment differences were detected, pairwise multiple comparisons (LSmeans) were made using a Tukey HSD test. In addition, treatments [cropping system: SRM (same row mixture), ARM (alternate rows mixture) and PS (pure stand)] were compared using the "LSMestimate" statement. Significance was declared at $p < 0.05$. Residual analysis diagnostic plots were used to check for assumptions for Gaussian distribution (normality, homogeneity of error variances) and to identify the most suitable solutions if violations were identified (Kozak and Piepho, 2017).

Although, generally considered as a random effect in statistical analysis (as it is difficult to repeat location effects year after year), locations were considered as a fixed effect in the current study. At University of Guelph, research stations at Elora and Woodstock are routinely used for field experiments. There is 210 CHU difference between these locations; in general, if planted in the same time, beans grown at WRS will mature ~1 week earlier. However, in order to apply the results over wider bean production area instead of limiting the results to Elora and Woodstock in 2017 or 2018, the average of the locations was also presented.

After identifying significant genotype effects, diallel analysis was performed for the seven measured traits. The analysis was done using the Plant Breeding Tools (PBTools) software (IRRI, 2014) based on the Griffing model 2 [pure stands (parents) and mixtures (F$_1$s)] (Griffing, 1956) method 1 (fixed effects). To determine the relative importance of the General Mixing Ability [GMA of pure lines, similar to the General Combining Ability (GCA) of parents in genetic studies] and Specific Mixing Ability [SMA of binary mixtures, similar to the Specific Combining Ability (SCA) of F$_1$s in genetic studies] effects, the GMA/SMA (GCA/SCA) variance ratios were calculated as $2MS_{GMA}/2MS_{GMA} + MS_{SMA}$ (Baker, 1978). A ratio closer to unity (1) indicates higher importance of the GMA effects.

The relationships among traits (raw data) at the experiment as well as treatment levels (cropping system) were analyzed by Pearson's correlation in SAS. Significant relationships among a number of traits suggested that multivariate (principal component, PCA) analysis (LSmeans) would be a suitable method for data reduction. After calculation (princomp in SAS), trait eigenvalues, and cultivar scores were exported to Microsoft Excel to produce Genotype x Traits (GT) biplots where angles between variable vectors indicate the level of association between them.

Soil Analysis and Weather Conditions at ERS and WRS in 2017 and 2018 Bean Growing Seasons

Daily weather data for two experimental years (2017 and 2018) at two locations (ERS and WRS) for the bean growing season (May to October) were collected at the nearest weather stations (available at: http://climate.weather.gc.ca/climateData/dailydata_e.html).

Prior to planting in 2018, a standard soil sample probe (Oakfield Classic Soil Probe) was used to randomly collect 10–16 soil samples at 0–9 inch (22.86 cm) depths within each replication to create a bulk sample. After hand mixing, representative samples were sent for analysis to the SGS Agri-Food Laboratories Inc., Guelph, ON.
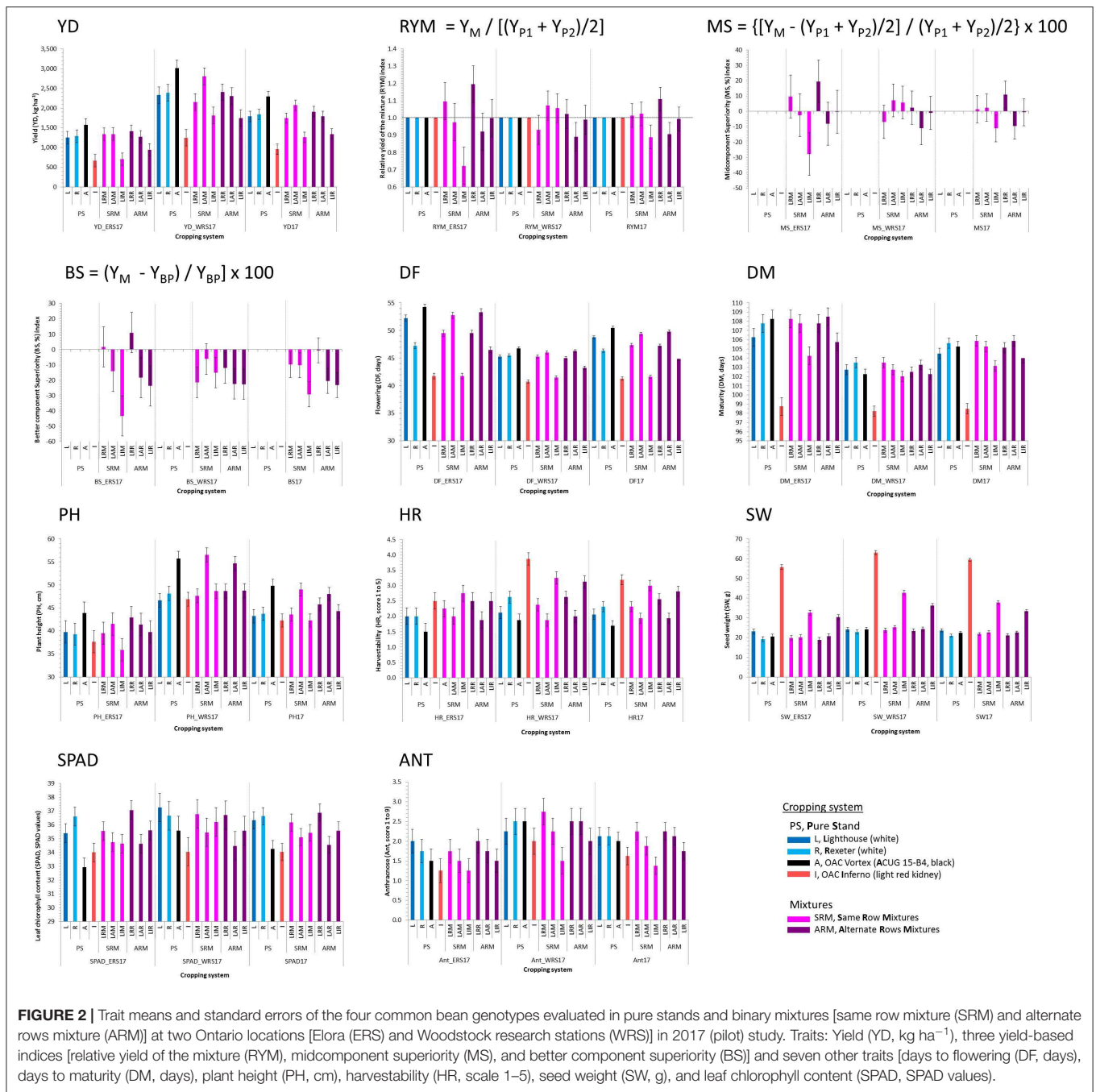
RESULTS

Soil Properties and Environmental Conditions

The plot soils were analyzed before planting the field trials in 2018. The soils at the three fields had different physico-chemical properties (**Table S1**). The field at the first planting at the ERS (1ERS18) had highest content of N (both nitrate and ammonium), K, Mg, and Ca as well as cation exchange capacity (CEC). On the other hand, soil at the WRS (WRS18) had highest content of P, Zn, Mn, Cu, and Fe. All of the other parameters had very similar values for the three fields.

The beans were in the field 105 days at the WRS and 114 days at the ERS in the 2017 pilot study. In 2018, beans were in the field 105 days at the WRS and 125 days at the ERS first planting and 133 days at the ERS second planting, respectively. During the bean growing season (June - September/October), the weather conditions (daily temperature and precipitation) at ERS varied considerably in two experimental years (**Figure S2**). However, due to the incomplete weather data for the WRS site direct comparison between the two sites was not possible for the whole bean growing seasons. As expected, in both years the warmer location was WRS resulting in earlier maturity. This site also received more rainfall in 2017 (more complete data). It received 259.8 mm precipitation (July 89.1 mm, August 108.2 mm) compared to 154.8 mm (July 32.4 mm, August 44.7 mm) rain at ERS for the periods with data collected for both sites, Although more fragmentary, 2018 data shown that WRS received more rainfall for overlapping periods in June (23.6 mm WRS and 0.8 mm ERS) and August (43.1 mm WRS and 26.2 mm ERS) (**Figure S2**).

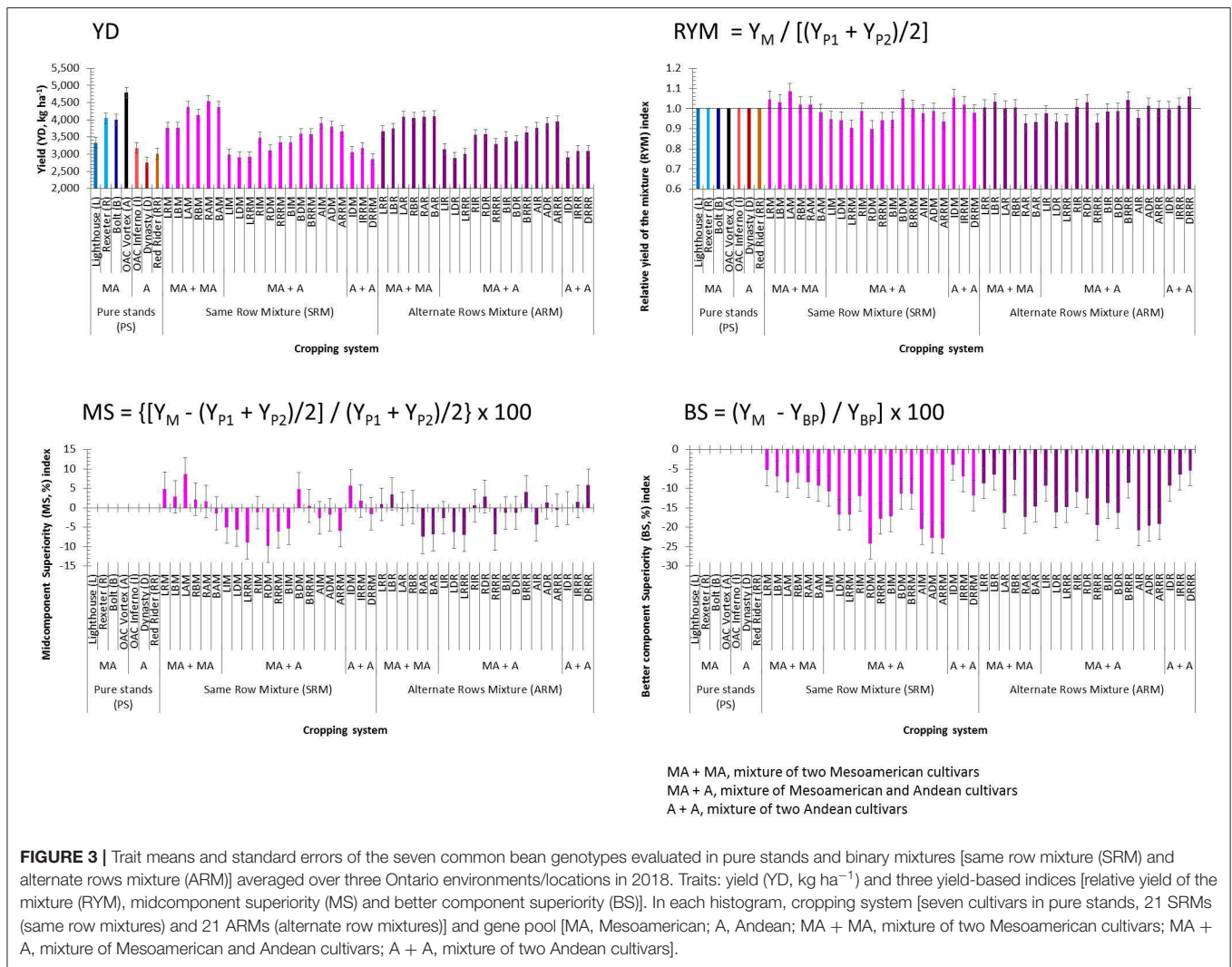## Feasibility of Growing Bean Cultivar Mixtures

In 2017, significant interactions between genotypes and locations were identified for flowering, maturity, harvestability, and seed weight. The presence of the interactions for some traits implies that different patterns of responses occurred for the pure stands and the mixtures at the two locations. Significant differences among four genotypes and their mixtures were identified in

FIGURE 2 | Trait means and standard errors of the four common bean genotypes evaluated in pure stands and binary mixtures [same row mixture (SRM) and alternate rows mixture (ARM)] at two Ontario locations [Elora (ERS) and Woodstock research stations (WRS)] in 2017 (pilot) study. Traits: Yield (YD, kg ha^{-1}), three yield-based indices [relative yield of the mixture (RYM), midcomponent superiority (MS), and better component superiority (BS)] and seven other traits [days to flowering (DF, days), days to maturity (DM, days), plant height (PH, cm), harvestability (HR, scale 1–5), seed weight (SW, g), and leaf chlorophyll content (SPAD, SPAD values).

both locations for the majority of the analyzed traits, except anthracnose, and yield-based indices (**Table S2**). Similarly, the location effect was significant for most of the traits, except for harvestability, SPAD, and yield-based indices. Beans evaluated at the WRS matured earlier and had higher yields compared to the mixtures grown at the ERS (**Figure 2**).

The yields of the bean mixtures were mostly similar to the averages of their component cultivars but, they were not always consistent at the two locations. The values of the RYMs varied between locations indicating inconsistent relative mixing

efficiencies of the bean biblends. A consistent yield advantage of the mixture, in terms of RYMs, was observed for mixtures between two white bean cultivars Lighthouse and Rexeter. Although statistically not significant, yield increases of 2.3% (WRS) and 19.5% (ERS) in LRR (ARM biblend) and 9.7% (ERS) in LRM (SRM) mixtures that were measured compared to monocrops of Lighthouse and Rexeter (**Figure 2**), indicated that it would be advantageous to grow mixtures compared to the pure lines. The yield and RYM values of the SRMs were higher compared to ARMs at WRS in 2017, however, the differences

FIGURE 3 | Trait means and standard errors of the seven common bean genotypes evaluated in pure stands and binary mixtures [same row mixture (SRM) and alternate rows mixture (ARM)] averaged over three Ontario environments/locations in 2018. Traits: yield (YD, kg ha^{-1}) and three yield-based indices [relative yield of the mixture (RYM), midcomponent superiority (MS) and better component superiority (BS)]. In each histogram, cropping system [seven cultivars in pure stands, 21 SRMs (same row mixtures) and 21 ARMs (alternate row mixtures)] and gene pool [MA, Mesoamerican; A, Andean; MA + MA, mixture of two Mesoamerican cultivars; MA + A, mixture of Mesoamerican and Andean cultivars; A + A, mixture of two Andean cultivars].

between mixture types (SRM vs. ARM) for these measures were not significant (**Table S3**).

Performance of Bean Cultivar Mixtures

In 2018, significant interactions between genotypes and locations were identified for all analyzed traits. This confirmed results of the initial mixture experiments conducted in 2017 indicating different patterns of responses for the pure stands and the mixtures over the three environments. Similarly, a significant location effect was identified for most of the analyzed traits except for yield and plant height. Significant differences among seven bean cultivars and mixtures were identified for all analyzed traits, except for the plant height at the WRS (**Table S4**).

Yield and Yield-Based Indices

Beans evaluated at the WRS matured earlier and had higher yields compared to the mixtures grown in two plantings at the ERS. As expected, small seeded (Mesoamerican) beans yielded on average 1,058 kg ha^{-1} more compared to the large seeded (Andean) genotypes (**Table S5**). Among the seven genotypes evaluated in

pure stands, the highest mean yield value (averaged over the three environments) was obtained for the small seeded black bean cultivar OAC Vortex (4,787 kg ha^{-1}) and the lowest (2,760 kg ha^{-1}) was in the dark red kidney cultivar Dynasty (**Figure 3**). On average, the SRM biblends yielded slightly higher compared to the ARMs, but the difference (on average, 10 kg ha^{-1}) was not significant. However, within each type of mixture, Mesoamerican (M) biblends were significantly higher yielding compared to both Andean (A) and between gene pool (Mesoamerican + Andean, MA) mixtures (**Table S5**). The highest mean yield value (averaged over three environments) for the SRMs was found in the biblend of two small seeded genotypes Rexeter and OAC Vortex (RAM, 4,548 kg ha^{-1}). The lowest yield mean value for the SRMs was found in a biblend of two large seeded cultivars Dynasty and Red Rider (DRRM, 2,851 kg ha^{-1}). In ARMs, the highest yielding was a biblend of two small seeded genotypes OAC Vortex and Bolt (BAR, 4,107 kg ha^{-1}). The lowest yielding ARM was a mixture between two large seeded cultivars, Dynasty and OAC Inferno (2,908 kg ha^{-1}). In general, between gene pool mixtures (both SRMs and ARMs) had intermediate yield

values that were closer to the values obtained for the pure small seeded beans. Similar yield trends were observed in separate environments/locations (**Table S6**).

For the yield-based indices, significant differences between SRM and ARM type of the bean biblends were identified only for the BS (better component superiority) index (**Table S5**). Averaged over three environments, the values of the RYM (relative yield of the mixture) index higher than unity and with positive MS (midcomponent superiority) indices were identified for five out of six Mesoamerican SRM mixtures (**Figure 3**). The highest RYM was observed in biblend of two small seeded cultivars OAC Vortex and Lighthouse (LAM, 1.087) indicating better performance (8.7% higher yield) in the mixture compared to the cultivar components evaluated in pure stands. Two out of three Andean SRM biblends outperformed monocropped cultivars. The IDM mixture had a 5.6% better yield compared to the cultivars OAC Inferno and Dynasty grown in pure stands. In ARM biblends, three out of six Mesoamerican (LRR, LBR, and RBR) and two out of three Andean (IRRR and DRRR) combinations had higher yields than their component cultivars evaluated as monocrops. In addition, two SRMs (BDM and BRRM) and four ARMs (RIR, RDR, BRRR and ADR) overyielded monocropped cultivar components (**Figure 3**). However, all mixtures had negative values for the BS index, indicating that none of the biblends outperformed the better cultivar component in the mixture, for both the SRM and ARM formats (**Figure 3**). Similar trends for all three indices were observed in separate environments (**Table S6**).

Other Characteristics

Significant differences between Mesoamerican and Andean genotypes in pure stands were identified for all additional traits. On average, Mesoamerican genotypes flowered (+6.5 days) and matured (+4.2 days) later, were taller (+4.9 cm), but had better harvestability (−0.434), lighter seeds (−41.4 g), and higher leaf chlorophyll contents (+2.7 SPAD values) compared to the Andean genotypes evaluated in pure stands (**Table S5**). The cultivar Lighthouse had the longest flowering (46.4 days) and maturity (100.7 days) but the highest SPAD reading (39.0 SPAD values). The black bean cultivar OAC Vortex was the tallest (60.4 cm) while the white cultivar Rexeter had the lightest seeds (21.1 g). The best (lowest) harvestability score was recorded in cultivar Bolt (1.5). The Andean cultivar Dynasty had the earliest flowering time (37.8 days) and the lowest SPAD reading (34.2 SPAD values) while the cranberry cultivar Red Rider was the shortest plant (48.8 cm) and had the earliest maturity (90.1 days, same as the small seeded cultivar Bolt) (**Figure 4**).

For the mixtures, the SRMs matured earlier (−0.25) and had poorer harvestability scores (+0.12) compared to the ARMs. However, there were no significant differences between the two biblend types for flowering, plant height, seed weight, and SPAD (**Table S5**). In the SRM biblends, the highest values for flowering (46.5 days, LRM), maturity (99.1 days, LRM), plant height (59.4 cm, LAM), harvestability (3.27 score, BAM), and SPAD reading (39.0 SPAD values, BAM) but the lowest for seed weight (21.3 g, LRM) were observed in Mesoamerican mixtures. On the

other hand, the lowest values for flowering (38.3 days, DRRM) and SPAD reading (34.2 SPAD values, DRRM) but the highest value for seed weight (64.3 g, IDM) were recorded in Andean SRMs. Maturity (90.6 days) and harvestability (1.75 score, lower is better) had the lowest values in the BRRM while the LRRM biblend had the shortest plant (50.2 cm), both between gene pool (Mesoamerican + Andean) biblends (**Figure 4**).
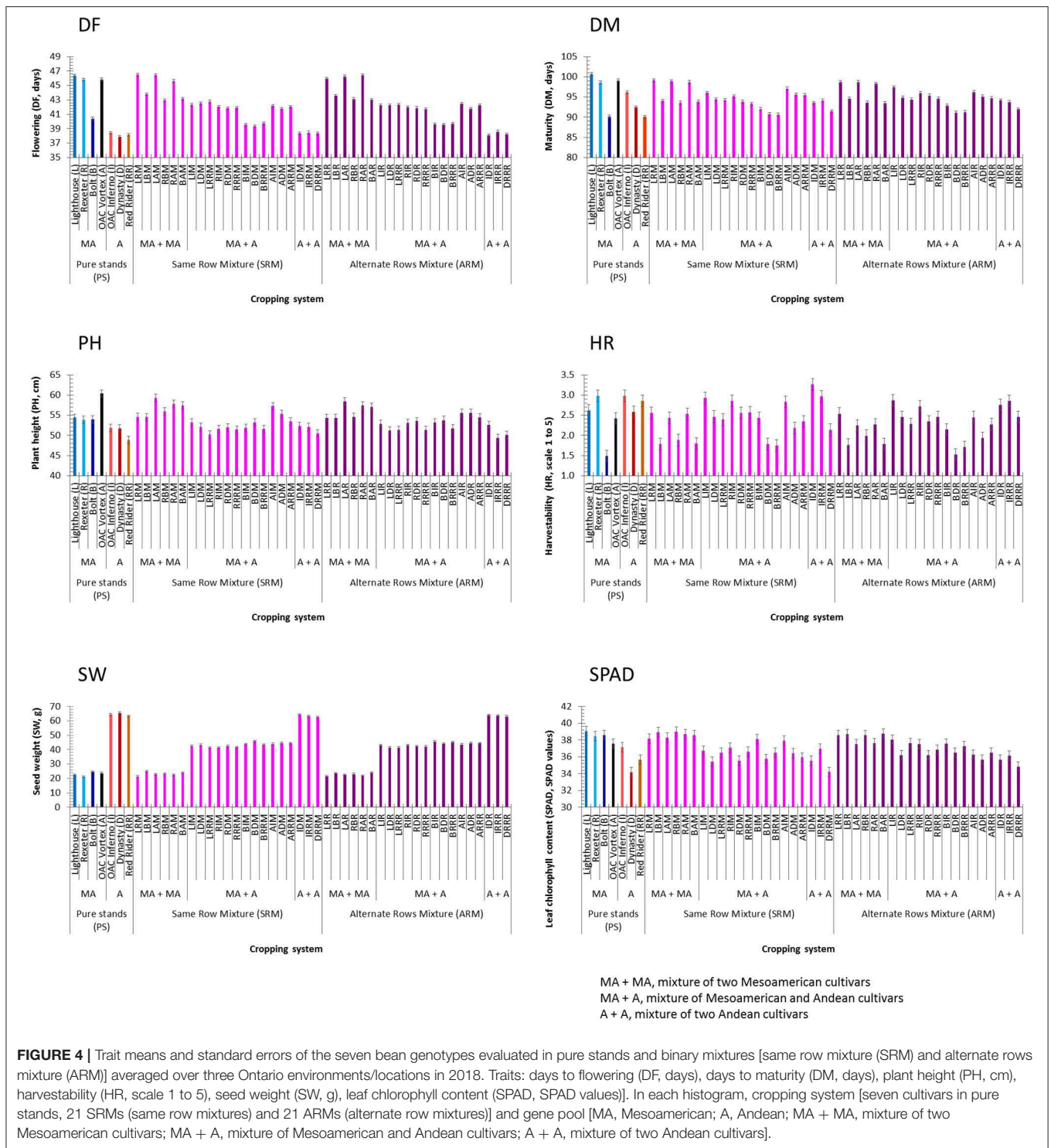
In the ARM biblends, the highest values for flowering (46.45 days, RAR), maturity (98.7 days, LRR and LAR), plant height (58.4 cm, LAR) and SPAD reading (38.7 SPAD values, BAR) but lowest value for seed weight (21.5 g, LRR) were identified in Mesoamerican mixtures. The highest value for seed weight (63.9 g) but the lowest values for flowering (38.1 days, IDR), plant height (49.4 cm, IRRR), and for SPAD reading (34.8 SPAD values, DRRR) were observed in Andean ARMs. The lowest value for maturity (91.1 days) was found in the BDR (Mesoamerican + Andean) mixture. Both, the lowest and the highest values for harvestability [1.53 score (BDR) and 2.86 score (LIR)] were observed in between gene pool (Mesoamerican + Andean) mixtures (**Figure 4**). Similar trends in these traits were identified when environments were analyzed separately (**Table S6**).

Relationships Among the Traits

Exploratory correlation analysis with raw data identified significant relationships among the evaluated traits (not shown). The majority of the correlations were confirmed using LSmeans ($N = 49$) averaged over the three environments (**Table 2**) or for each environment/location separately (**Table S7**). Significant positive associations were identified between the three environments for all analyzed traits except the RYM index (**Table S7**). Some traits were associated with a number of other traits. For example, yield was significantly positively correlated with flowering, plant height and SPAD reading but in an inverse relationship with seed weight. Flowering was positively correlated with yield, maturity, plant height and SPAD reading but negatively correlated with seed weight (**Table 2**, **Table S7**). The SPAD reading was significantly positively associated with yield and flowering but was negatively related with seed weight. In contrast, the RYM index was not significantly correlated with any of the analyzed traits.

These correlations suggested that there are complex relationships among the traits and a principal component analysis (PCA), based on the correlation matrix, was used to reduce the dimensionality of the data. Using the Kaiser criterion (Kaiser, 1958), 10 principal components (PC) were identified that had eigenvalues >1 and cumulatively explained 96.3% [48.92% (PC1) to 1.44% (PC10)] of the variation for evaluated traits (**Table 3**, **Figure S3**). The first two PCs explained 65.8% of the variation. Averaged over three environments, PC1 had large positive associations with yield, flowering, plant height, and SPAD and was negatively associated with seed weight. PC2 had large positive associations with maturity and harvestability (**Figure 5**).

Significant associations among traits, analyzed in different locations, were indicated by their groupings in a genotype-trait (GT) biplot. For example, all four seed weight measurements (mean environment, WRS18, 1ERS18, and 2ERS18) clustered

FIGURE 4 | Trait means and standard errors of the seven bean genotypes evaluated in pure stands and binary mixtures [same row mixture (SRM) and alternate rows mixture (ARM)] averaged over three Ontario environments/locations in 2018. Traits: days to flowering (DF, days), days to maturity (DM, days), plant height (PH, cm), harvestability (HR, scale 1 to 5), seed weight (SW, g), leaf chlorophyll content (SPAD, SPAD values)]. In each histogram, cropping system [seven cultivars in pure stands, 21 SRMs (same row mixtures) and 21 ARMs (alternate row mixtures)] and gene pool [MA, Mesoamerican; A, Andean; MA + MA, mixture of two Mesoamerican cultivars; MA + A, mixture of Mesoamerican and Andean cultivars; A + A, mixture of two Andean cultivars].

together in a quadrant opposite to the negatively associated yield trait. In contrast, yield evaluated at WRS was positioned further from the yield values measured at the ERS (two plantings). Significant negative correlations between yield and harvestability at WRS, ERS first planting and environmental average are reflected by their positions in different quadrants. However, there

was no associations observed between these traits at ERS, second planting (**Figure 5**).

In the GT biplot, the genotypes were divided into three distinct clusters. Three large seeded Andean cultivars and their mixtures (three SRMs and three ARMs) clustered together (left side of the plot). Similarly, the four small seeded Mesoamerican

TABLE 2 | Correlations among analyzed traits averaged over three Ontario environments in 2018 (LSmeans, $N = 49$).

Traits	RYM	DF	DM	PH	HR	SW	SPAD[a]
YD (Yield, kg ha^{-1})	NS[b]	0.6340**	0.3965**	0.8693**	−0.3877**	−0.7422**	0.6284**
RYM (Relative yield of the mixture index)		NS	NS	NS	NS	NS	NS
DF (Flowering, days)			0.8022**	0.6945**	NS	−0.8886**	0.6447**
DM (Maturity, days)				0.5834**	0.4112**	−0.5073**	0.4359**
PH (Plant height, cm)					NS	−0.6968**	0.5454
HR (Harvestability, scale 1 to 5)						0.4009**	NS
SW (Seed weight, g)							−0.7976**

[a] SPAD, leaf chlorophyll content (SPAD values).
[b] NS, not significant; ** significant at the 0.01 level.

genotypes and their mixtures (six SRMs and six ARMs) grouped together (right side). Interestingly, mixtures with both types [composed of between gene pool (Mesoamerican + Andean) genotypes] grouped close to each other and in a third cluster that was placed in the middle between the two within gene pool groups (mixture of two Mesoamerican or two Andean) cultivars (**Figure 5**).

Yield Advantage of Bean Cultivar Mixtures

The results of the current work indicated a yield advantage for some of the bean cultivar mixtures (**Figure 6**). Significant positive correlations were identified between the yields of the mixtures and yields of the midcomponent (average of two cultivar components evaluated in pure stands) averaged over three environments ($r = 0.9373$; $p < 0.0001$; $N = 42$; **Figure 6A**) as well as in each environment/location evaluated separately (**Figure S4A**).

When the mixture yield superiority over the yield of the midcomponent (yield of the mixture minus yield of the average of the two components in pure stands) was regressed over the yield of the mixture, a significant linear relationship was observed averaged over the three environments (**Figure 6B**) as well as in each environment/location separately, except at the ERS for the first planting (**Figure S4B**).

However, when the mixture yield superiority over the yield of the better component (yield of the mixture minus yield of the better component in a pure stand) was regressed over the yield of the mixture, no significant linear relationships was observed (**Figure 6C**, **Figure S4C**). Averaged over the three environments, only three mixtures (RBM, RBR, and DRRR) yielded more compared to the better yielding cultivar component, (**Figure 6C**).

The advantage of growing bean cultivars in mixtures instead of monocultures, was also assessed in comparison to the yield of the lower yielding component. When the mixture yield superiority over the yield of the lower yielding component (yield of the mixture minus yield of the lower yielding components in pure stands) was regressed over the yield of the mixture, a significant linear relationship was observed (**Figures 6D, 4D**). In general, mixtures yielded more than the lower yielding cultivar component [ranging from 5.4 kg ha^{-1} (LRRR) to 1141.0 kg ha^{-1} (ADR) in the mean environment]. Averaged over the three environments, only three between gene pool mixtures [LIM (−185.4 kg ha^{-1}), LRRM LIM (−90.1 kg ha^{-1}) and LIR LIM

(−41.6 kg ha^{-1})] yielded lower than the low-yielding cultivar component (**Figure 6D**).

Identification of the Best Performing Mixtures

Mixture Selection Based on the Yield and RYM Index

To identify the best performing mixture, yield and RYM index were plotted against each other [similar to (Nyikako et al., 2014)] for each environment/location and averaged over the three environments (**Figure 7**). In general, mixtures between higher yielding Mesoamerican genotypes placed in the two top quadrants while mixtures between lower yielding Andean genotypes were always in the bottom two quadrants.

The best performing mixtures (high yield and RYM >1) were identified in upper right quadrant. Based on the three-environment average data, 14 mixtures [seven SRM (RAM, LAM, RBM, LRM, LBM, BDM, and BRRM) and seven ARM (ADR, BRRR, RDR, RIR, RBR, LRR, and LBR)] had combined high yields [ranging from 3,555.8 kg ha^{-1} (RIR) to 4,547.7 kg ha^{-1} (RAM)] with RYM values >1 [ranging from 1.002 (BRRM) to 1.087 (LAM)]. Eight mixtures were biblends between Mesoamerican genotypes and six were between gene pool mixtures (**Figure 7**). Mixtures between white bean cultivars Lighthouse and Rexeter, Lighthouse, and Bolt, Rexeter and Bolt as well as Bolt (white) and Red Rider (cranberry) were good performers in both SRM and ARM. The mixtures LAM, LBM, and BRRR had the most stable performances across all environments (they consistently placed in the top right quadrant in all three environments/locations as well as averaged over the three environments).

Four out of the six Andean mixtures had good RYM values [ranging from 1.014 (IRRR) to 1.060 (DRRR)] but had low yields, averaged over the three environments (**Figure 7**). However, their performances were inconsistent among environments (**Figure S5**). Only the DRRR mixture [ARM biblends between Dynasty (dark red kidney) and Red Rider (cranberry)] consistently placed in the bottom right quadrant [yield lower than the average of the mixtures but RYM index over one (from 1.008 at the ERS first planting to 1.128 at the ERS second planting].

Although the majority of between gene pool biblends were placed on the left side quadrants with high (top) or low (bottom) yields and values of the RYM index below one, some of these

Order	Eigenvalue	Percentage	Cumulative percentage
1	15.6528	48.92	48.92
2	5.3982	16.87	65.78
3	2.8346	8.86	74.64
4	2.2004	6.88	81.52
5	1.1662	3.64	85.16
6	1.1500	3.59	88.76
7	0.8091	2.53	91.29
8	0.6411	2.00	93.29
9	0.5027	1.57	94.86
10	0.4612	1.44	96.30
11	0.3060	0.96	97.26
12	0.2364	0.74	98.00
13	0.1820	0.57	98.56
14	0.1464	0.46	99.02
15	0.1132	0.35	99.38
16	0.0694	0.22	99.59
17	0.0545	0.17	99.76
18	0.0247	0.08	99.84
19	0.0184	0.06	99.90
20	0.0116	0.04	99.93
21	0.0080	0.02	99.96
22	0.0057	0.02	99.98
23	0.0028	0.01	99.99
24	0.0021	0.01	99.99
25	0.0013	0.00	100.00
26	0.0004	0.00	100.00
27	0.0003	0.00	100.00
28	0.0002	0.00	100.00
29	0.0001	0.00	100.00
30	0.0001	0.00	100.00
31	0.0000	0.00	100.00
32	0.0000	0.00	100.00

mixtures had both high yields and good RYMs. For example, ARM biblend between small seeded white cultivar Bolt and large seeded cranberry cultivar Red Rider (BRRR) had both high yields and high RYMs [range from 1.032 (WRS) to 1.054 (ERS, planting 2)] in all three environments (**Figure S5**) as well as in the three environment average (**Figure 7**). This mixture also performed well as the SRM biblend having high values for yield and RYM at WRS and three-environment average.

Mixture Selection Based on Diallel Analysis

The performances of the bean SRM and ARM mixtures were further evaluated in separate diallel analyses and significant differences among seven bean cultivars and their mixtures for all measured traits were identified. This confirmed the Glimmix results that there was variability among the genotypes for the tested traits in three Ontario environments. The total genotypic variation of the data was further partitioned into general mixing ability (GMA) and specific mixing ability (SMA) of pure lines and

their binary mixtures, respectively. Mean squares (MS) for GMA were greater in magnitude than the SMA MS and GMA/SMA (Baker's) ratios were close to unity, which indicated that additive effects were more important than dominance in the expression of these traits (**Table 4**).

Significant GMA effects were observed for all seven measured traits. SMA had significant effects only for yield and maturity in SRM and seed weight in ARM biblends. Individual GMA and SMA analyses were also conducted for each environment/location (**Table S8**). Significant genotype × environment interactions (G × E) were observed for the majority of the traits, except for the SPAD in SRM and seed weight in the ARM biblends.

Both positive and negative estimates of GMA effects were observed among cultivar components of the mixtures (pure stands) for all analyzed traits (**Tables 5**, **6**). For example, the highest positive GMA effect on yield was observed in a small seeded black cultivar OAC Vortex, which also had the highest value for this trait. Similarly, the large seeded kidney bean cultivar Dynasty had a negative GMA value for the trait and had the lowest yield. Therefore, cultivar OAC Vortex was the best general mixer with the maximum positive GMA effects on yield (673.5 in SRM and 564.6 in ARM) but the poorest general mixer for plant height, having the highest positive GMA effect (if short plants are desirable) on this trait, averaged over three environments (**Tables 5**, **6**). On the other hand, with the maximum negative values of the GMA effects on yield (−400.2 in SRM and −345.3 in ARM biblends), cultivar Dynasty was the poorest general mixer for this trait, and also for SPAD, but increased seed weight (+12.3 in SRM) and reduced flowering (−2.0).

In the SRM biblends, 10 mixtures had positive SMA values for yield (**Table 5**). The most positive SMA effect was observed in the low (Lighthouse, GMA = −147.2) × high (OAC Vortex, GMA = 673.5) LAM mixture (SMA = 273.5) and the most negative SMA effect for yield was identified in the high (Rexeter, GMA = 214.7) × low (Dynasty, GMA = −400.2) RDM mixture (SMA = −365.4). The most positive SMA effect for flowering was observed in the low × low BRRM mixture (SMA = 0.298), for the maturity in the high (OAC Inferno, GMA = 0.281) × low (Red Rider, GMA = −2.071)] IRRM mixture (SMA = 1.138), for plant height in the low × low IRRM mixture (SMA = 1.837), for harvestability in the high × high IDM mixture (SMA = 0.420), for seed weight was found in the low × low LBM mixture (SMA = 1.387) and for the SPAD readings in the low × low IRRM mixture (SMA = 0.982). The most negative SMA effect for flowering was found in the high × low LIM biblend (SMA = −0.240), for maturity in the high × high RIM biblend (SMA = −1.2930), for plant height in the high × low LRRM biblend (SMA = −1.517), for harvestability in the high × high DRRM biblend (SMA = −0.344), for seed weight in the high × high DRRM biblend (SMA = −1.276)and for the SPAD readings in the high × low LIM biblend (SMA = −1.089) (**Table 5**). Similar trends in SRM biblends were observed when analyses were performed separately for each environment/location (**Table S9**).

In the ARM biblends, 12 mixtures had positive SMA values for yield (**Table 6**). The most positive SMA effects was observed in the low (Dynasty, GMA = −345.3) × low (Red Rider, GMA =

FIGURE 5 | Genotype-trait (GT) biplot analysis for seven common bean genotypes and their mixtures in three Ontario environments/locations in 2018. Cumulatively, first two principal components (PC) explained 65.8% variability. Genotypes separation into Andean (A, left), Mesoamerican (MA, right) and combined (MA + A, middle) is indicated. Traits: yield (YD, kg ha⁻¹), relative yield of the mixture (RYM) index, flowering (DF, days), maturity (DM, days), plant height (PH, cm), harvestability (HR, scale 1 to 5), seed weight (SW, g) and leaf chlorophyll content (SPAD, SPAD values). Environments/locations: mean environment [trait symbol with a year (e.g. YD18)], Woodstock research station (WRS18), Elora research station planting 1 (1ERS18) and planting 2 (2ERS18). Additional information (scree plot, eigenvectors and genotype scores) can be found in **Figure S3**.
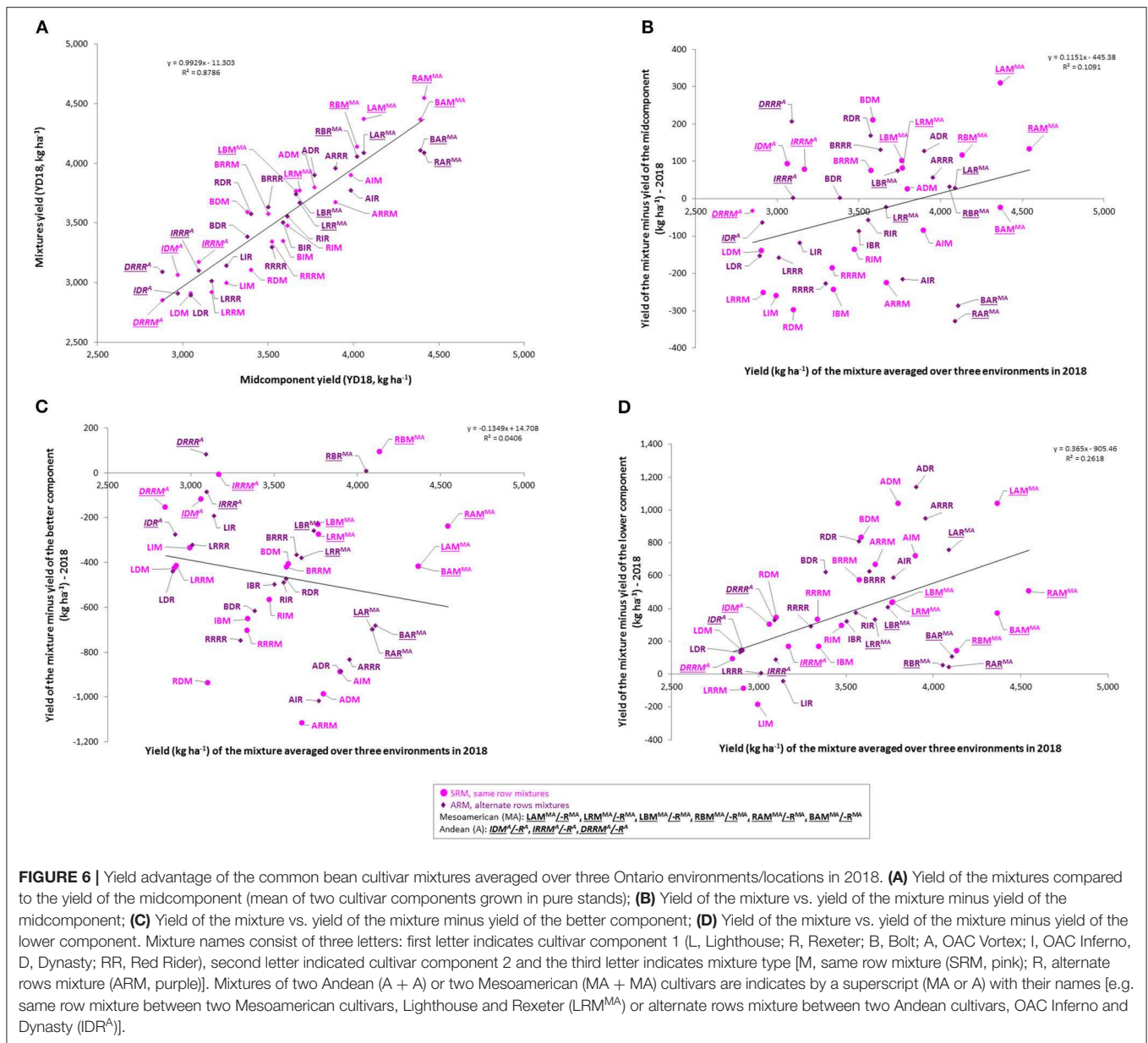
FIGURE 6 | Yield advantage of the common bean cultivar mixtures averaged over three Ontario environments/locations in 2018. **(A)** Yield of the mixtures compared to the yield of the midcomponent (mean of two cultivar components grown in pure stands); **(B)** Yield of the mixture vs. yield of the mixture minus yield of the midcomponent; **(C)** Yield of the mixture vs. yield of the mixture minus yield of the better component; **(D)** Yield of the mixture vs. yield of the mixture minus yield of the lower component. Mixture names consist of three letters: first letter indicates cultivar component 1 (L, Lighthouse; R, Rexeter; B, Bolt; A, OAC Vortex; I, OAC Inferno, D, Dynasty; RR, Red Rider), second letter indicated cultivar component 2 and the third letter indicates mixture type [M, same row mixture (SRM, pink); R, alternate rows mixture (ARM, purple)]. Mixtures of two Andean (A + A) or two Mesoamerican (MA + MA) cultivars are indicates by a superscript (MA or A) with their names [e.g. same row mixture between two Mesoamerican cultivars, Lighthouse and Rexeter (LRM^{MA}) or alternate rows mixture between two Andean cultivars, OAC Inferno and Dynasty (IDR^{A})].

−246.5) DRRR mixture (SMA = 177.1) and the most negative SMA effects for yield was found in the high × low RRRR mixture (SMA = −242.0). The most positive SMA effect for flowering was found in the high × high RAR mixture (SMA = 0.579), for maturity in the low × low BRRR mixture (SMA = 0.954), for plant height was observed in the high × high LAR mixture (SMA = 1.209), for harvestability in the high × high LIR mixture (SMA = 0.146), for seed weight in the low × high BIR mixture (SMA = 0.952) and for SPAD in the high × high BAR biblend (SMA = 0.704). The most negative SMA effect for flowering was observed in the high × low RRRR biblend (SMA = −0.364), for maturity in the high × high RIR biblend (SMA = −0.969), for plant height in the high × low LDR biblend (SMA = −1.625), for harvestability in the low × low BDR biblend (SMA

= −0.275), seed weight in the low × high LDR biblend (SMA = −1.384) and for SPAD in the high × low AIR mixture (SMA = −0.793) (**Table 6**). Similar trends for the ARM biblends were observed when the analyses were performed separately for each environment/location (**Table S10**).

Best Performing (Yielding) Mixtures

Significant association was identified between values of seven measured traits and their mixing abilities (GMA of cultivar components and SMA of biblends) in both types of the mixtures (**Figure 8**, **Figure S6**). For yield, correlation was higher in SRM (r = 0.682) compared to the ARM (r = 0.598) biblends.

When evaluated in pure stands, three Mesoamerican genotypes (Rexeter, Bolt, and OAC Vortex) were higher yielding
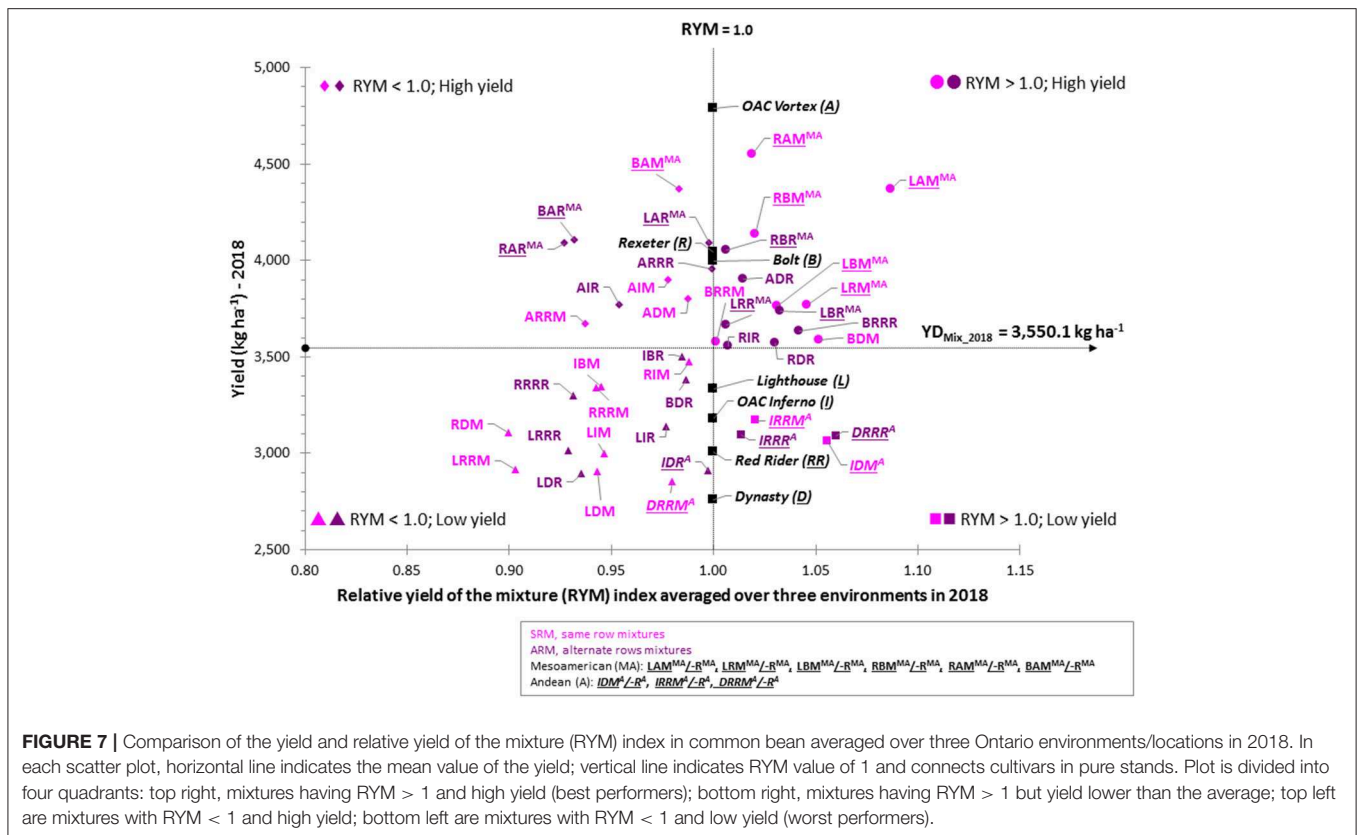
FIGURE 7 | Comparison of the yield and relative yield of the mixture (RYM) index in common bean averaged over three Ontario environments/locations in 2018. In each scatter plot, horizontal line indicates the mean value of the yield; vertical line indicates RYM value of 1 and connects cultivars in pure stands. Plot is divided into four quadrants: top right, mixtures having RYM > 1 and high yield (best performers); bottom right, mixtures having RYM > 1 but yield lower than the average; top left are mixtures with RYM < 1 and high yield; bottom left are mixtures with RYM < 1 and low yield (worst performers).

compared to the average of all seven genotypes (3,587 kg ha^{-1}) and had positive general mixing ability (GMA). In contrast, Lighthouse and all three Andean cultivars (OAC Inferno, Dynasty, and Red Rider) had negative GMA effects and had lower yields compared to the average yield of all seven genotypes (**Figure 9**).

A combination of high yield, RYM index >1 (RYM >1) and positive SMA was used to identify mixtures that outperformed (in yield) cultivar components when evaluated in pure stands. All 12 biblends formed among the four Mesoamerican genotypes had higher yields compared to the average yield of all biblends [3,555.1 kg ha^{-1}(SRMs) and 3,545.1 kg ha^{-1}(ARMs). In addition, five SRMs (LRM, LBM, LAM, RBM, and RAM) and four ARMs (LRR, LBR, LAR, and RBR), respectively, had positive SMA effects and values of the RYMs >1 (**Figure 9**). Although high yielding, three mixtures [BAM (SRM), RAR (ARM), and BAR (ARM)] showed negative SMA effects and RYM index values below one and one ARM biblend (LAR) had positive SMA effects but did not outperform cultivar components in pure stands (RYM <1). Pure Andean biblends (three SRMs and three ARMs) were lower yielding compared to the mixture averages. However, two SRMs (IDM and IRRM) and two ARMs (IRRR and DRRR) performed better than their cultivar components in pure stands (RYM >1) and showed positive SMA effects. Yields of the between gene pool biblends were variable; only five SRMs (BDM, BRRM, AIM, ADM, and ARRM) and six ARMs (RIR, RDR, BRRR, AIR, ADR, and ARRR) had yields higher than the averages of the two mixture types. Among them only two

of the SRMs (BDM and BRRM) and four of the ARMs (RIR, RDR, BRRR, and ADR) biblends had positive SMA effects and RYMs >1.

Based on these three parameters (above average yield, RYM >1 and positive SMA effects) seven SRMs (LRM, LBM, LAM, RBM, RAM, BDM, and BRRM) and seven ARMs (LRR, LBR, RBR, RIR, RDR, BRRR, and ADR) could be selected as better performers compared to their cultivar components evaluated in pure stands. Among them, four biblends (LR, LB, RB, and BRR) were good performers both as SRM and ARM. The best SRM was biblend between the two Mesoamerican cultivars, white Lighthouse and black OAC Vortex (LAM), having the highest positive SMA effect (273.5), second highest yield among the SRMs (4,368.9 kg ha^{-1}) and a potential yield increase of 8.7% based on the RYM index (1.087). Among the ARMs, the best performer was a between gene pool BRRR mixture (white Bolt and cranberry Red Rider) with the SMA highest in ARM biblends (150.8), high yield (3,633.5 kg ha^{-1}) and a potential yield increase of 4.2% (RYM = 1.042).

DISCUSSION

A common criticism of modern field crop production is that it relies on highly related cultivars propagated in monocultures that are selected for performance under ideal conditions but are poised for collapse under stress. A study of Canadian beans revealed that many of the small seeded Mesoamerican cultivars have common parents, and large seeded (Andean) cultivars,

TABLE 4 | Analysis of variance for general mixing ability (GMA) and specific mixing ability (SMA) of the bean same row (SRM) and alternate rows (ARM) binary mixtures evaluated in three Ontario environments/locations in 2018.

Source of variation	Degrees of freedom	MS[a]						
		Yield (kg ha^{-1})	Flowering (days)	Maturity (days)	Plant height (cm)	Harvestability (scale 1–5)	Seed weight (g)	Chlorophyll (SPAD values)
SRM								
Environment (E)	2	14,145,440.00[NS]	306.86**	832.21**	31.46[NS]	75.34**	345.90**	422.28**
Rep [E]	9	4,395,491.00**	1.62**	4.12*	50.11**	0.84**	22.45**	32.48**
Genotype (G)	27	4,062,830.00**	94.86**	107.10**	99.06**	2.38**	2,869.94**	24.76**
G × E	54	331,753.40**	1.81**	8.67**	26.37**	0.47**	7.66**	3.80[NS]
Residuals	277	176,223.10	0.42	1.79	9.84	0.22	4.04	2.79
GMA	6	16,751,368.00**	425.53**	452.89**	402.15**	9.04**	12,892.50**	98.28**
SMA	21	437,533.60**	0.38[NS]	8.30*	12.46[NS]	0.48[NS]	6.36[NS]	3.75[NS]
GMA × E	12	981,533.40**	6.61**	23.69**	73.17**	1.04**	19.20**	5.08*
SMA × E	42	146,102.00[NS]	0.44[NS]	4.38**	13.00[NS]	0.31[NS]	4.36[NS]	3.43[NS]
Baker's ratio[b]		0.9872	0.9996	0.9909	0.9847	0.9741	0.9995	0.9813
ARM								
Environment (E)	2	11,013,773.00[NS]	283.24**	935.28**	37.09[NS]	66.05**	380.85**	377.74*
Rep [E]	9	3,274,656.00**	1.73**	4.36*	42.23**	0.70**	13.72**	48.95**
Genotype (G)	27	2,938,212.00**	94.28**	95.68**	84.19**	2.21**	2,918.35**	19.47**
G × E	54	403,834.20**	1.76**	9.21**	26.67**	0.59**	5.11[NS]	3.94**
Residuals	277	188,995.40	0.40	1.86	8.62	0.26	3.85	2.17
GMA	6	12,368,638.00**	422.00**	413.67**	362.56*	8.26**	13,112.08**	82.91**
SMA	21	243,804.60[NS]	0.65[NS]	4.82[NS]	4.66[NS]	0.49[NS]	5.85*	1.34[NS]
GMA × E	12	1,199,459.00**	6.30**	25.76**	83.08**	1.11**	13.63**	8.29**
SMA × E	42	176,513.00[NS]	0.47[NS]	4.48**	10.55[NS]	0.44**	2.67[NS]	2.69[NS]
Baker's ratio		0.9902	0.9992	0.9942	0.9936	0.9712	0.9998	0.9920

[a]MS, Mean square.

[b]Baker's ratio of GMA to SMA variance, calculated as $2MS_{GCA}/2MS_{GCA} + MS_{SCA}$.

*, **Significant at the 0.05 and 0.01 probability level, respectively; NS, not significant.

have relatively narrow genetic diversity (Navabi et al., 2014). The use of cultivar mixtures has been suggested as a means of increasing genetic diversity in the production field, and achieving better disease control, increasing of crop productivity and, resiliency to stress and disease resistance (Browning and Frey, 1969; Wortmann et al., 1996; Kristoffersen et al., 2020). Increased plant diversity may also increase ecosystem functioning (Loreau et al., 2001; Letourneau et al., 2011; Bybee-Finley and Ryan, 2018). However, although widely used in the tropics and some temperate regions, intracropping is not a common agricultural practice in developed countries. Currently, the most successful use of the cultivar mixtures is for disease control in small cereals (Bowden et al., 2001; Tratwal and Bocianowski, 2018; Giunta et al., 2020). The aim of the current study was to determine the agronomic (including crop yield) and ecosystem effects of increasing bean crop diversity by using cultivar mixtures instead of monocultures in cropping systems in Ontario environments.

Genotype Selection and Mixture Formation

To maximize the diversity of the mixtures in the current study, genotypes that originated from both Mesoamerican and Andean gene pools differing in their architecture, phenological traits, and resistance to several bean diseases were selected as mixing components. All cultivars had high yield potentials, and this was their first use in mixture studies.

Although widely used in intercropping, the replacement series design confounds the effects of the plant size, plant density, and species proportion (Connolly, 1986; Snaydon, 1991; Banik, 1996; Jolliffe, 2000; Connolly et al., 2001a,b; Bybee-Finley and Ryan, 2018). In this study, a "fixed-ratio mixture design" (Federer, 1993) was used where both cultivars were considered as main crops. In all mixtures, two cultivar components (same number of seeds) were jointly evaluated relative to their response in pure stands. This gave a single and accurate performance result for each mixture. However, it is possible that improved productivity for one cultivar may have resulted in a yield reduction of the other component, but this assessment was not available with the current study design. In particular, differences in plant size and architecture between Mesoamerican and Andean cultivar (Kelly, 2001) components could have affected the performance of the MA mixtures, both negatively (through shading for example), and positively (through nitrogen fixation).

TABLE 5 | Estimated general mixing ability (GMA) for traits analyzed in seven bean genotypes and specific mixing ability (SMA) of their same row binary mixtures (SRM) averaged over three Ontario environments/locations in 2018.

GMA/SMA	Genotypes	Traits[a]						
		YD	DF	DM	PH	HR	SW	SPAD
Pure stands								
GMA_Cultivars	1. Lighthouse (L)	−147.22	2.409	2.248	0.435	0.020	−9.194	0.638
	2. Rexeter (R)	214.67	1.890	1.442	0.254	0.138	−9.895	0.591
	3. Bolt (B)	259.18	−0.703	−2.553	0.396	−0.557	−7.678	0.872
	4. OAC Vortex (A)	673.48	1.908	2.188	3.618	−0.059	−8.183	0.510
	5. OAC Inferno (I)	−268.18	−1.735	0.281	−0.960	0.408	11.609	−0.002
	6. Dynasty (D)	−400.22	−1.982	−1.534	−1.197	0.003	12.276	−1.678
	7. Red Rider (RR)	−331.71	−1.787	−2.071	−2.547	0.047	11.065	−0.932
	LSD (Gg$_i$-G$_j$)[b]	112.52	0.173	0.359	0.841	0.125	0.539	0.448
Mixtures								
SMA_Same Row mixtures (SRM)	1_2 (LRM)	134.47	0.218	0.780	0.291	−0.075	−0.071	−0.038
	1_3 (LBM)	147.24	0.102	−0.432	0.249	−0.089	1.387	0.505
	1_4 (LAM)	273.51	0.117	−0.339	1.818	0.059	−0.325	0.083
	1_5 (LIM)	−243.27	−0.240	−1.182	−0.061	0.008	−0.375	−1.089
	1_6 (LDM)	−167.04	0.196	−1.033	−0.867	−0.004	−0.292	−0.613
	1_7 (LRRM)	−273.58	0.293	−0.663	−1.517	−0.110	−1.114	−0.325
	2_3 (RBM)	86.90	−0.170	−0.043	1.638	−0.145	0.522	0.444
	2_4 (RAM)	138.51	−0.073	0.300	0.457	−0.017	0.026	0.555
	2_5 (RIM)	−34.70	−0.013	−1.293	−1.506	−0.152	−0.940	−0.483
	2_6 (RDM)	−365.42	−0.017	−0.811	−0.770	−0.038	−0.369	−0.524
	2_7 (RRRM)	−65.87	−0.169	−0.858	0.330	−0.061	0.088	−0.236
	3_4 (BAM)	−120.85	0.020	−0.622	−0.284	−0.010	−0.632	0.183
	3_5 (BIM)	−249.28	−0.004	−0.646	−1.106	0.189	−0.616	0.145
	3_6 (BDM)	180.10	−0.007	0.017	0.589	−0.115	0.692	−0.454
	3_7 (BRRM)	40.55	0.298	0.471	−0.020	−0.179	−0.346	−0.350
	4_5 (AIM)	−53.19	0.094	−0.122	1.005	0.045	0.256	0.364
	4_6 (ADM)	63.40	−0.118	0.193	−0.692	−0.196	0.147	0.407
	4_7 (ARRM)	−215.63	−0.063	0.647	−1.159	−0.073	1.150	−0.813
	5_6 (IDM)	217.50	0.150	0.017	0.695	0.420	0.089	0.177
	5_7 (IRRM)	253.12	−0.044	1.138	1.837	0.085	0.142	0.982
	6_7 (DRRM)	−61.38	0.086	0.286	0.348	−0.344	−1.276	−0.067
	LSD$_{0.05}$ (s$_{ij}$-s$_{ik}$)[c]	318.26	0.490	1.014	2.378	0.354	1.524	1.267
	LSD$_{0.05}$ (s$_{ij}$-s$_{kl}$)[d]	297.71	0.459	0.949	2.224	0.331	1.426	1.186

[a] Traits: YD, yield (kg ha^{-1}); DF, flowering (days); DM, maturity (days); PH, plant height (cm); HR, harvestability (scale 1–5); SW, seed weight (g); SPAD, leaf chlorophyll content (SPAD values).
[b] Least significant difference for GMA for comparing genotypes (pure stands).
[c] Least significant difference for SMA for comparing mixtures with one common cultivar component.
[d] Least significant difference for SMA for comparing different mixtures (no cultivar component in common).

The current work was restricted to two cultivar mixtures with a constant single (1:1) density, which allowed the use of diallel analysis to test mixing ability of cultivar components as well as performance of the mixtures. Kiær et al. (2009) found significant increases in the relative mixing effects with the number and diversity of the component cultivars in a meta-analysis of grain yields in cereal mixtures. However, Frey and Maldonado (1967) did not find an association between relative yield and the number of cultivars in oat mixtures, analyzed over two planting dates; in their study mixtures containing two or five genotypes were equally effective.

Reasons to Choose a Cultivar Mixture Over the Monoculture in Industrialized Agriculture
Potential Buffering Effect

One of the advantages of intercropping systems is a buffering effect against various biotic and abiotic factors (Finckh et al., 2000). In the current study, environmental buffering was reflected in the finding that significant SMA × E effects were observed only for a few traits for the mixtures [maturity (SRM and ARM) and harvestability (ARM)],

TABLE 6 | Estimated general mixing ability (GMA) for traits analyzed in seven bean genotypes and specific mixing ability (SMA) of their same row binary mixtures (ARM) averaged over three Ontario environments/locations in 2018.

GMA/SMA	Genotypes	Traits[a]						
		YD	DF	DM	PH	HR	SW	SPAD
Pure stands								
GMA_Cultivars	1. Lighthouse (L)	−149.59	2.223	2.286	0.362	0.063	−9.458	0.905
	2. Rexeter (R)	214.10	1.919	1.585	0.434	0.163	−9.736	0.581
	3. Bolt (B)	225.75	−0.660	−2.474	0.321	−0.542	−7.696	0.828
	4. OAC Vortex (A)	564.60	2.042	1.675	3.400	−0.108	−8.379	0.073
	5. OAC Inferno (I)	−262.98	−1.720	0.372	−1.105	0.336	11.979	−0.185
	6. Dynasty (D)	−345.34	−1.997	−1.368	−0.959	−0.014	11.861	−1.488
	7. Red Rider (RR)	−246.53	−1.808	−2.075	−2.454	0.102	11.428	−0.714
	LSD (Gg$_i$-G$_j$)[b]	116.53	0.170	0.366	0.787	0.136	0.526	0.395
Mixtures								
SMA_Alternate Rows Mixtures (ARM)	1_2 (LRR)	45.92	−0.145	−0.050	−0.100	−0.016	0.195	0.109
	1_3 (LBR)	40.12	0.101	−0.157	−0.279	−0.123	0.122	−0.287
	1_4 (LAR)	95.82	0.011	−0.223	1.209	−0.035	0.027	−0.533
	1_5 (LIR)	40.93	−0.214	−0.169	0.148	0.146	0.122	0.366
	1_6 (LDR)	−179.21	0.105	−0.930	−1.625	0.037	−1.384	−0.322
	1_7 (LRRR)	−170.63	0.040	−0.806	0.121	−0.224	−0.869	0.363
	2_3 (RBR)	98.27	−0.054	−0.457	−0.017	0.069	0.041	0.079
	2_4 (RAR)	−229.85	0.579	0.091	0.226	−0.148	−0.484	−0.184
	2_5 (RIR)	89.09	−0.160	−0.969	0.326	−0.121	0.241	−0.060
	2_6 (RDR)	141.31	−0.008	0.188	0.471	−0.167	−0.149	−0.014
	2_7 (RRRR)	−242.01	−0.364	0.228	0.107	−0.179	0.109	−0.147
	3_4 (BAR)	−237.04	−0.301	−0.713	−0.275	0.090	−0.507	0.704
	3_5 (BIR)	−10.50	0.044	0.055	0.122	0.021	0.952	−0.181
	3_6 (BDR)	−79.55	0.321	−0.055	0.788	−0.275	−0.538	0.056
	3_7 (BRRR)	150.76	0.256	0.954	0.454	−0.245	0.927	0.032
	4_5 (AIR)	−158.48	0.259	−0.751	−0.807	−0.121	−0.574	−0.793
	4_6 (ADR)	81.38	−0.213	−0.152	−0.354	−0.272	0.536	−0.014
	4_7 (ARRR)	109.55	0.097	0.138	0.125	−0.075	0.903	−0.047
	5_6 (IDR)	−43.22	−0.119	0.235	0.968	0.139	−0.405	0.127
	5_7 (IRRR)	4.30	0.150	0.442	−0.620	0.065	−0.239	0.011
	6_7 (DRRR)	177.09	0.094	0.432	−0.142	0.019	−0.671	−0.044
	LSD$_{0.05}$ (s$_{ij}$-s$_{ik}$)[c]	329.60	0.481	1.035	2.226	0.384	1.487	1.116
	LSD$_{0.05}$ (s$_{ij}$-s$_{kl}$)[d]	308.31	0.450	0.968	2.082	0.357	1.391	1.044

[a] Traits: YD, yield (kg ha^{-1}); DF, flowering (days); DM, maturity (days); PH, plant height (cm); HR, harvestability (scale 1–5); SW, seed weight (g); SPAD, leaf chlorophyll content (SPAD values).
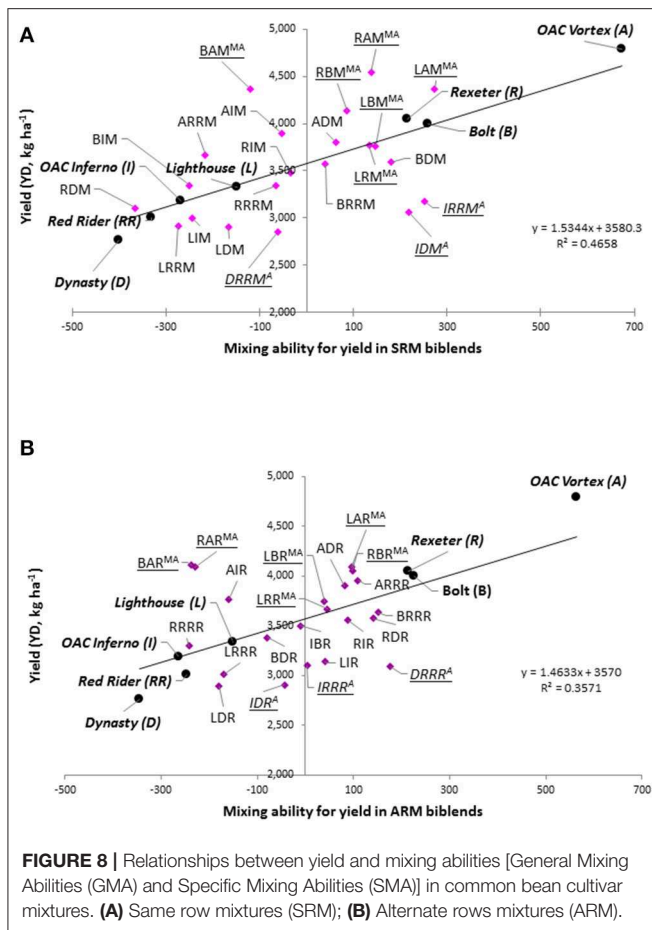[b] Least significant difference for GMA for comparing genotypes (pure stands).
[c] Least significant difference for SMA for comparing mixtures with one common cultivar component.
[d] Least significant difference for SMA for comparing different mixtures (no cultivar component in common).

compared to the cultivars in pure stands for which significant environmental effects were observed for all of the traits. A graphic illustration of the buffering potential was observed in some plots at the ERS (2017) which were flooded shortly after the planting and resulted in reduced establishment in the rows with the black bean cultivar OAC Vortex but did not affect the rows seeded with the navy bean Lighthouse. During the season the Lighthouse plants filled in the gaps in the mixed plots so that by the end of the season there was canopy closure (data not shown) and overall yields in the mixture plots were similar to the two parent means. This type of compensatory effect is

similar to the yield protection seen in mixtures of bean cultivars grown in Palmira, Colombia (Panse et al., 1989) where it was observed that mixtures had a greater potential to compensate for plant establishment losses caused by inferior seed quality of individual genotypes. Panse et al. (1989) concluded that intergenotypic compensation was a major determinant for a positive mixture effect on yield. However, further analysis including yield, field germination, and number of plants at harvesting for each cultivar component in the mixtures used in the current study would be necessary for a proper assessment of potential buffering (compensatory) effect in mixtures of

FIGURE 8 | Relationships between yield and mixing abilities [General Mixing Abilities (GMA) and Specific Mixing Abilities (SMA)] in common bean cultivar mixtures. **(A)** Same row mixtures (SRM); **(B)** Alternate rows mixtures (ARM).

Lighthouse and OAC Vortex as well as bean cultivar mixtures in general.

Better Disease Resistance - Could Not Be Determined

Anthracnose is a seed-borne fungal disease that can significantly reduce yield and quality in all bean market classes. Ontario is characterized by frequent warm and humid summers that make ideal conditions for a rapid fungal spread. This could result in anthracnose outbreaks and Ontario bean fields are regularly monitored for disease symptoms (OMAFRA, 2018; Conner et al., 2020) and treated with fungicides to prevent outbreaks.

In the current study, bean genotypes were scored for the anthracnose symptoms under the field conditions. However, potential advantage of bean cultivar mixtures over monocrops in resistance to anthracnose could not be assessed in the present study. There was no significant difference between mixtures and pure stands in 2017 and no disease symptoms were detected in 2018. To obtain necessary environmental conditions for fungal development, a future work should include screening bean cultivar mixtures in anthracnose disease nursery.

Yield Advantage Was Identified in Some Cultivar Mixtures

Both positive and negative effects of mixing bean cultivars on yield were observed in the current study. Similar findings were reported for the mixtures in other crops. For example, working with cowpea cultivar mixtures, Okonya and Maass (2014) noticed that mixtures grown in marginal environments were more stable than a single cultivar but not all mixtures yielded more than a single cultivar. A significant positive correlation was identified between yield of the mixtures and yield of the midcomponent. These findings are similar to Gallandt et al. (2001) and suggested that mixture yield could be predicted from the average yield of the two cultivar components grown in pure stands.

In general, the two types of common bean cultivar mixtures used in the current study, namely the same row mixtures SRMs) and the alternate row mixtures (ARMs) were not significantly different (average of SRMs vs. average of ARMs) for the traits that were evaluated. However, some individual mixtures were significantly different in the SRM vs. the ARM configuration. There were also differences between Mesoamerican and Andean (M vs. A) mixtures, probably due to different plant architecture and root systems (Singh et al., 2019; Strock et al., 2019). The lowest yield in the ARM configuration was identified in mixture between the two large seeded colored kidney bean cultivars Dynasty and OAC Inferno.

In general, between gene pool mixtures (both SRMs and ARMs) had intermediate yield values that were closer to the small seeded beans. This agrees with findings of Essah and Stoskopf (2002) who observed that mixtures of four diverse barley cultivars (evaluated as ARMs and SRMs at ERS over two years) yielded similar to the midvalue. Only two of the 12 gene pool biblends overyielded the monocrops, by 13 and 14%, respectively.

Similar to Diaz et al. (2018), significant positive correlations among the three environments/locations for all analyzed traits (except the RYM index) were identified in the current study. In general, the strongest associations for the majority of the traits were observed between the two plantings at the ERS, illustrating the importance of the fit of genotypes, singly or in mixtures, to their environment.

Some trait associations were specific for the plant materials and environments used in this study but, some confirmed previously identified correlations. For example, in a number of studies, earliness is negatively associated with productivity (Kamfwa et al., 2015; Polania et al., 2016; Diaz et al., 2017). Similarly, in the current study, yield was significantly positively correlated with flowering and maturity, except at the WRS. At this location, yield was negatively associated with maturity. Similar contrary findings between environments were reported in a number of previous studies (Asfaw et al., 2017; Polania et al., 2017; Diaz et al., 2018) emphasizing the importance of environmental effects. In addition, a positive correlation between yield and SPAD confirmed relationships between these traits identified previously in bean (Guler and Ozcelik, 2007).

SPAD readings are used as a measurement of relative chlorophyll content in leaves, which relates to photosynthetic capacity. Higher SPAD values are often associated with a higher photosynthetic rate and potentially higher yield.
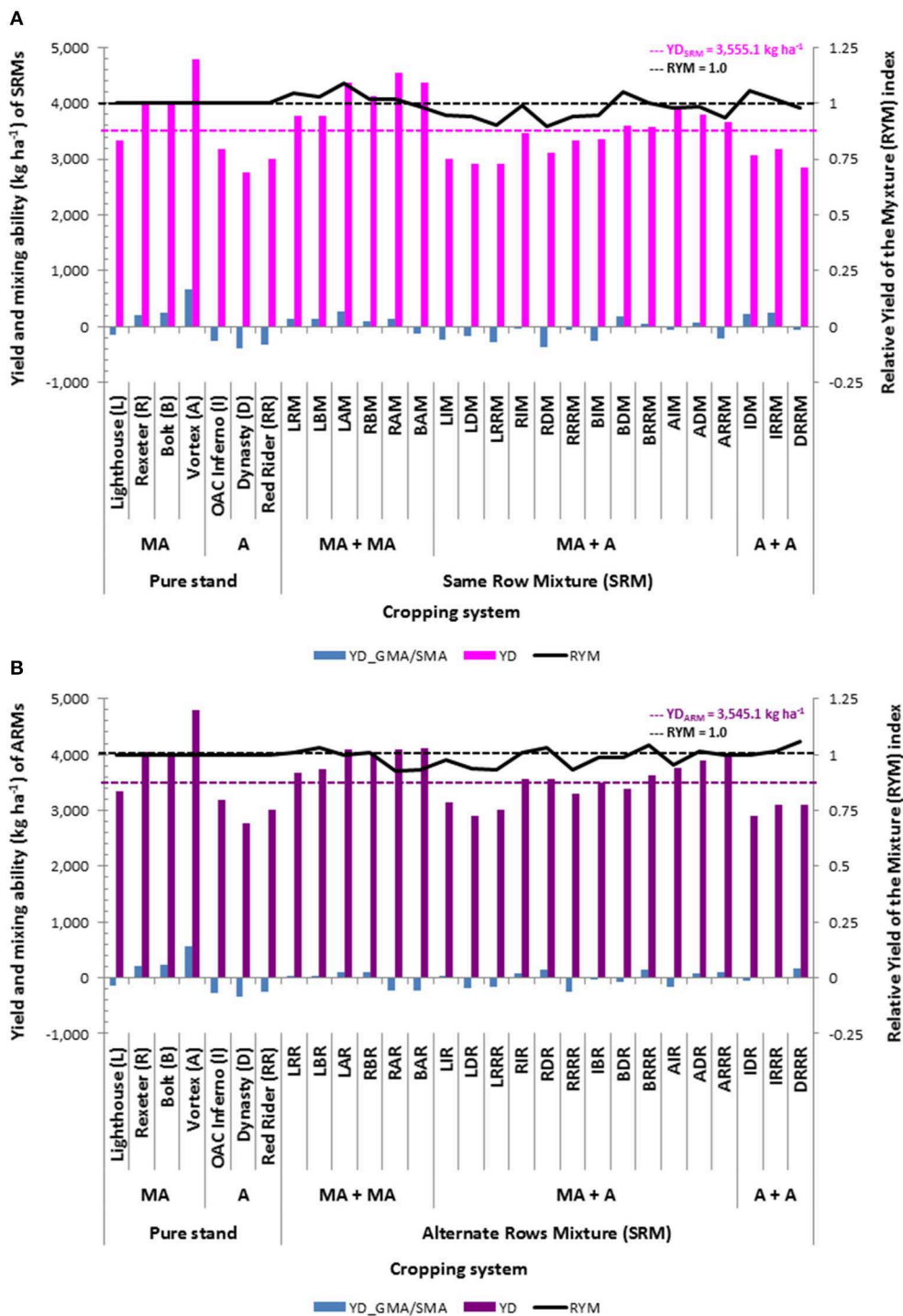
FIGURE 9 | Selection of the best mixture of common bean cultivars based on the yield, relative yield of the mixture (RYM) index and mixing ability. **(A)** Same row mixtures (SRM); **(B)** Alternate rows mixtures (ARM).

In the current study, yield was negatively correlated with seed weight. In some studies, positive correlations were identified in various environmental conditions (Mukeshimana et al., 2014; Polania et al., 2016; Assefa et al., 2017; Diaz et al., 2018) and in the others, both positive and negative correlations were identified (Asfaw et al., 2017).

Based on the RYM Index, Some Mixtures Outperformed Monocrops

To evaluate the bean cultivar mixtures, a combined crop response was of interest and use of the RYM index was appropriate to determine mixing efficiencies of particular combinations. There were some synergistic effects of intracropping on total productivity, as the total yield and RYM index of some bean mixtures at the 50:50 seed rate exceeded the average yields of the cultivars grown in pure stands. No particular influence of environment on the RYM index was noted. It appeared that specific mixtures had RYM indices that were optimal for specific environments.

In mixtures consisting of components with very similar resource utilization patterns, competition rather than cooperation can be expected. In this case, mixtures generally yield less than the higher yielding monocrop (Rao and Willey, 1980). The bean cultivars that were chosen for the study had similar maturities to ensure that they could be harvested together but that choice may also have resulted in competition among the components. However, not all mixtures had RYM values >1.0. Averaged over three environments/locations, the RYMs ranged from 0.900 (RDM) to 1.087 (LAM) in the SRMs and from 0.927 (RAR) to 1.060 (DRRR) in the ARMs. Also, in the present study the RYM index also showed a positive relationship with the sum of the yields of the two cultivar components in the mixture, indicating that the nature of the interaction between components was mainly facilitation, rather than competition (Li et al., 1999; Loreau and Hector, 2001).

It may be possible to design mixtures with non-competitive growth patterns. For example, Mesoamerican and Andean genotypes have different root distribution patterns (Strock et al., 2019). Henry et al. (2010) evaluated three bean multilines each composed of two bean RILs with contrasting root architectures in seven environments in Honduras, in soils with varying phosphorus levels and moisture availabilities. Production of the multilines was not associated with a yield penalty, even though variation in belowground competition among bean genotypes was identified.

Diallel Analysis Enabled Identification of Bean Cultivar(s) Having the Best Mixing Abilities

Higher values of General Mixing Abilities (GMA) MS compared to Specific Mixing Abilities (SMA) MS and GMA/SMA ratios closer to unity, indicated the importance of additive effects in the expression of traits evaluated in the current study (Griffing, 1956). The finding that significant GMA effects were observed for all seven measured traits indicated that significant differences existed among the cultivar components of the mixtures and suggests that there was at least one (or more) cultivar(s) that demonstrated superior mean performances in the mixture combinations. OAC Vortex, Bolt and Rexeter were genotypes that were frequently members of mixtures with high yields, whereas, Dynasty and Red Rider had poor GMA values for yield.

SMA was significant only for yield and maturity in SRM and ARM biblends. The lack of significant SMA effects for most traits suggests that there is a lack of complementary phenotypic variability among the genotypes for the traits that were evaluated.

When diallel mating is used in a breeding program, at least one parent should have good general combining ability and the best combination must be the one presenting the highest SCA (Griffing, 1956). In the current study, mixtures with high SMA for the analyzed traits were obtained from mixing cultivar components with various types of GMA effects (high × high, high × low, and low × low general mixers) and were different in SRM and ARM biblends. For example, OAC Inferno and Red Rider, which had a poor GMA values, combined in a mixture with the highest SMA. The results indicate that good mixture combinations will need to be determined experimentally, on a case-by case basis.

The existence of significant mixture × E interactions (observed for majority of the traits, except for the SPAD in SRM and seed weight in ARM biblends) suggested that the mixtures responded differently in different environments. In the current study, it probably resulted from the significant GMA × E (all traits). In general, small seeded Mesoamerican beans (Lighthouse, Rexeter, Bolt, and OAC Vortex) had positive GMA values for most of the traits except seed weight. These genotypes generally contributed positively to the yield and SPAD readings of the mixtures but had negative effects on other traits resulting in delays in flowering and lengthening the time to maturity. In contrast, the large seeded Andean cultivars (OAC Inferno, Dynasty, and Red Rider) had negative effects on the yields and SPAD readings of the mixtures and their inclusion as mixture components resulted in reductions in days to flowering and maturity, as well as increases in seed weight and harvestability. Therefore, between gene pool mixtures combined the potential for higher yield and SPAD readings and reduced harvestability from Mesoamerican component with positive effects of the Andean component to increase seed weight and phenological traits (flowering and maturity).

Selection of the Best Performing Mixture

This work confirmed that the selection of the best performing mixtures needs to be carried out using several approaches including direct mixture performance (trait-based), or index- or diallel-based selection. Different superior mixtures will be identified, based on the selection method used, and all of them have some limitations.

Based on the performance (yield) a small seeded black cultivar OAC Vortex was the best performer. This cultivar was the highest yielding genotype among all monocrops and mixtures. Mixtures of two high yielding genotypes usually cannot overyield the monocrops. In addition, trait expression (values) could potentially be different in mixtures and pure stands due to their plasticity (Barot et al., 2017).

Among 50+ indices (Weigelt and Jolliffe, 2003) available for the assessment of the intercropping, that compare mixture yield or biomass data with component cultivars evaluated in pure stands, only few were applicable to the current study. Since no information from individual components of the mixtures was collected, it was not possible to use indices such as LER, competition, or economic evaluation. The economic evaluation of the bean mixtures would be interesting, especially for the

mixtures between gene pools, since the large seeded beans have the higher market price (http://www.omafra.gov.on.ca/english/stats/crops/index.html). However, careful use of the index-based selection is recommended. It is a ratio-based index (Jasienski and Bazzaz, 1999) and high RYM values (RYM >1) can also be obtained blending low yielding genotypes.

In general, diallel analysis of mixture yield data complemented well the index evaluation approach used in the current study. All three indices (RYM, MS, and BS) used were derived from the measured yield data. High yield is the major selection criteria in the most bean breeding programs but good architecture, appropriate phenological characteristics and disease resistance should also be considered in the selection. Although information regarding additional traits can be obtained by diallel analysis (as in the current study), the disadvantage is in its restricted use for two-component mixtures (analogous to its use for bi-parental crosses) and requires that all genotype combinations are tested. A benefit of utilizing a diallel analysis to determine GMA, is that the information can be used to select pairs of cultivars as the best mixture components. There is also possibility to predict performance of more complex mixtures by applying analysis of mixing ability from the two-component mixtures to three-, four-, and five-component mixtures as Lopez and Mundt (2000) have shown in wheat.

The selection of the best performing mixture should be performed with at least two criteria, including an index or diallel analysis in combination with trait (yield) values. In the current study, based on the yield (above average), RYM index (RYM >), and mixing ability (positive SMA effect) averaged over three locations, different best performing mixtures were identified in the two mixture types. For the SRMs the LAM mixture (Mesoamerican, white Lighthouse, and black OAC Vortex) was the best. Among the ARM biblends the BRRR mixture (Mesoamerican, white Bolt and Andean, cranberry Red Rider) was identified as the best performer.

In addition to the high yield, significant other objectives of the University of Guelph bean breeding program, are early maturing genotypes with a good architecture (good harvestability) and resistance to various diseases, especially anthracnose and common bacterial blight, for the Ontario environment. Based on the seven traits that were measured in the current study, as mixture components, cultivars should have positive effects (GMA) on yield, and some market classes seed weight and photosynthetic efficiency (SPAD). These would be complemented by cultivars that have negative effects (GMA) on flowering (early), maturity (early), and harvestability (smaller values are associated with better standability). Information about the performance of cultivars for these attributes in mixture would allow bean breeders to select appropriate cultivars to combine in bean cultivar mixtures with a high commercial potential.

In order to maximize crop yields in mixtures, Hamblin and Zimmermann (1986) suggested that breeding should focus on selection in a mixed cropping system instead of selecting individual components. This approach would require a whole new level of testing for interactions in cultivar and agrochemical trials and would require different approaches to breeding crops. Litrico and Violle (2015) introduced a new system of breeding "from ideotypes to ideomixes," while Gaudio et al. (2019) reviewed various approaches for modeling mixtures in annual crops.

Adoption of the Bean Cultivar Mixture Cropping System

To justify the study and to ensure a balanced view of this research topic, questions such as how the cultivar mixtures may address production constraints and/or help bean growers achieve high yield potential need also to be discussed. Although the importance of cultivar mixtures to increase and stabilize yield, reduce disease pressure and enhance diversity have been reported (Wolfe, 1985; Creissen et al., 2016), increased cost to growers and seed cleaning/processing plants could also be anticipated. Some of the agronomic considerations and potential disadvantages of cultivar mixtures have been discussed previously (Castro, 2001; Barot et al., 2017). One of the reasons that bean growers have not embraced mixtures for crop production is seed uniformity demanded by marketers, processors (canning), and consumers. Common bean seeds also undergo extensive cleaning, which in some years result in seed coat breakage or splitting.

The current work tested bean biblends formed by mixing two cultivar components based on the same number of seeds and evaluated as SRM and ARM biblends. A number of mixtures had high and stable yields over three Ontario environments/locations. However, the adaptation of intracropping practices to modern agriculture may have some difficulties including potential incompatibility with mechanization (Reiss and Drinkwater, 2018; Snyder et al., 2020).

Planting
Step one (same for both mixture types) would include seed counting (additional step for grower). SRM would require mechanical mixing of selected cultivar components (additional step) prior to planting. In ARM biblends, each component is planted in a separate row, which may require planter adjustment as an additional step.

In-Field Management
In general, no additional cost for fertilizers, weed management, and pest/disease control would be required. Moreover, mixture cropping could reduce use of the nitrogen fertilizer if one of the components is a highly efficient nitrogen fixer. As a part of the pilot study, plants were dug, and roots were examined for the nodule formation. Lighthouse had a greater number of viable (pink) nodules compared to OAC Inferno (data not shown) probably associated with the better nitrogen fixing ability.

Harvesting
Basically there is no additional cost associated with the harvesting. In the current study, cultivar components were selected to differ up to 10 days in maturity and the harvesting was done when the later component in the mixture was mature. This may however prolong harvest and potentially reduce yield of the earlier component due to possibility of pod shattering.

Processing

There might be some additional cost associated with the processing (more seed splits with large seeded beans; need to separate the components). However, some mixtures can be marketed as a specialty crops (small seeded white/black or large seeded kidneys). In addition, there is no need to sort bean mixtures of the same market class; most are actually combined during the processing and marketing. In particular, the three mixtures between white beans (Lighthouse/Rexeter, Lighthouse/Bolt, and Rexeter/Bolt) that had excellent and stable yields could be immediately utilized by growers. The mixtures between Lighthouse/Bolt and Rexeter/Bolt could also potentially provide crop resistance to a broader range of diseases, since Lighthouse and Rexeter are resistant to common bacterial blight and Bolt is resistant to anthracnose (https://www.gobeans.ca/head2head.php).

Farmer's Acceptance and Mixture Marketing

To be accepted by bean growers, the advantages of mixture cropping over the monocropping should be significant, stable and without additional production cost. Yield increase up to 8% obtained for some mixtures in the current study is the first step toward implementing growing common bean mixture in Ontario environments. However, additional questions would be mixture advertising and marketing.

In conclusion, the study represents the first field evaluation of bean cultivar mixtures in Ontario environments. Initial, one year two location study results indicated that it is feasible to grow bean mixtures instead of monocultures. Results of the second year confirmed some advantages (plot-based) of growing beans in mixtures instead of monoculture in southern Ontario environments.

The research has the potential to provide a theoretical basis for the use of precision agriculture tools to plant fields with mixtures instead of monocultures. It could lead to greater in-field diversity in the crop and in the above and below ground ecosystems that might provide greater buffering capacity and resiliency to the cropping system. General adoption of the use of cultivar mixtures, instead of pure lines, would transform cropping system practices and supporting research. For the latter, it might require different approaches to breeding crops and a whole new level of testing for interactions in cultivar and agrochemical trials and require different approaches to breeding crops.

The experiment needs to be expanded and repeated over sites and years in order to estimate the stability of the positive interactions in mixtures, to define those factors that are responsible for variation in yield, understand the mechanisms that produce advantages in intercropping systems as well as to identify a broad range of good cultivar combinations. More research is needed to understand competition/complementarity between cultivar components in the mixtures. Direct comparison of mixing effects on ecosystem parameters such as soil microbial diversity is also needed. Additional testing the performance of bean mixtures within the same market classes (e.g., within whites, blacks or kidneys); these types of mixtures might find immediate acceptance from Ontario bean producers; they could be marketed as mixtures with a uniform appearance but with enhanced resilience to disease or other environmental challenges. Finally, to be accepted by Ontario bean producers/processors/consumers, bean mixture cropping system requires testing over several years/locations under more conditions relevant to the Ontario farming systems, preferably in large field settings with bean growers' co-operation.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

KP designed the project. KP, TS, and YR conceived and planned the work. TS and LS set up experiments and performed field work. Crop data were collected by LS and YR. YR analyzed the data and wrote first draft of the manuscript. KP revised and proofread the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2020.00126/full#supplementary-material

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