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Phenotypic Variability for Leaf and Pod Color within the Snap Bean Association Panel

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Abstract. **The color of vegetables is an important factor in consumer food choices and in cultivar choice by growers and processors for production. In absorbing a broad spectrum of light, leaves support plant development by influencing factors such as biomass accumulation, chlorophyll content, and reproductive growth. The edible organ of the snap bean (*Phaseolus vulgaris* L.) is the pod, and its color is not only one of the most important traits for commercial consideration, but also influences phytonutrient content. Although chlorophyll provides the base color, other compounds such as carotenoids and flavonoids may affect leaf and pod color. Darker yellow- or blue-green pods are preferred for processing, but there is more leeway for fresh market, with lighter-colored pods being acceptable. This research characterized leaf and pod color variation in the 378-member Snap Bean Association Panel. Leaf and pod colors were measured with a colorimeter using the L*a*b* scale, which was then transformed to L* (lightness), C* (chroma), and H° (hue angle) for analysis. Both green and wax bean accessions had predominantly green leaves, even though both exterior and interior colors of pods varied by accession. The leaves at the upper level in the canopy were lighter than lower and middle-level leaves. C* of leaves was similar across environments but leaves from the field were greener than leaves of greenhouse-grown plants when converted to Royal Horticultural Society (RHS) values, even though they had similar H°. L* did not differ for corresponding leaf positions of both field and greenhouse leaves. Purple pods were darker (lowest L*) and yellow pods were lighter (highest L*). Although wax beans had similar exterior and interior colors, accessions with purple exterior of pods had green interiors. Green pods were generally two times higher for L* and lower in C* compared with leaves. Pod interior L* was darker than exterior in both years. Pod exterior L* was not significantly different among accessions, whereas pod interior L* differed significantly between years. Broad sense heritabilities ranged from 0.69 to 0.88 for L*, 0.12 to 0. 87 for C*, and 0.81 to 0.89 for H°. Although greater variation was observed in pods than leaves, lower heritability was determined. Moderate correlations between leaf L* and the interior and exterior pod L* implies that it would be possible to select for pod color on the basis of leaf color, with verification using standard cultivars.**

The nutritional content of vegetables and fruits is related in part to the chlorophyll, carotenoids, and flavonoids that they contain. These, in turn, may serve as pigments that influence their color. Often, the more intense the color, the greater the amount of the compound responsible for that color. Color is important to consumers because the decision to purchase may be made based on the appearance of the fruit or vegetable (Swegarden et al. 2019). Other preferred criteria are flavor, texture, and nutritional content, but consumers often are not able to judge these until after purchase (Ngamwonglumlert et al. 2020). Color is important not only for consumers but also for producers because improving the color of the vegetable or fruit is effective in increasing its commercial value. In fresh fruits and vegetables, green color is directly correlated to chlorophyll content, purple to red colors may be produced by anthocyanins or betacyanins, and yellow to orange to red colors may be related to betaxanthins or carotenoid content. In senescing leaves and ripening fruits, an increase in yellow to red colors indicates not only carotenoid accumulation but also chlorophyll catabolism and reuse elsewhere in the plant (Hu et al. 2020).

Snap bean (*Phaseolus vulgaris* L.) pod color is considered a critically important trait, particularly for cultivars used for processing (Myers and Kmiecik 2017). Pod color can range from the light yellow-green of wax beans to various shades of green in snap beans, to deep purple pods of specialty types. Greater variability in color is permissible for fresh market snap beans, but those used in processing need to be relatively dark green. Even with fresh market snap beans, the trend is toward darker-green pods in contemporary cultivars. There is no universal standard color for processed snap beans and each processor has their own proprietary specifications. As such, there is a need to quantify snap bean pod color to better address the needs of the industry.

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Color refers to the part of the electromagnetic spectrum that is visible to the human eye, as various physical and optical properties can significantly alter a person's perception of color. Visible color wavelengths are between 400 and 700 nm, varying according to the ability of an object to reflect light. Although color depends on wavelength of the electromagnetic spectrum, the human eye perceives color differently, with greater sensitivity to some spectra relative to others. As a result, various means have been developed to describe color that is more amenable to human perception.

There are many different color standards and scales used to classify color. Commission Internationale de l'Éclairage (CIE) L*a*b* is a widely used color space for agricultural and food-based research. It is based on a three-dimensional representation that allows a point anywhere in a spherical color space to represent a particular color. It is considered an absolute color space if a white point is specified. L* indicates how light the subject is with value ranging from black (0) to white (100). The a* axis extends from red (+a*) to green (−a*), and the b* axis is orthogonal to L* and a* and ranges from blue (−b*) to yellow (+b*) (Commission Internationale de l'Éclairage 2004). Another color space often used to represent colors is CIE L*C*H° (lightness, chroma, and hue angle). Chroma (C*) and hue angle (H°) are better correlated with human vision compared with directly measured color space values and provide more information to observers (Sigge et al. 2001; Vega-Gálvez et al. 2009). L*a*b* may be converted directly into L*C*H°. The color space is three-dimensional and cylindrical with the L* axis orthogonal to the rotation of H°. C* is specified as the intensity or dominance of any H° of color with the same brightness and is represented by distance from the lightness axis along an orthogonal axis. H° refers to the positions of a color at a visible wavelength around the cylinder. The hue angle is defined as: 0° (360°) - red, 60° - yellow, 120° - green, and 240° - blue (Kasajima 2019).

In plants, color charts such as Munsell and RHS have been widely used to represent various color spaces. One of the oldest and most widely used, the RHS color system, uses C* as determined by the light intensity and color expressions calculated using all three color space values. This contrasts with L*C* H°, where C* and H° are calculated using only a* and b*. The RHS standard reference consists of four color groups and a total of 202 colors, each of which has four different (A, B, C, and D) levels of brightness (Voss 1992).

The objectives of our research were to study the color variation of leaves in different positions and pods of plants grown under both greenhouse and field conditions using the Snap Bean Association Panel (SnAP). We sought to characterize color and estimate the heritabilities of these parameters for leaves and pods.

Material and Methods

Plant material. Seeds of 378 accessions from the SnAP were used in this study. The SnAP consists of different snap bean market classes including yellow (wax) beans, Blue Lake, European small sieve, Refugee, and romano beans mostly of American or European origin (Saballos et al. 2022). Most accessions had determinate bush (type I) growth habit, but a few were half-runners (type III) and another subset had pole (type IV) growth habit.

Greenhouse trials. The SnAP was planted in the Oregon State University, Corvallis, OR, USA, greenhouse in Dec 2018, with data collection in 2019 (Table 1). The population was divided into five groups based on flowering date, and groups were planted sequentially 2 to 5 d apart to distribute the data collection workload. Five seeds of each accession were sown in each pot, followed by thinning to three plants. The 10-cm-diameter pots were supplemented with 3.5 g of Osmocote 14N–6.1P–11.6K controlled release fertilizer in soilless media (Sungro Horticulture, Agawam, MA, USA). Bamboo stakes were used to support each plant when required. They were irrigated at least once a week, and more often when the medium was dry. The greenhouse temperature was set to 18 °C night and 25 °C day. Supplemental lighting was provided for 16 h per day by 1000-W metal halide high-intensity discharge lamps (Sun System 3; Sunlight Supply, Woodland, WA, USA).

Field trials. The field trials were planted in the summers of 2018, 2019, and 2020 at the Oregon State University Vegetable Research Farm near Corvallis, OR (lat. 44.573778°N, long. 123.236750°W) (Table 1). Accessions were planted within 1 day in alphabetical order in 2018; however, they were arranged into five planting blocks of ∼75 accessions each based on flowering date and were planted into a single plot per group with six accessions ('Brittle Wax', 'Normandie', 'Oregon 5630', 'Provider', 'Renegade', and 'Roma II') repeated in all blocks. Planting was distributed into blocks across planting dates to facilitate data collection. Soil was a Chehalis silty clay loam (fine-silty, mixed, superactive, mesic Cumulic Ultic Haploxeroll). Pelleted 16N–7P–13.3K fertilizer was banded under each row before planting, and seeds were sown using a hand-propelled belt planter. Seeds of the *persistent color* types were treated with captan fungicide (Bonide Products Inc., Oriskany, NY, USA) before sowing to improve emergence, but other seeds were untreated. Sixty seeds were used for each variety, which were sown ∼2.5 cm deep in rows 5 m long and 76 cm between rows, with in-row spacing averaging 8 cm between plants. The determinate beans did not require support, whereas indeterminate climbing beans were trellised using metal wires hung on T-posts in the field. The plants were irrigated using overhead sprinklers with ∼25 mm of water applied per week.

Color analysis. The leaf color of the field (2018) and greenhouse plants was measured using a colorimeter (Minolta BC-10; Konica Minolta Sensing Americas, Ramsey, NJ, USA), which recorded color parameters using the CIE L*a*b* scale. A white card supplied by the manufacturer was used to establish the white point for each session of use. Three plants per accession were used for the measurement, and the first three trifoliate leaves of each plant were measured three times. The lowest leaf was designated as lower, the middle leaf as middle, and the third leaf as upper. Although leaf color measurements were recorded in the field in 2018 for 65 accessions, they were taken from 376

Table 1. Year, environment, plant tissue, and accession number used in a study of snap beans in the Snap Bean Association Panel grown in the greenhouse and field in Corvallis, OR, USA.

Yr	Environment	Plant part	No. of accession
2018	Field	Leaf	65
2018–19	Greenhouse	Leaf	376
2019	Field	Pod	378
2020	Field	Pod	378

accessions in the greenhouse in 2019 (Table 1). For pod color measurements, five pods at harvest maturity were randomly collected from the plot of each accession, and the external and internal colors of the pods were measured after pods were cut in half and the seeds removed. Harvest maturity was defined as when pods had achieved full size, but seeds had not fully developed. This developmental stage corresponds to 50% 1–4 sieve size pods used by the processing industry to maximize quality and yield during harvest of large podded snap bean cultivars. Pod colors were recorded from field-grown plants of 378 accessions in 2019 and 2020. C* and H° were calculated from L*a*b* data as: $C^*_{ab} = [a^{*2}+b^{*2}]^{1/2}$ and $H^\circ = \tan^{-1}(b^*/a^*)$.

L*a*b* color values were also converted to RHS scales and visualized with Red-Green-Blue (RGB) equivalent colors using equations and an Excel (version 4.0, Microsoft Corp., Redmond, WA, USA) macro developed by Lattier and Contreras (2020) (Supplemental Tables 1 and 2).

Experimental design and data analysis. For the analysis of phenotypic data of leaves obtained both in the field and in the greenhouse, a completely random design was used, and an augmented design was used for pods. For the augmented design, blocking with six accessions repeated in each was as described previously under field trials. For leaf position in single environments, the linear model was Y = μ + leaf position + accession + accession × leaf position. This was modified to Y = μ + leaf position + accession + environment + leaf position/environment + environment × accession + accession × leaf position + environment × accession × leaf position when two or more environments were analyzed. Pod data were obtained from more than one environment with the linear model being Y = μ + accession + year + block(year) + year*accession using plot means obtained from analysis of variance (ANOVA) within each environment. ANOVA was performed in R [version 4.2.2 (R Core Team 2022)] using a mixed model with accessions being fixed and other effects being random for pods, and all effects were fixed for leaves. Statistical differences among means were analyzed using Fisher's least significant difference test with $\alpha = 0.05$. Spearman's rank correlation was used to analyze relationships among leaf and pod colors using PROC CORR in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA).

Heritability analysis. Heritabilities were calculated using the Singh et al. (1993) equation for inbred lines in single or multienvironment variety trials. The estimation of variance components was based on ANOVA mean squares, where error mean square, or accession × year mean square was subtracted from the accession mean square to obtain an estimate of genetic variance for each trait. Equations were H: $\sigma G^2/(\sigma G^2+ \sigma\varepsilon^2)$ (leaf color variables, single environment) and H: $\sigma G^2/(\sigma G^2+ \sigma GE^2+ \sigma\varepsilon^2)$ (pod traits ≥2 environments). The expected mean squares are in Supplemental Table 3.

Results

L*, a*, b*, C*, and H° color data. Three-dimensional plots of L*a*b* for leaves (Fig. 1) revealed a greater degree of diversity along the b* axis compared with L* or a*. The greatest degree of color variation was for the gradation of yellow color along b*. In the greenhouse environment where more accessions were evaluated, as leaves became more yellow, they also were lighter (Fig. 1A and B). Accessions generally had lighter, more yellow leaves in the upper leaf position, and darker leaves with little variation in color at mid and lower positions as shown by the stratification of color-coded points associated with leaf position (Fig. 1A and B). L*a*b* pod exterior and interior colors were encoded with their RGB equivalent to reveal three main clusters (Figs. 2A, 2B, 3A, and 3B). The largest cluster was composed of pods of various shades of green, a second cluster represented wax beans with pale yellow-green pods, and a third small cluster typified purple-podded accessions. In both years, the single point between the three clusters was the 'Oregon Giant Pole', which has light green pods with purple striping. Intermediate to the green bean and the wax bean clusters were the light-green–colored Refugee beans. In the purple pod cluster, 'Romano Purpiat' had high a* (more red) in 2019 and 'Amethyst' had the lowest a* (more green) in 2020. Because of these extremes, these two accessions were located some distance away from other members of the purple clusters (Fig. 2).

Pod interior color revealed that anthocyanin pigments in purple pods did not carry over to the interior because the plot had only two clusters with all green and purple pods grouped into the larger cluster while wax beans remained in a second cluster (Fig. 3). Even though yellow pod color parameters were more widely dispersed in 2020 compared with 2019, exterior and interior color patterns exhibited the same relative distribution in each year.

L*, C*, and H° were used for more in-depth analyses of leaves and pods (Tables 2 and 3, Figs. 4 and 5, Supplemental Tables 1 and 2). Leaf position and accession color parameters were highly significant ($P < 0.001$) for both greenhouse and field (Table 2). Accession × leaf position interaction was significant for all color parameters in the greenhouse, and it was significant for L*, and the level of significance changed for C* and H° ($P < 0.01$ and $P < 0.05$, respectively) in the field. Adjusted coefficients of determination (r^2) for all color parameters were higher in the greenhouse leaf data than field leaf data. L* ranged from 33.0 to 43.9 for field leaf measurements, compared with 28.3 to 48.9 for greenhouse measurements (Supplemental Table 1). When comparing the same accession across environments, greenhouse-grown leaves were darker ($\bar{x} = 33.9$) than field-grown leaves ($\bar{x} = 37.9$). For the 65 accessions common to both environments, main effects were highly significant. Environment × accessions interaction was highly significant ($P < 0.001$) for all color parameters while accessions × leaf positions, and environment × accessions × leaf positions interactions were at the $P < 0.01$ for L* and at the $P < 0.05$ for H° (Table 3).

For C*, the range of 14.1 to 31.9 for field-grown leaves was smaller than greenhouse-grown leaves (11.7–35.0) among 65 accessions (Supplemental Table 1). For the same set of accessions, the mean H° was similar for both environments and centered around 120° (118.3° for field-grown leaves and 124.2° for greenhouse-grown leaves). Greenhouse-grown leaf H° ranged from 111.2 to 134.0. The leaves with the lowest H° had yellow-green colors, and leaves with the highest H° had blue-green colors. 'Calgreen' (field) and 'US Refugee #5' (greenhouse) had the lowest H° (~112° and 111°, respectively) for each leaf position with their leaves more yellow-green than other accessions (Supplemental Table 1).

The significant differences in L* associated with leaf position in both greenhouse and field was mainly due to the upper leaf position being lighter. H° was highly significant for all leaf positions in the field and it was significantly different for upper leaf

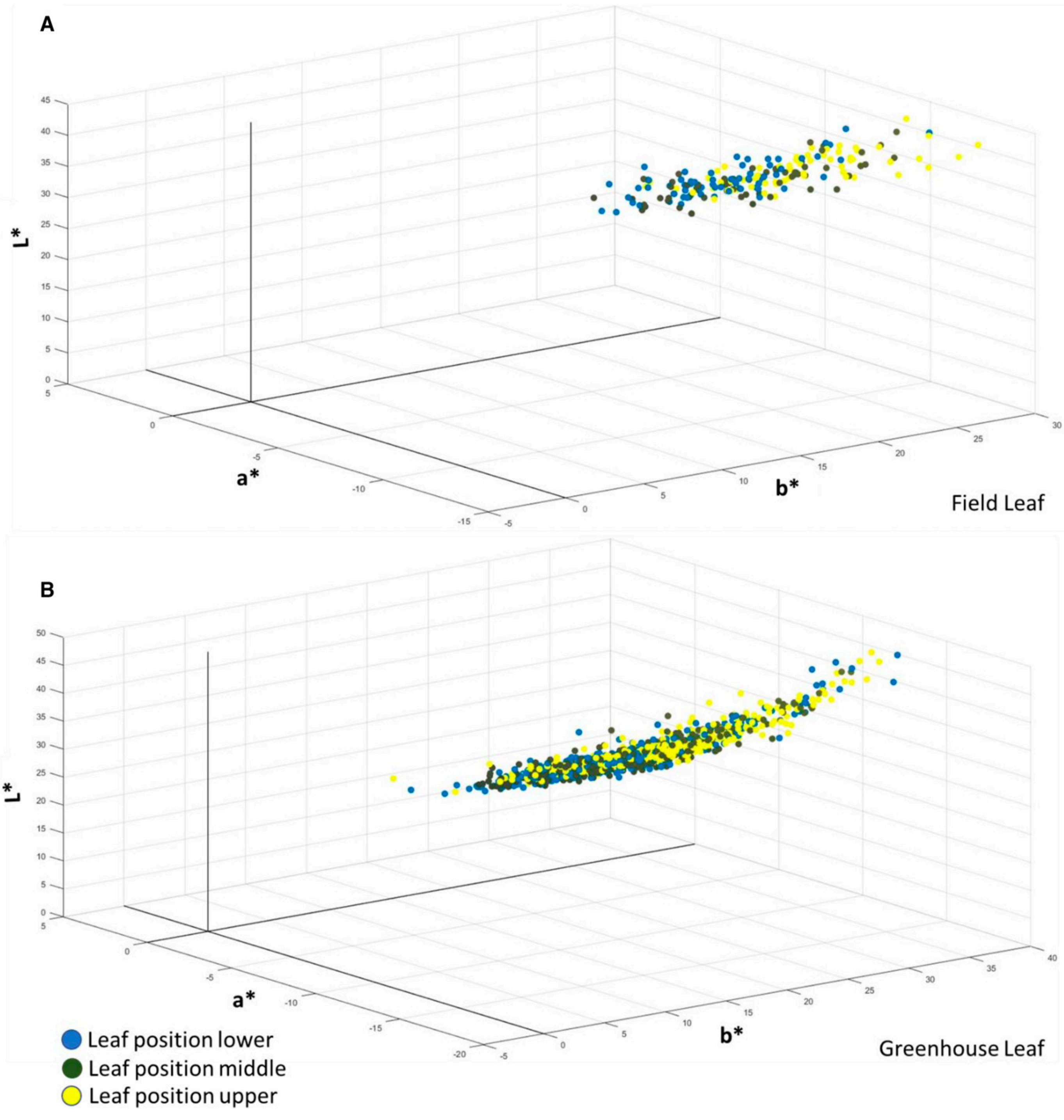

Fig. 1. Three-dimensional plot of L*a*b* color data for leaves of accessions in the Snap Bean Association Panel snap bean diversity panel obtained in Oregon State University greenhouse and field locations near Corvallis, OR, USA. (**A**) Leaves of field (65 accessions) and (**B**) greenhouse (376 accessions) with the three leaf positions. Dots represent individual accession color averages [blue: first (lower) leaf position, green: second (middle) leaf position, yellow: third (upper) leaf position]. L* = lightness; a* = red (+) and green (−); b* = blue (−) and yellow (+).

position in the greenhouse. When more accessions were included, the significance levels in leaf position were lower for C* (Fig. 4). Across all accessions, the lower leaf position was darker green in both environments although middle leaves were not significantly different from lower leaves (Fig. 4, Supplemental Table 1).

Examples of the extremes in leaf color in the field were 'Catania', which had darker leaves in every leaf position (L* = 33.0, 33.2, and 35.7, respectively, from lowest to highest position), compared with 'Navarro' (romano; 40.8, 41.9, and 41.1, lowest to highest) and 'Gold Mine' (wax; 40.3, 42.9, and 43.9) that had lighter leaves (Supplemental Table 1). Similarly, 'Catania' leaves were relatively dark in the greenhouse, but 'EZ Harvest' had some of the darkest leaves (28.6, 29.2, and 32.4 from lowest to highest leaf position) in the greenhouse, whereas 'US

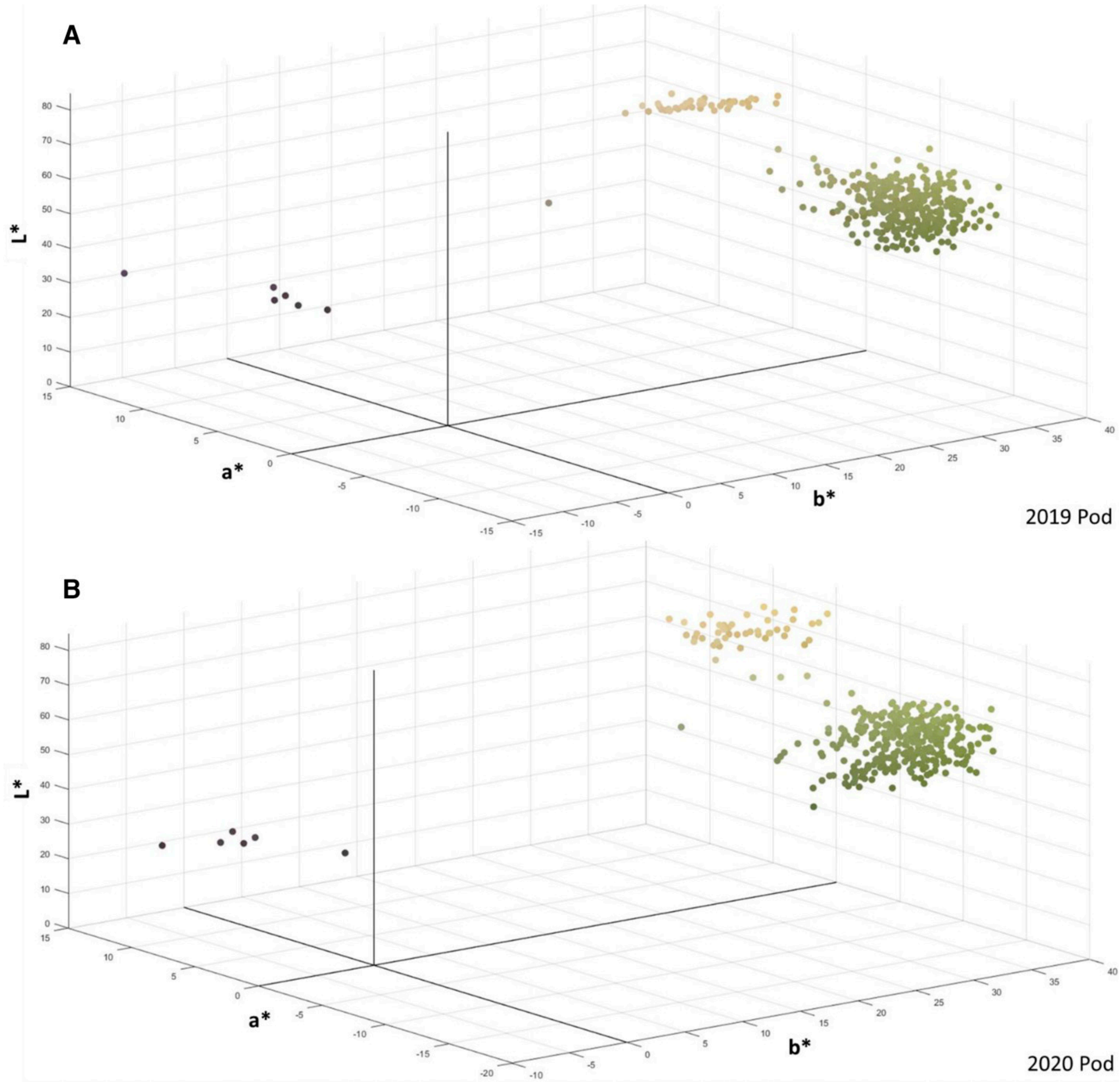

Fig. 2. Three-dimensional plot of L*a*b* (represented by equivalent RGB colors) for pod exterior color from the Snap Bean Association Panel snap bean diversity panel obtained at the Oregon State University Vegetable Research Farm, Corvallis, OR, USA. Each dot represents a single snap bean accession. (**A**) 2019 and (**B**) 2020. L* = lightness; a* = red (+) and green (−); b* = blue (−) and yellow (+); RGB = Red-Green-Blue.

Refugee #5' was lighter (46.8, 46.3, and 48.8 from lowest to highest leaf position). If breeders want to develop plants with dark green leaves, these varieties can provide genetic resources.

RHS color groups showed a similar trend to L*, C*, and H°, but were not as sensitive to differences compared with L*C* H° (Supplemental Table 1). All leaves were classified as green based on H°, but some were in the yellow-green color group ($\bar{x}$ = 118.8°) based on RHS color groups. The green RHS color group had darker leaves than the yellow-green RHS group, whereas the yellow-green group had the highest chroma compared with the green group. Comparing the leaves grown in the greenhouse with corresponding accessions grown in the field, the plants in the yellow-green group were darker and greener and this was apparent based on the RHS data (Supplemental Table 1).

For pod colors, accessions and year were highly significant ($P < 0.001$) for all parameters, except L* exterior and interior ($P < 0.01$) were not significant across years (Table 4). Block was nested in years was highly significant for L* exterior ($P < 0.001$) and L* interior ($P < 0.01$) while accession and year interaction were significant for H° exterior and interior ($P < 0.001$). L* (both exterior and interior) and C* (exterior) had a wider

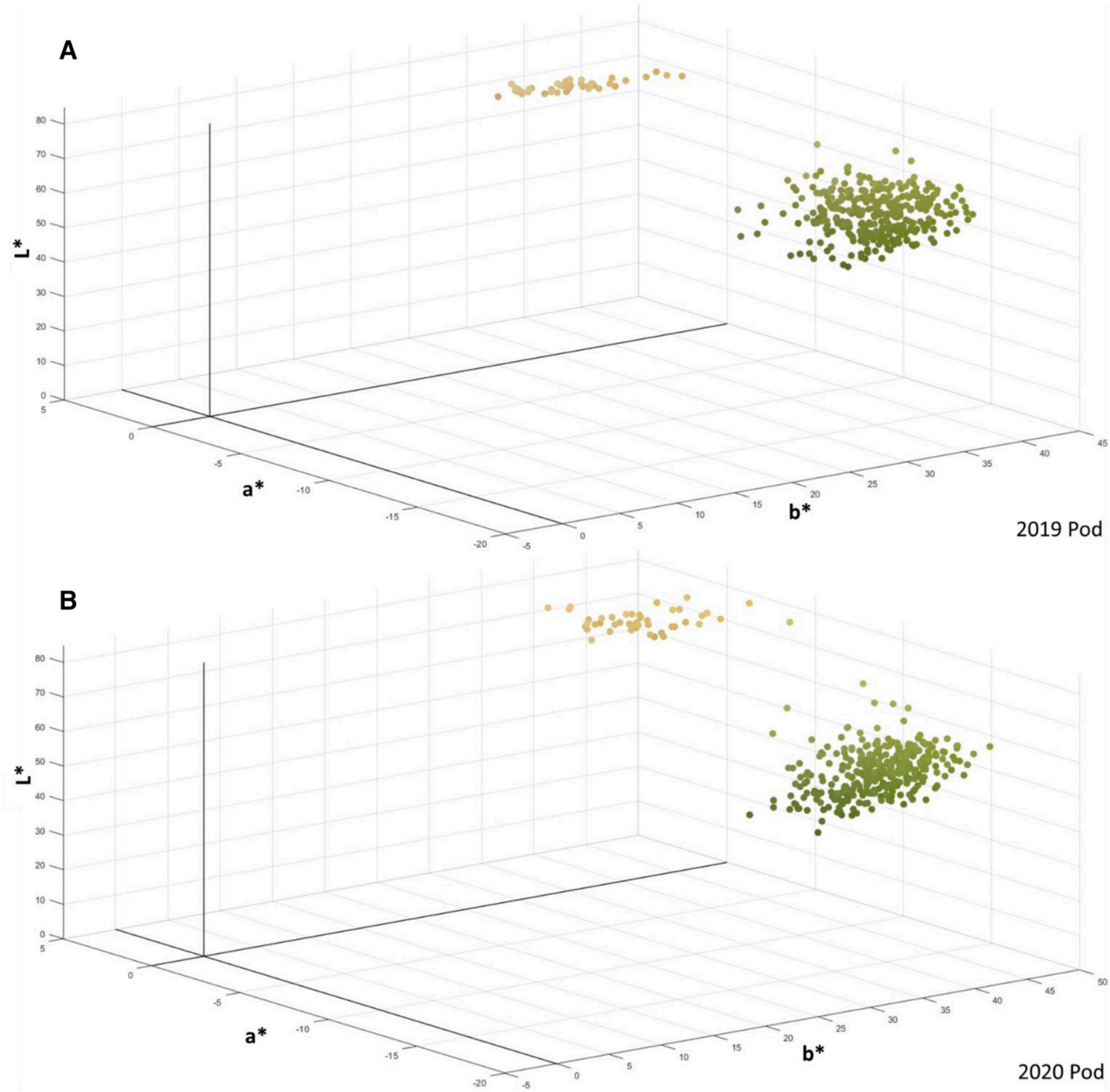

Fig. 3. Three-dimensional plots of L*a*b* (represented by equivalent RGB colors) for pod interior color from the Snap Bean Association Panel snap bean diversity panel obtained at the Oregon State University Vegetable Research Farm, Corvallis, OR, USA. (**A**) 2019 and (**B**) 2020. L* = lightness; a* = red (+) and green (−); b* = blue (−) and yellow (+); RGB = Red-Green-Blue.

range in 2020 than in 2019 and accessions had greener pods in 2020 than 2019 (Supplemental Table 2, Fig. 5). All color parameters except L* exterior in pods were significantly different between years (Fig. 5). The accessions with purple exterior pod color had the lowest L*, and wax bean pods had lighter-colored pods (highest L*) in both years.

The mean L* of pod exterior (essentially identical across years: 62.3 in 2019 and 62.4 in 2020) was almost twice that of L* for greenhouse leaves (35.0), supporting the observation that green and wax pods were consistently lighter than leaves of corresponding accessions. Even though pod interior (L* = 57.9 in 2019 and 58.4 in 2020) was darker than exterior, they remained lighter than leaves. Pod exterior had a wider range of C* and was generally higher than leaves, indicating that the pods had greater color saturation. The range of H° was also wider for both the exterior and interior of pods compared with leaves. As would be expected with pod colors ranging from yellow-green to purple, pod exterior H° was predominately in the yellow-green range (99.8–118.6), but some accessions had values in the violet-pink range (121.7–341.3). H° for pod exteriors varied for some accessions from year to year, with 106 classified as green in both years (slightly greener in 2020); the remainder showed

Table 2. Analysis of variance for leaf color (L*, C*, and H°) measured in the field (2018) and greenhouse (2019) for 65 snap bean accessions grown at the Oregon State University greenhouse and 376 snap bean accessions grown in the Vegetable Research Farm, Corvallis, OR, USA.

			L*[i]	C*	H°
Data set	Source	*df*		MS[ii]	
Field	Leaf position	2	108***	642***	227***
	Accession	64	27***	70***	42***
	Accession × Leaf position	128	6***	12**	8*
	Error	390	4	8	6
	Adjusted r^2		0.48	0.55	0.44
	H[iii]		0.78	0.83	0.81
Greenhouse	Leaf position	2	875***	1,622***	687***
	Accession	375	60***	124***	67***
	Accession × Leaf position	746	7***	16***	9***
	Error	2,011	5	12	6
	Adjusted r^2		0.58	0.56	0.57
	H[iii]		0.88	0.87	0.87

[i] L* = lightness; C* = chroma (saturation); H° = hue angle (tint of color).
[ii] MS = mean square.
[iii] H = broad sense heritability. * = significant at $P < 0.05$; ** = significant at $P < 0.01$; and *** = significant at $P < 0.001$.

Table 3. Analysis of variance for leaf color (L*, C*, and H°) measured in the field (2018) and greenhouse (2019) for only 65 snap bean accessions common to both environments grown at the Oregon State University greenhouse and Vegetable Research Farm, Corvallis, OR, USA.

		L*[i]	C*	H°
Source	*df*		MS[ii]	
Accession	64	43***	114***	55***
Environment	1	4,510***	180***	9,703***
Leaf position (Environment)	4	37**	7	8
Environment × Accession	64	12***	29***	28***
Accession × Leaf position	128	6**	13**	8*
Environment × Accession × Leaf position	128	6**	11*	8*
Error	753	4	9	6
Adjusted r^2		0.65	0.52	0.69

[i] L* = lightness; C* = chroma (saturation); H° = hue angle (tint of color).
[ii] MS = mean square. * = significant at $P < 0.05$; ** = significant at $P < 0.01$; and *** = significant at $P < 0.001$.

more substantial variation in yellow-green in 2019 to darker green in 2020. However, more accessions showed variation for pod interior H° with only 57 accessions green in color in both years. 'Embassy' and 'Bountiful' had the greenest pods in both years by exterior color; however, their interior was yellow-green in 2019 but recorded as green in 2020 (Supplemental Table 2). 'Catania', 'Surfing', and 'Orient' had the greenest pod interiors in 2019, but only 'Orient' was in the green group in 2020. Although 'Embassy' had the greenest pods based on RHS colors, 'FR 266' was the greenest by H° in both years and followed by 'Flavio' and 'Greensleeves' (Supplemental Table 2). Similarly, the interior H° of 'FR 266' and 'Flavio' was the greenest in both years.

Pod H° was most similar to the middle leaf position H° in the field, but most similar to the upper leaf position in the greenhouse among 65 accessions common to both environments. With the full set of accessions, the association between leaf and pod H° was greatest for the upper leaf position with exterior pod color and the middle leaf position and interior pod H°. Even though pod exteriors were lighter by RHS values than leaves in most cases 'Galveston' had darker pods in the field, and 'Bogey', 'Tendergreen', and 'Ulysses' had darker pods than leaves in the greenhouse (Supplemental Tables 1 and 2).

When the diversity panel was grouped by pod color and snap, romano, and *pc* types, little variation for leaf L* was observed among green snap, romano, and *pc* types, but they were significantly darker than the purple romano, as well as both snap and romano wax types (Table 5). The H° of leaves of the romano accession with purple pods were significantly lighter green than leaves of all other types and leaves with wax color were significantly lighter than green *pc* and snap types, but not significantly different from green podded romano and purple-podded snap types. In contrast to leaves, pods classified by color differed significantly for all color parameters. For exterior pod color within the green color class, L* differed significantly among *pc*, romano, and snap types, whereas for C*, *pc* types were significantly different from others within the green class (Table 6). Like exterior color, wax types had the lightest interior L*, and their H° was similar to exterior pod color (Supplemental Table 2). Interior C* was highly saturated compared with the exterior and green romano and snap types were significantly different as compared with other types.

Broad sense heritability. Genotypic and environmental variance estimates were used to calculate broad sense heritability (*H*) for each color parameter. With few exceptions, *H* was generally high for various color parameters (Tables 2 and 4). Greenhouse-grown plants had consistently higher *H* for leaf parameters than field-grown plants, but the differences were not great (Table 2).

For pods, *H* for L* and H° was similar to the leaves, whereas *H* of C* was lower in pods than leaves (Tables 2 and 4). *H* was highest for pod exterior H° (0.89), followed by interior H° (0.87), exterior L* (0.76), and interior L* (0.69). *H* of C* was low to moderate with differences between pod exterior and interior (0.40 and 0.12, respectively).

Relationship among color parameters. The relationship of all variables was examined using Spearman's rank correlation (Tables 7 and 8; Supplemental Tables 4 and 5). For leaves, L* and C* were positively and strongly correlated, whereas H° was strongly but negatively correlated with L* and C*. The correlation within greenhouse-grown leaf color parameters among 65 accessions was higher than within field-grown leaf color parameters. L* and C* were moderately and positively correlated for leaf color parameters between the field and greenhouse. H° was negatively and weakly correlated with these two parameters (Supplemental Table 4).

Pod exterior and interior were generally strongly correlated for color parameters (Table 7), but even though the correlations were similar, correlation among pod exterior traits was higher than among pod interior color parameters. For the 65 accessions, the correlation between pod exterior and interior was higher within ($r \sim 0.89$) compared with between years ($r \sim 0.63$). For pod exterior, L* and C* were not correlated, but H° showed a strong negative correlation with L*. A similar pattern was

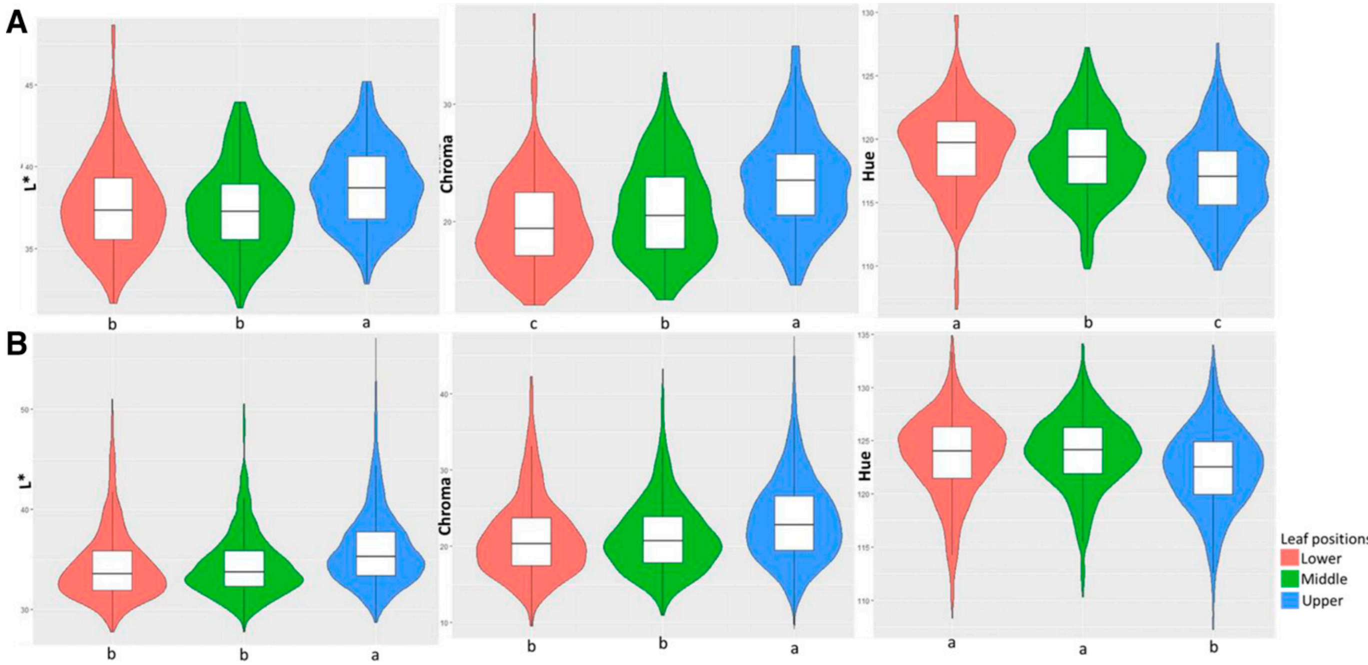

Fig. 4. Violin plots summarizing analysis of variance results for leaf color by leaf positions of accessions in the Snap Bean Association Panel snap bean diversity panel. (**A**) Leaf parameters for 65 accessions grown in the field. (**B**) Leaf parameters for 376 accessions grown in the greenhouse. Colors represent the different leaf positions [orange = first (lower) leaf, green = second (middle) leaf, and blue = third (upper) leaf]. L* (lightness), chroma (saturation), and hue (tint of color) are represented. For each trait, leaf positions with the same letter are not significantly different as determined by Fisher's least significant difference ($P \leq 0.05$).

observed for pod interior with a moderate negative correlation between L* and H°.

Pod interior and exterior L* was moderately correlated with leaf color parameters, whereas pod interior and exterior C* was not correlated. The correlation between field leaf and pod interior and exterior H° was highly significant (r = 0.23** and 0.27**) in 2019 but not significant in 2020 (r = 0.05 and 0.03) (Supplemental Table 4).

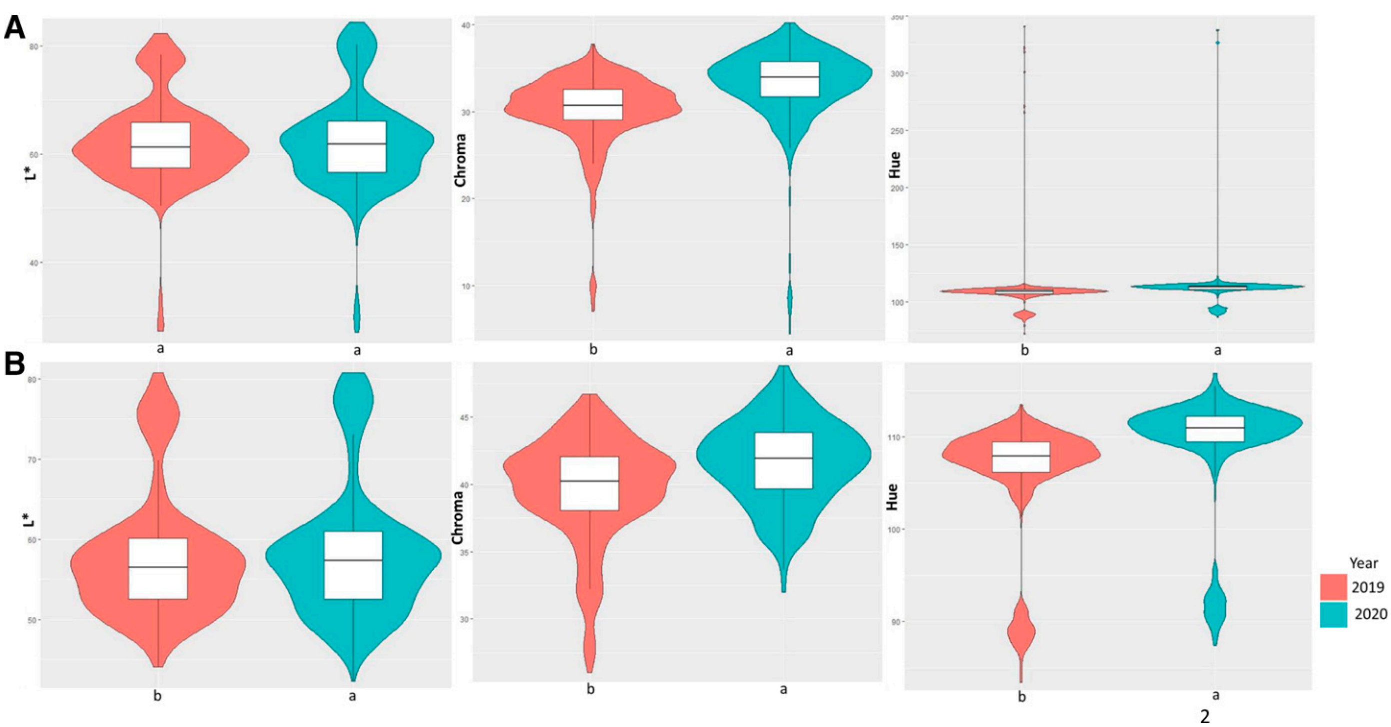

Fig. 5. Violin plots from analysis of variance of pod color measured in the field for accessions in the Snap Bean Association Panel snap bean diversity panel. (**A**) Exterior and (**B**) interior color partitioned by year (orange = 2019 and green = 2020). Color parameters include L* (lightness), chroma (saturation), and hue (tint of color). For each trait, years with the same letter are not significantly different as determined by Fisher's least significant difference ($P \leq 0.05$).

Table 4. Analysis of variance for pod data measured in the field in 2 years (2019 and 2020) for 378 snap bean accessions grown at the Oregon State University Vegetable Research Farm, Corvallis, OR, USA.

		L*[i](e)	C*(e)	H°(e)	L*(i)	C*(i)	H°(i)
Source	*df*	MS[ii]					
Accession	377	149***	33***	1,309***	135***	18***	89***
Year	1	0.03	1,727***	2,913***	43**	751***	1,750***
Block (Year)	8	28***	2	0.6	21**	4	0.3
Accession × Year	377	4.6**	4*	40***	1.1	8**	1.7***
Error	40	2	2	0.7	4	3	0.5
Adjusted r^2		0.97	0.90	0.99	0.94	0.77	0.99
H[iii]		0.76	0.40	0.89	0.69	0.12	0.87

[i] L* = lightness; C* = vividness; H° = tint of color; an (e) or (i) following the trait label corresponds to pod exterior and interior, respectively.
[ii] MS = mean square.
[iii] H = broad sense heritability. * = significant at $P < 0.05$; ** = significant at $P < 0.01$; and *** = significant at $P < 0.001$.

An analysis using only green podded processing and dual-purpose cultivars did produce statistically significant but weaker associations between leaf and pod color parameters (Supplemental Table 5). The correlation among pod color L* and H° was lower, whereas L* and C* was higher. Comparing leaves and pods, L* for pods was moderately correlated with L* for leaves, but correlations between leaves and pods for C* and H° were not significant.

Table 5. Means for leaf color parameters grouped by pod color and market type for 376 snap bean accessions of the Snap Bean Association Panel diversity panel grown in the greenhouse.

Color	Type[i]	L*[ii]		C*		H°	
Green	*pc*	34.4	c[iii]	20.3	d	123.4	a
	Romano	35.5	bc	21.6	d	122.0	ab
	Snap	35.2	c	21.6	d	122.8	a
Purple	Romano	41.2	a	32.7	a	116.5	c
	Snap	36.0	abc	22.2	cd	122.1	ab
Wax	Romano	39.0	a	28.7	ab	119.2	b
	Snap	36.8	ab	24.4	bc	120.9	b

[i] *pc* = *persistent color* snap beans, Romano = flat pod types, and Snap = normal green pod color.
[ii] L* = lightness; C* = chroma; H° = hue angle.
[iii] Means followed by the same letter within a column are not significantly different as determined by Fisher's least significant difference ($P \leq 0.05$).

Table 6. Means for pod color parameters grouped by color and market type for 378 snap bean accessions grown in the field in the Snap Bean Association Panel diversity panel.

		Pod exterior						Pod interior					
Color	Type[i]	L*[ii]		C*		H°		L*		C*		H°	
Green	*pc*	58.3	d[iii]	31.0	b	111.7	c	52.0	d	39.7	b	110.4	a
	Romano	65.2	b	34.1	a	110.5	c	60.2	bc	42.4	a	109.6	a
	Snap	60.6	c	32.6	a	110.9	c	55.6	d	41.4	a	109.6	a
Purple	Romano	32.1	e	13.6	d	321.9	a	66.6	b	35.8	c	109.6	a
	Snap	29.4	e	8.6	d	295.0	b	56.1	cd	37.9	bc	109.8	a
Wax	Romano	80.8	a	27.5	c	92.3	d	78.6	a	37.7	bc	92.2	b
	Snap	78.7	a	28.2	c	90.1	d	76.2	a	36.1	c	90.1	b

[i] *pc* = *persistent color* snap beans, Romano = flat pod types, and Snap = normal green pod color.
[ii] L* = lightness; C* = chroma; H° = hue angle.
[iii] Means followed by the same letter within a column are not significantly different as determined by Fisher's least significant difference ($P \leq 0.05$).

When the sample size was increased, the correlations were generally similar, although there was some variation in the magnitude (Table 8). Correlations among leaf and pod parameters were slightly reduced except those among C* pod exterior and leaf parameters increased to become low but statistically significant. C* pod exterior also shifted from nonsignificant to weakly but significantly correlated for external and internal pod H° and became nonsignificant with pod interior L* (Table 8).

Discussion

In this study, variation in color was documented in leaves and pods of snap beans. The extremes for pod color were represented by purple pods with the lowest L* and wax beans with the highest L*. Refugee beans had the next highest L* and pale green colors intermediate between wax and green pods (Supplemental Table 2). Romano beans as a group also had relatively light green pods (Table 6). A previous study found that wax bean pods had the highest L* color values and lowest carotenoid content among BeanCAP accessions (Myers et al. 2019). The purple and wax podded beans had a dull color (low C*), and green pods had higher saturation. In general terms, lighter green pod colors may be present in heritage cultivars and those used for fresh market, whereas those used in processing tend to be darker green. The general trend over time has been to breed for darker-green pods. Dark green pod interior color in particular is a desirable quality trait in both fresh market and processing green beans.

The range in color parameters was greater for pods than for leaves, indicating that genetic variation was greater relative to environmental variation. There are two possible explanations for this observation. One is that leaves are essential for photosynthesis and must be green to do so. Variation in leaf color may be limited by reduction or loss of fitness when photosynthesis is too high or low. As an example of an extreme case, wax bean pods lack functional chloroplasts (Myers et al. 2019), and if this trait was expressed in leaves, the condition would be lethal to the plant. Second, human selection has likely been a driver of variation through preservation of novel pod colors. In contrast, there has likely been little conscious selection for leaf color.

Pod color varied qualitatively as well as quantitatively. Purple pod color is qualitatively controlled and requires the expression of *P* and *V* for purple color in seeds, flowers, and vegetative plant parts and *Prp* for expression in pods (Miklas 2022). Pale yellow-green wax pods are conditioned by *y* (Currence 1931).

Table 7. Spearman's rank correlation of combined leaf and pod measurements (L* or lightness, C* or chroma, and H° or hue angle) recorded in the field in 2018, 2019, and 2020 and in the greenhouse in 2019, for 65 snap bean accessions grown at the Oregon State University Vegetable Research Farm and greenhouse in Corvallis, OR, USA.

Variable[i]	L*(l)	C*(l)	H°(l)	L*(e)	C*(e)	H°(e)	L*(i)	C*(i)
C*(l)	0.91***[ii]							
H°(l)	−0.83***	−0.85***						
L*(e,)	0.48***	0.40***	−0.45***					
C*(e)	−0.05	−0.12	0.12	0.13				
H°(e)	−0.30***	−0.33***	0.31***	−0.78***	−0.08			
L*(i)	0.48***	0.42***	−0.43***	0.91***	0.20**	−0.72***		
C*(i)	0.02	−0.09	0.00	0.17*	0.67***	−0.11	0.13	
H°(i)	−0.32***	−0.35***	0.34***	−0.73***	−0.03	0.92***	−0.69***	−0.14

[i] Codes for trait labels: (e), pod exterior; (i), pod interior; and (l), leaf.
[ii] Probability > |*r*| under Ho: Rho = 0. * = significant at $P < 0.05$; ** = significant at $P < 0.01$; and *** = significant at $P < 0.001$.

The third qualitatively controlled trait is persistent color (*pc*), a member of the STAY-GREEN family that arrests chlorophyll catabolism with the green color persisting even though photosynthesis no longer happens (Myers et al. 2018; Thomas and Ougham 2014). The *pc* gene has a number of pleiotropic effects, chief among them is uniform green pods valued by processors, but such cultivars show decreased germination and emergence unless seed is fungicide treated (Cirak and Myers 2021). We found that *pc* types had distinctive interior and exterior pod colors, particularly for L* and C* (Table 6).

The quantitative variation that we observed for green pod color has not been well characterized genetically. Two genes (*pa* and *pg*) have been described as producing pale green foliage (Miklas 2022), but it is unclear whether these affect pod color and are present in any of the genotypes used in this study.

Although genetic composition and genetic background were the major influences in color of the accessions included in this study, environmental variation as well as plant growth stage played a role. Environmental factors like temperature, light intensity, water stress, and plant nutrition may have affected color. We observed differences between the greenhouse and field for colors as well as variation from year to year in the field, and although there was significant genotype by environment interaction for both pods and leaves, the interaction was generally greater in leaves than in pods. A possible influence on pod color is stage of maturity, because pods, with the exception of *pc* types, become more yellow-green after reaching physiological maturity. Because the pods were harvested from the same position at processing maturity in our study and no definite relationship between pod color and ripening time was observed, it was determined that the effect of the environment was less. In terms of plant development, leaf position had a major influence on leaf color. This is to be expected where young leaves at the top of the canopy do not have fully developed chloroplast populations, and old leaves lower in the canopy may be declining into senescence, whereas midcanopy leaves are at their full potential for chloroplast development and photosynthesis.

The use of a subset of accessions replicated in blocks to create the augmented design gave us insight into the variability in leaf and pod color in space and time. These were analyzed in an ANOVA of only the replicated accessions (data not shown). Although the six accessions ('Brittle Wax', 'Normandie', 'OR 5630', 'Provider', 'Renegade', and 'Roma II') were significantly different from one another, the five blocks were not statistically significant in 2019, and significantly different only for L* and chroma for pods in 2020. The range for L* was 60 to 66.6 and that for chroma was 32 to 33.8, which was small compared with the range of the entire SnAP. In the case of L*, blocks one and two were similar but significantly different from blocks three through five, which were similar to each other. For chroma, six of the 10 possible pairwise comparisons were significant, but no discernable patterns or gradients were evident. Our general conclusion was that spatial variation for color traits was small relative to that observed for genotype, year, and leaf position in the case of leaves.

Stage of pod growth and development as well as exposure to sunlight will alter green bean pod color. Accessions with the *pc* trait show very uniform pod color, whereas normal green podded types may vary in color depending on position in the canopy and orientation to the sun. Surfaces exposed to sunlight are generally

Table 8. Spearman's rank correlation of combined leaf and pod measurements (L* or lightness, C* or chroma, and H° or hue angle) measured in the field in 2018, 2019, and 2020 and in the greenhouse in 2019, for 376 snap bean accessions grown at the Oregon State University Vegetable Research Farm and greenhouse in Corvallis, OR, USA.

Variable[i]	L*(l)	C*(l)	H°(l)	L*(e)	C*(e)	H°(e)	L*(i)	C*(i)
C*(l)	0.93*** [ii]							
H°(l)	−0.90***	−0.94***						
L*(e)	0.35***	0.38***	−0.37***					
C*(e)	−0.19**	−0.18**	0.20**	−0.13*				
H°(e)	−0.26***	−0.29***	0.28***	−0.79***	0.14**			
L*(i)	0.40***	0.44***	−0.43***	0.91***	−0.05	−0.73***		
C*(i)	−0.06	−0.06	0.05	0.15**	0.59***	−0.08	0.12*	
H°(i)	−0.30***	−0.34***	0.34***	−0.75***	0.21***	0.92***	−0.75***	−0.10

[i] Codes for trait labels: (e), pod exterior; (i), pod interior; and (l), leaf.
[ii] Probability > |*r*| under Ho: Rho = 0. * = significant at $P < 0.05$; ** = significant at $P < 0.01$; and *** = significant at $P < 0.001$.

darker green than shaded surfaces. In our study, we took two color measurements on each pod on opposite sides, which were averaged for analysis.

In 2019, no correlation was observed between L* and C* in pod interior and exterior. However, in 2020, significant but weak correlations among pod exterior traits and moderate correlations among pod interior traits were observed. Some accessions that were in the yellow-green group in 2019 were in the green group in 2020. The lighter color of some accessions in 2019 may have been caused by flood waters, before planting, carrying herbicide residues from a hazelnut planting into some bean plots. The time of measurement after pods were harvested is important for interpreting these data, and between the residual herbicide and the longer period of evaluation in 2019, the second-year data are probably more representative of actual colors.

Leaf color data from field and greenhouse were analyzed separately because of the large difference in number of accessions in each environment and when analyzed with the 65 accessions common to both environments, the accessions by environment interaction was highly significant (Table 3). For leaf color interactions, accession mean rank remained the same although the magnitude differed, whereas pod measurements showed crossover interaction. This suggests that leaf color could be selected in a single environment, but for those traits showing crossover interaction, the response was genotype specific and would need to be evaluated in multiple environments.

The H° calculated from a* and b* does not exactly match the RHS color chart, which represents colors on a broader scale. Part of the reason for this is that RHS color groups are related to L* as well as a* and b*. Although H° can provide a more accurate representation of color, RHS may be easier to conceptualize. Pod H° ranged from 71.4 to 341.3 in 2019 but from 86.2 to 337.9 in 2020. In recombinant inbred lines obtained by crossing Andean (snap) and Mesoamerican (dry) type beans, the H° was between 74.01 and 121.7 (Clavijo Michelangeli et al. 2013). The results of this study are similar to our results when purple-podded accessions are omitted (Supplemental Table 2). In another study, the average H° of ‘Booster’ was 108.1 (Coste et al. 2005), which is similar to the average H° of ‘Booster’ in our study (109 in 2019 and 111.2 in 2020).

H was less influenced by the environment for both pod and leaf colors, but a larger environmental variance and reduced phenotypic variation led to lower *H* for pod C*. The low *H* for C* suggests that this parameter has lower utility for breeding for pod color than other color parameters. *H* was high for pod color among Spanish bean accessions (García-Fernández et al. 2021), and we found similar results except for C* in our study.

This research was initiated by the question: “Is it possible to use leaf color to indirectly select for pod color?” Our snap bean breeding program develops cultivars for processing and requires a relatively dark green pod color as typified by the cultivar Oregon 5630. Many experimental lines are rejected because the pod color is too light, and efficiency of the program could be increased if it were possible to eliminate these lines before flowering and before they were entered into replicated processing trials. Pod exterior L* showed highly significant but moderate correlations with leaf L*, C*, and H° (r = 0.48, 0.40, and −0.45, respectively). Pod C* did not show significant correlation with leaf color traits, whereas H° had a low but significant correlation. These results suggest that darker-green leaf color could be used to select for darker pod color but would need to be supplemented by directly comparing pod color with standard cultivars.

The data from our studies show that color space values are complex traits in snap bean and although qualitative genetic control was apparent, much of the variation is quantitative. Environment has a significant effect on color and needs to be accounted for in breeding schemes. Broad sense heritability was high and similar between pod and leaf color traits, but narrow sense heritabilities would be expected to be lower. Correlations among related pod and leaf traits were generally moderate and might be used for indirect selection with direct verification. Genetic analysis is needed to determine the genetic control of quantitative variation in green leaf color.

Data Availability

SnAP passport data can be found at ScholarsArchive@OSU (https://doi.org/10.7267/dj52wd79f).

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