Phenolic profiles and nutritional quality of four new mungbean lines grown in northern Australia

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Abstract
Four new lines of mungbean cultivated under northern Australian cropping conditions (19.8462°S, 147.2448°E) were characterised for yield, seed characteristics, nutritional contents, phytochemical profiles and phenolic acid contents. Their performance was compared to a commercially grown mungbean line (Jade-AU). The seed yield for three of the new lines (AVTMB 1, 3 and 4) was 14% higher compared to Jade-AU. However, the seed size of AVTMB 1 was also significantly smaller compared to the other lines and Jade-AU. in vitro sprouting acceptability scores ranged between 80% and 100%, with no significant difference between lines. Ash content was highest (4.26% w/w) for lines with smallest seed (e.g., AVTMB 1), while higher protein content was recorded for AVTMB 1 followed by AVTMB 4, AVTMB 3 and Jade-AU, and lowest in AVTMB 2. The seed coat colour of AVTMB 1 and 4 was significantly different (lighter green) than other lines and Jade-AU, whereas the flour colour did not vary significantly. Total polyphenolic content, antioxidant capacity and total monomeric anthocyanin content did not vary significantly between lines. Phenolic acid and flavonoid profiling by HPLC showed the predominant constituents to be vitexin (which averaged between 115 and 149 μg/g for different lines), isovitexin (132–174 μg/g) and catechin (94–105 μg/g). There were statistically significant differences between the mungbean lines for several individual phenolic acids, including p-hydroxybenzoic, vanillic, caffeic, sinapic, ferulic and cinnamic acids, as well as for the flavonoid glycoside vitexin. In most cases, with the highest concentrations of these compounds were found in AVTMB 4 or AVTMB 1. Several of these new mungbean lines show potential for commercial cropping in the drier, hotter regions of northern Australia due to their yield and seed quality performance.

KEYWORDS
nutritional composition, phenolic acid, phenolics and antioxidant capacity, Vigna radiata

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1 | INTRODUCTION

Mungbean (Vigna radiata) has traditionally been considered a relatively minor crop in the Australian pulse sector, with around 120,000 tonnes harvested annually (Chauhan & Williams, 2018). However, its popularity among producers has increased considerably in recent years, with the market value of Australian mungbean industry rapidly approaching $100 million p.a. (Australian Mungbean Association, 2017), with 90% of the harvested crop exported overseas. Indeed, the Australian mungbean industry is considered to be a world leader in the development and adoption of industry-wide standards, in order to deliver the highest quality produce possible (Australian Mungbean Association, 2017). This opens a huge potential to increase the production of mungbean in Australia, both for export purposes and to supply increasing demand in the domestic food market.

Upon receipt in grain depots, the mungbean crop is classified into No. 1, processing and manufacturing grades, allowing mungbean growers to maximise their returns through high-quality and value-added products. This also adds value to the Australian mungbean domestic and export market, as such standards ensure the highest quality beans are matched with the highest paying buyers in an increasingly competitive market space. In addition to seed size, hardness and protein content, visual qualities such as lustre and bean colour are primary quality attributes for Australian mungbean (AEGIC, 2017). In general, seed with an even, bright green colouration will attract a premium price (Skylas, Blanchard, & Quail, 2017).

Mungbean seed can be used in soups or dhal, milled to produce flour for various culinary uses, dried and roasted as snack products, or germinated and consumed as sprouts (Dahiya et al., 2015). Although the use of high-quality beans as salad sprouts provides a significant opportunity for value-adding to the mungbean crop (Guo, Li, Tang, & Liu, 2012), only 5% of Australian produce is currently used for this purpose (Australian Mungbean Association, n.d.).

The uses of mungbean continue to diversify, driven by opportunity for generating income at various points along the value chain. Hence, an increasing number of mungbean-based value-added products are appearing on market shelves worldwide. Maintaining the high quality of both the mungbean seed and its value-added products is fundamental for the successful commercial future of the mungbean crop.

One of the major anticipated demands for this crop in the future is related to its high-protein content, leading to the potential for meat replacement products (Alexeev, Alexeeva, Enaleva, Tupolskikh, & Shumskaia, 2020). In addition to providing a valuable source of protein, mungbean also contains high levels of antioxidant and phenolic compounds (Ganesan & Xu, 2018; Hou et al., 2019). The major phenolic acids present in mungbean have been reported as trans-ferulic acid, caffeic acid and coumaric acid, while the flavonoids present in the highest concentrations are isovitexin, vitexin and catechin (Hou et al., 2019; Meenu, Sharma, Guha, & Mishra, 2016). These and other bioactive compounds present in mungbean have been reported to possess a wide range of physiological activities, including scavenging-free radicals (Tiwari et al., 2013), increasing insulin sensitivity (Yao et al., 2013), reducing plasma triglyceride levels (Tachibana et al., 2013), inhibiting the growth of pathogenic microbes (Hafidh et al., 2013), reducing hypertension (Hsu, Lu, Chang, & Hsu, 2011) and inflammation (Venkateshwarlu, Reddy, & Dilip, 2016), and exerting anti-cancer effects. Increased research activities into the potential health benefits of mungbean (e.g., Amaral, Ferreira, Silva, Neves, & Demonte, 2017; Hou et al., 2019; Hou et al., 2020) have led to increased consumer awareness and interest in this crop and its derived products as functional foods (Sehrawat et al., 2020), raising the profile and market value of this commodity.

In Australia, the majority of the mungbean crop is cultivated in central to southern Queensland and northern New South Wales, as the optimum temperature for growth is between 27°C and 30°C (Bangar et al., 2019). In addition to the higher temperatures experienced in northern Australia, maintaining stable yield and quality under the highly variable climatic conditions typically experienced during the summer growing season is one of the major challenges for the rainfed production of mungbean in these areas. In order to increase production levels of this crop, there is a need to develop new mungbean lines suited to drier and warmer environmental conditions (HanumanthaRao, Nair, & Nayyar, 2016; Kaur, Bains, Bindumadhava, & Nayyar, 2015), which could potentially allow mungbean production to extend into the northern Australian landscapes. In this work, we characterise four new lines of mungbean grown on farm production trials under northern Australian cropping conditions, with a focus on their yield, physical characteristics, nutritional content and phenolic acid profiles. Their performance is compared to a current commercial line of mungbean (Jade-AU) grown under the same conditions.

2 | MATERIALS AND METHODS

2.1 | Seed material

The mungbean seed material comprised four new lines from AgriVentis Technology Ltd Australia (AVTM 1–4), which had previously been grown in small-scale field trials in 2017 (Rockhampton, Queensland) and 2018 (Biloela, Queensland). For comparison, we used a well-established commercial line (Jade-AU) grown under the same conditions. Marketed by the Australian Mungbean Association, Jade-AU is a large-seeded, shiny green line typically grown in the region between central Queensland and northern New South Wales. Compared to other large-seeded lines such as Crystal, it displays improved yield and increased resistance to powdery mildew.

2.2 | Growing conditions and harvest

The mungbean lines were grown on a commercial farm in northern Queensland as a sugarcane break crop. The farm site was 25 km SW of Home Hill, adjacent to the Burdekin river (19.8462° S, 147.2448° E) (Figure 1), with a sandy clay loam soil type (type 6Umb in the DPI...
classification system). As this is north of the potential range currently deemed suitable for mungbean cropping (Figure 1), our aim was to demonstrate the versatility of these new lines for extending this crop to the northern regions of Queensland and potentially northern Australia more broadly.

Between 6 and 18 ha of each new line (1 ha for Jade-AU) was sown into seed beds (prepared with 100 kg/ha of diammonium phosphate) between 25 and 27 August 2019 as a spring season crop. Pre-emergent and post-emergent herbicides were utilised for weed control, alongside two sprays of micronutrients. Furrow irrigation commenced after the soil water deficit reached the refill point. This period of the year is generally dry (Figure S1), with just 7 mm of cumulative rainfall received throughout the growing trial (BOM, 2020). The daily minimum and maximum temperatures ranged between 7.7°C and 38.7°C, respectively, with a mean maximum temperature of 30.3°C (BOM, 2020).

Upon commencement of senescence, the crop was chemically defoliated (200 g/L of diquat dibromide monohydrate) on 25 November and harvested on 27 November 2019 (93 days after sowing). Five within-field replicates were collected for each variety for subsequent analysis.

2.3 | Seed quality

Thousand kernel weight (TKW) was determined using an IC-VA seed counter (AIDEX Co, Japan) (n = 3 replicates). Seed sprouting tests were conducted by the Agricultural Testing Laboratory for Seed and Grain (AgEtal), Toowoomba, Qld, following standard methods for the Primary Production and Processing Standard for Seed Sprouts (FSANZ, 2010). Resultant sprouts were classified as acceptable or unacceptable for human consumption and also tested for the presence of three common bacterial contaminants (Escherichia coli, Salmonella and Listeria).

For each field replicate (n = 5 per line), approximately 20 g of seed material was ground to a fine powder (Breville Coffee & Spice Grinder; Botany, NSW). Moisture content was determined according to AOAC Official Method 925.10; all subsequent results were expressed on a dry weight basis. Seed material and flour colour was quantified in triplicate using a Konica Minolta chroma meter (CR-400), reported as CIE values of brightness (L*), yellowness (b*) and red/green colouration (a*).

Nitrogen and carbon content were determined on a LECO TruMac Series Carbon and Nitrogen Analyser (LECO, USA). Protein content was obtained by multiplying the nitrogen content by the standard conversion factor of 6.25 (Skylas, Blanchard, & Quail, 2017). Ash content was measured by combustion in a muffle furnace (ModuTemp; Midvale, WA) at 500°C for 8 h (Kalra, 1997).

2.4 | Measurement of antioxidant capacity, phenolics and anthocyanins

Polar compounds were extracted following the protocol previously reported by our laboratory (Johnson et al., 2020b), using 1 g of flour and a final volume of 15 ml. Extractions and subsequent assays were performed in duplicate. Ferric reducing antioxidant potential (FRAP), cupric reducing antioxidant potential (CUPRAC), total polyphenolic content (TPC) and total monomeric anthocyanin content (TMAC) were determined as previously described (Johnson et al., 2020b). The results for FRAP and CUPRAC were expressed in Trolox equivalents (TEs), TPC results in gallic acid equivalents.
(GAEs) and TMAC results in equivalents of cyanidin-3-glucoside (cyd-3-glu).

Although previous researchers have investigated both free and bound phenolic fractions in other pulse crops (Xiang, Zhang, Apea-Bah, & Beta, 2019), only the free phenolic fraction was investigated in the present study. The principal reason for this is that phenolics bound to cell wall structures have reduced bioavailability (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004), meaning that the free phenolic compounds are likely to be the primary contributors to any observed beneficial health effects.

All reagents used were analytical grade. Methanol, hydrochloric acid and sodium carbonate were purchased from Chem Supply; all other reagents were obtained from Sigma-Aldrich Australia.

2.5 | FTIR spectroscopy

Fourier transform infrared (FTIR) spectra (4,000–400 cm⁻¹; 24 scans at 4 cm⁻¹ resolution) were collected from the mungbean flour in triplicate using a Bruker Alpha FTIR spectrophotometer (Ettlingen, Germany) fitted with an attenuated total reflectance (ATR) module, as previously described (Johnson et al., 2020a).

2.6 | Phenolic profiling by HPLC

Ten millilitre of each methanol extract was concentrated (in duplicate) using a rotary evaporator at 27°C, reconstituted in 1 ml of methanol and syringe filtered (Livingstone 0.45 μm PTFE). Phenolics were separated using an Agilent 1100 HPLC system (Waldbonn, Germany) with a reversed phase C₁₈ column (Agilent Eclipse XDB-C₁₈; 150 × 4.6 mm; 5-μm pore size) and guard cartridge (Gemini C₁₈ 4 × 2 mm). The injection volume was 5 μL, with a mobile phase comprising 0.01-M phosphoric acid and methanol at a flow rate of 1 ml/min. The gradient was modified slightly from that previously reported (Johnson, Collins, Walsh, & Naiker, 2020), beginning at 20% methanol and ramping linearly to reach 100% methanol by 20 min. The total run time was 25 min, with a post-run equilibration time of 7 min.

Phenolic acids and selected flavonoids (Figure 2) were identified and quantified using commercial analytical standards (Table 1), aside from isovitexin, which was quantified as equivalents of vitexin. Although hydroxybenzoic acids are typically quantified at a detection wavelength of 280 nm (Xiang, Zhang, Apea-Bah, & Beta, 2019), a wavelength of 210 nm was found to provide improved linearity and detection limits for most of these standards in this work (Table 1). This wavelength corresponds to the primary λmax of most benzoic acids and has also been used by several previous researchers for their quantification (Chirinos et al., 2008; Pereira, Câmara, Cacho, & Marques, 2010). The typical coefficient of variations associated with the HPLC analysis, as measured by triplicate injections of quercetin, gallic acid and p-coumaric acid standards, was 2.5%, 0.73% and 0.25%, respectively.

2.7 | Data analysis

Yield and seed quality data were analysed by Genstat v19 (VSN International, UK). All other statistical analysis was performed in IBM SPSS.
FTIR spectra were analysed in Unscrambler X 10.5 (Camo ASA, Oslo, Norway). Where applicable, results are presented as mean ± 1 standard deviation.

3 | RESULTS AND DISCUSSION

3.1 | Yield and physical seed characteristics

The graded seed yield (Table 2) ranged from 0.87 to 1.32 t/ha. Higher seed yield was recorded for AVTMB 1 (1.32 ± 0.10 t/ha), AVTMB 3 (1.32 ± 0.14 t/ha) and AVTMB 4 (1.32 ± 0.15 t/ha), followed by Jade-AU (1.16 ± 0.16 t/ha). A significantly lower yield was found for AVTMB 2 (0.87 ± 0.13 t/ha). The growers and exporter were asked to qualitatively rank their preference of these lines at the point of harvest, with the observed 14% higher yield and seed quality (size, uniformity and colour) for three of the new lines (AVTMB 1, AVTMB 3 and AVTMB 4) being reported as the most attractive characteristics. For these reasons, the grower also expressed preference in choosing these lines for planting in the following season.

The long-term average yield for the mungbean crop in Australia is approximately 0.9 t/ha, with annual averages ranging from 0.6 to 1.1 t/ha between 2005 and 2017 (Chauhan & Williams, 2018). However, in 2019—the year that this trial was conducted—the Australian mungbean industry average yield was exceptionally low due to severe drought stress, ranging from 0.3 to 0.5 t/ha (Lyon, 2019). Hence, the high mean yields found in the present study (>1.3 t/ha) demonstrate the viability of mungbean cropping in the northern Australian region, if grown under irrigation. Several of the new lines demonstrating greater adaptation to warmer temperatures may provide improved and more stable yield under the subtropical and tropical north Australian environments, consistent with their yield performance in earlier seasons at Biloela, Rockhampton and Georgetown (Queensland) (unpublished data).

The seed size (TKW) of the five lines ranged from 61.6 to 70.6 g/1,000 seeds (Table 2). One of the higher yielding mungbean lines (AVTMB1) recorded a significantly smaller seed size (61.6 g/1,000) compared to all other new lines and Jade-AU. However, the sprouting rates were quite comparable between lines (Table 2). Combined with the absence of microbial contamination across all samples, this indicates high suitability of all lines for spraying purposes.

3.2 | Proximate nutritional composition

In terms of proximate composition (Table 2), there was a significant difference between lines in moisture content at harvest (one-way ANOVA, $F_{4,20} = 14.452, P < 0.001$), ash content ($F_{4,20} = 10.799, P < 0.001$), protein content ($F_{4,20} = 7.358, P = 0.001$), carbon content ($F_{4,20} = 15.077, P < 0.001$) and the C:N ratio ($F_{4,20} = 27.743, P < 0.001$). The moisture content ranged between 10.1% in line AVTMB 2 to 16.0% in AVTMB 4, with Jade-AU also possessing quite

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**TABLE 1** Quality-of-analysis parameters associated with the phenolic acid and flavonoid standards

| Compound | Retention time (min) | Wavelength (nm) | Slope | Calibration $R^2$ |
|----------|----------------------|-----------------|-------|------------------|
| Hydroxybenzoic acids | | | | |
| Gallic acid | 2.47 | 210 | 40.1 | 0.9999 |
| Protocatechuic acid | 4.00 | 250 | 15.0 | 1 |
| $p$-Hydroxybenzoic acid | 5.79 | 250 | 25.5 | 1 |
| Gentisic acid | 6.08 | 210 | 39.7 | 1 |
| Vanillic acid | 6.36 | 210 | 28.4 | 1 |
| Isovanillic acid | 6.58 | 210 | 32.2 | 1 |
| Syringic acid | 6.68 | 210 | 30.5 | 1 |
| Hydroxycinnamic acids | | | | |
| Chlorogenic acid | 5.25 | 320 | 13.2 | 0.9999 |
| $p$-Coumaric acid | 8.25 | 320 | 32.3 | 1 |
| Caffeic acid | 6.43 | 320 | 25.0 | 0.9999 |
| Sinapic acid | 8.47 | 320 | 23.6 | 1 |
| trans-Ferulic acid | 8.54 | 320 | 25.5 | 0.999 |
| Cinnamic acid | 12.50 | 280 | 44.5 | 1 |
| Flavonoids | | | | |
| Catechin | 4.86 | 280 | 52.9 | 1 |
| Vitexin | 8.76 | 320 | 8.1 | 1 |
| Isovitexin | 9.34 | 320 | (8.1)$^a$ | (1)$^a$ |
| Quercetin-3-glucoside | 9.89 | 250 | 11.7 | 1 |

Note. All standards were calibrated across the range of 1–100 mg/L.

$^a$Quantified as equivalents of vitexin.
a low moisture content (11.7%). There is a small possibility that the different lines were at slightly different stages of maturity at the time of harvest, which could influence their moisture content, although all lines displayed the onset of senescence directly prior to the application of defoliant and subsequent harvest, indicating relatively similar stages of maturity. The ash content was also the lowest in AVTMB 2 (3.72%) and highest in AVTMB 1 (4.26%), a similar range to that found in previous work on commercial lines of Australian mungbean (Skylas, Blanchard, & Quail, 2017). The protein content of line AVTMB 2 (3.72%) and highest in AVTMB 1 (4.26%), a similar range to that found for the seed colour (Table 4), while the other parameters (a* and b*) were relatively similar to those found for the flour colour from Jade-AU and any other line, however, that of line AVTMB 1 was significantly darker compared to AVTMB 3 and 4 (Table 4).

3.3 | Colour

In terms of the seed coat colour, a significant difference between lines was found for luminosity (L*), with line AVTMB 4 found to be lighter overall and Jade-AU darker (Table 3). No significant differences were observed for a* (red-green colouration). The colour of line AVTMB 4 was significantly less yellow compared to AVTMB 2 (lower value of b*), but not to any other line. In general, lighter coloured samples (higher L* values) were positively correlated with increased yellowness (higher b*) (Pearson linear correlation: \( r_{75} = 0.435, P < 0.001 \)) and increased greenness (lower a*) (\( r_{75} = -0.335, P < 0.01 \)). Yellowness (higher b*) and greenness (lower a*) were also correlated (\( r_{75} = -0.437, P < 0.001 \)). Overall, there was no significant difference (at \( \alpha = 0.05 \)) between the seed colour of Jade-AU and the lines AVTMB 2 and 3 for any colour parameter, while the lines AVTMB 1 and 4 were both significantly lighter in colour compared to Jade.

The flour colour may also play an important role in the consumer acceptability of mungbean flour products (Liu et al., 2018); hence, it was also assessed in this study. The luminosity of the flour was much lighter than that found for the seed colour (Table 4), while the other parameters (a* and b*) were relatively similar to those found for the seed colour. In contrast to the correlations observed for mungbean seed coat colour, lighter coloured flours (higher L*) tended to have greater blueness (lower b*) (\( r_{75} = -0.587, P < 0.001 \)) and greenness (lower a*) (\( r_{75} = -0.396, P < 0.001 \)). Increased levels of yellowness (higher b*) were positively correlated with redness (higher a*) (\( r_{75} = 0.579, P < 0.001 \)). No significant differences were observed in the flour colour from Jade-AU and any other line, however, that of line AVTMB 1 was significantly darker compared to AVTMB 3 and 4 (Table 4).

3.4 | Antioxidant capacity, phenolics and anthocyanins

There was no significant difference between lines in the CUPRAC (one-way ANOVA, \( F_{4,20} = 0.589, P > 0.05 \)), FRAP (\( F_{4,20} = 0.530, P > 0.05 \)), TPC (\( F_{4,20} = 1.958, P > 0.05 \)) or TMAC content \( (F_{4,20} = 1.315, P > 0.05) \) (Table 2), reflecting that the overall free radical scavenging potential (i.e. antioxidant activity) was consistent across the mungbean lines.

3.5 | FTIR spectra

FTIR spectroscopy is a technique that is increasingly used to profile the chemical differences in grain and pulse crops (Do, Cozzolino, \( \ldots \))
The FTIR spectra of the flour produced from the five mungbean lines possessed major spectral peaks attributable to the OH stretch of water (centred at 3270 cm\(^{-1}\)), antisymmetric CH stretch of lipids (2,930 cm\(^{-1}\)), amide I bonds in protein (1,635 cm\(^{-1}\)), amide II (1,540 cm\(^{-1}\)), various carbohydrate features (1,480–1,180 cm\(^{-1}\)) and pyranose rings in starch/cellulose (\(\approx\)1,000 cm\(^{-1}\)) (Cozzolino, Roumeliotis, & Eglinton, 2014).

As the amplitude of spectra collected with an ATR module varies depending on the particle size and pressure applied to the sample on the ATR cell (Lee, Liong, & Jemain, 2017), the vertical offset of the spectra likely results from this variation. However, no differences in peak shape or position were identified, indicative of the overall similar phytochemical composition of the samples (Figure S2).

This observation was confirmed through principal component analysis (PCA) conducted on the entire mid-infrared spectral region (4,000–400 cm\(^{-1}\)). Following previous work on barley (Do, Cozzolino, Muhlhausler, Box, & Able, 2015) and mungbean (Johnson et al., 2020), the spectra were processed to the second derivative.

### Table 3

| Line     | Seed material | Flour |
|----------|---------------|-------|
| AVTMB 1  | 35.86 ± 0.78\(^{ab}\) | 76.87 ± 1.41\(^{b}\) |
| AVTMB 2  | 37.93 ± 1.35\(^{c}\) | 78.01 ± 1.22\(^{ab}\) |
| AVTMB 3  | 37.52 ± 1.02\(^{bc}\) | 80.44 ± 0.60\(^{b}\) |
| AVTMB 4  | 34.12 ± 1.27\(^{a}\) | 80.24 ± 1.10\(^{b}\) |
| Jade     | 38.56 ± 0.53\(^{c}\) | 78.84 ± 1.92\(^{ab}\) |

Note. Lines with the same superscript letter in each column were not significantly different from one another according to post-hoc Tukey testing at \(\alpha = 0.05\). L = 100 (white); L = 0 (black); +a = red; –a = green; +b = yellow; –b = blue.

### Table 4

| Compound                      | AVTMB 1               | AVTMB 2               | AVTMB 3               | AVTMB 4               | Jade-AU   | P value |
|-------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------|---------|
| Gallic acid                   | 0.78 ± 0.04           | 0.70 ± 0.25           | 0.65 ± 0.15           | 0.66 ± 0.16           | 0.65 ± 0.27 | 0.848   |
| Protocatechuic acid           | 1.18 ± 0.19           | 1.21 ± 0.20           | 1.30 ± 0.12           | 1.41 ± 0.41           | 1.42 ± 0.17 | 0.338   |
| p-Hydroxybenzoic acid         | 0.93 ± 0.29\(^{a}\)   | 0.83 ± 0.09\(^{ab}\)  | 0.81 ± 0.06\(^{ab}\)  | 0.81 ± 0.37\(^{b}\)  | 0.60 ± 0.19\(^{b}\) | 0.043*   |
| Gentisic acid                 | 0.75 ± 0.26           | 0.53 ± 0.09           | 0.68 ± 0.25           | 0.80 ± 0.25           | 0.45 ± 0.19 | 0.058   |
| Vanillic acid                 | 2.40 ± 0.12\(^{ab}\)  | 2.37 ± 0.53\(^{ab}\)  | 2.70 ± 0.17\(^{a}\)  | 3.00 ± 0.60\(^{b}\)  | 1.83 ± 0.42\(^{a}\) | 0.002**  |
| Isovanillic acid              | 2.00 ± 0.47           | 2.18 ± 0.19           | 2.52 ± 0.45           | 2.78 ± 0.63           | 2.16 ± 0.65 | 0.111   |
| Syringic acid                 | 31.0 ± 2.7            | 30.8 ± 5.1            | 28.7 ± 5.1            | 38.9 ± 8.5            | 30.4 ± 5.0  | 0.082   |
| Sum of hydroxybenzoic acids   | 38.3 ± 1.0            | 37.1 ± 4.4            | 39.2 ± 3.8            | 48.4 ± 9.9            | 38.0 ± 5.4  | 0.103   |
| Chlorogenic acid              | 0.82 ± 0.26           | 0.71 ± 0.16           | 0.77 ± 0.07           | 0.97 ± 0.37           | 0.73 ± 0.19 | 0.362   |
| p-Coumaric acid               | 0.70 ± 0.12           | 0.78 ± 0.21           | 0.82 ± 0.14           | 0.81 ± 0.17           | 0.57 ± 0.12 | 0.051   |
| Caffeic acid                  | 0.84 ± 0.18\(^{ab}\)  | 0.74 ± 0.10\(^{b}\)   | 0.85 ± 0.09\(^{ab}\)  | 0.92 ± 0.22\(^{a}\)  | 0.61 ± 0.09\(^{b}\) | 0.015*   |
| Sinapic acid                  | 0.17 ± 0.04\(^{a}\)   | 0.15 ± 0.03\(^{ab}\)  | 0.13 ± 0.04\(^{ab}\)  | 0.17 ± 0.05\(^{b}\)  | 0.11 ± 0.01\(^{b}\) | 0.036*   |
| trans-Ferulic acid            | 0.11 ± 0.04\(^{a}\)   | 0.20 ± 0.08\(^{a}\)   | 0.19 ± 0.02\(^{a}\)   | 0.22 ± 0.08\(^{a}\)  | 0.14 ± 0.06\(^{a}\) | 0.046*   |
| Cinnamic acid                 | 0.17 ± 0.02\(^{ab}\)  | 0.18 ± 0.03\(^{a}\)   | 0.19 ± 0.03\(^{a}\)   | 0.18 ± 0.03\(^{a}\)  | 0.13 ± 0.04\(^{b}\) | 0.017*   |
| Sum of hydroxycinnamic acids  | 2.64 ± 0.30\(^{b}\)   | 2.65 ± 0.40\(^{b}\)   | 2.90 ± 0.23\(^{a}\)   | 3.26 ± 0.82\(^{b}\)  | 2.29 ± 0.45\(^{b}\) | 0.049*   |
| Catechin                      | 93.8 ± 7.0            | 93.4 ± 3.5            | 94.2 ± 2.9            | 105.2 ± 19.6          | 98.4 ± 18.7 | 0.607   |
| Vitexin                       | 117.9 ± 12.5\(^{a}\)  | 132.0 ± 21.1\(^{b}\)  | 140.4 ± 14.3\(^{ab}\) | 148.9 ± 21.7\(^{b}\) | 114.9 ± 23.5\(^{b}\) | 0.029*   |
| Isovitexin                    | 143.8 ± 28.9          | 152.6 ± 26.9          | 163.0 ± 15.4          | 173.5 ± 27.6          | 132.0 ± 26.9 | 0.078   |
| Quercetin-3-glucoside         | 0.99 ± 0.27           | 0.88 ± 0.19           | 0.84 ± 0.17           | 0.89 ± 0.17           | 0.65 ± 0.12 | 0.071   |
| Apigenin                      | <LOQ                  | <LOQ                  | <LOQ                  | <LOQ                  | <LOQ       | n/a     |
| Sum of flavonoids             | 349 ± 36              | 370 ± 38              | 402 ± 37              | 429 ± 68              | 346 ± 67   | 0.103   |

Note. Values given in \(\mu\)g/g (mean ± SD from five replicates for each line). Lines with the same superscript letter in each row were not significantly different from one another according to post-hoc Tukey testing at \(\alpha = 0.05\). Abbreviation: LOQ, limit of quantification.

*\(P < 0.05\). **\(P < 0.01\). ***\(P < 0.001\).
using a Savitzky–Golay algorithm prior to performing the PCA. As shown in Figure 3, there was no obvious clustering of mungbean lines across PCs 1 and 2, indicating generally similar phytochemical composition between the five lines. Minor differences could be observed in the PCA plot on the lipid region of the spectra (2,900–2,820 cm⁻¹; data not shown). However, as no profiling of lipid composition was conducted here, the significance of this observation remains for future studies to clarify.

3.6 | Phenolic profiles

From the HPLC analysis of the mungbean sample extracts, 13 phenolic acids were identified (comprising seven hydroxybenzoic acids and six hydroxycinnamic acids), in addition to four flavonoids (Table 4). The predominant hydroxybenzoic acid present was syringic acid, followed by vanillic and isovanillic acid in much lower concentrations. Protocatechuic acid, p-hydroxybenzoic acid, gentisic acid and gallic acids were also found in low concentrations. All hydroxycinnamic acids were found in low concentrations, with chlorogenic, p-coumaric and caffeic acids found in the largest amounts, followed by cinnamic, sinapic and trans-ferulic acids. These trends broadly agreed with previous international studies profiling phenolic acids in mungbean (Hou et al., 2019; Meenu, Sharma, Guha, & Mishra, 2016; Yao et al., 2013). The predominant flavonoids were vitexin and isovitexin, followed closely by catechin. Quercetin-3-glucoside was found in low levels, while apigenin was below the limit of quantification.

Significant differences between the mungbean lines were found for two hydroxybenzoic acids (p-hydroxybenzoic acid and vanillic acid), four hydroxycinnamic acids (caffeic acid, sinapic acid, trans-ferulic acid and cinnamic acid) and one flavonoid (vitexin). For p-hydroxybenzoic acid, and sinapic acid, the content found in the AVTMB 1 cultivar was significantly higher than that found in Jade-AU (P < 0.05), with no significant differences found between the remaining cultivars and either AVTMB 1 or Jade-AU (P > 0.05). For both caffeic acid and vitexin, the content found in AVTMB 4 was

![FIGURE 3](image-url) Scores plot and loadings for the PCA conducted on the mungbean flour FTIR spectra
significantly higher than Jade-AU, while for vanillic acid, both AVTMB 3 and 4 were higher than Jade-AU. The cinnamic acid content of all lines aside from AVTMB 1 were higher than Jade-AU. Although the one-way ANOVA for trans-ferulic acid indicated a significant difference between lines (P < 0.05), post hoc Tukey testing was unable to determine which lines were significantly different to one another. For this phenolic acid, AVTMB 2 had the highest concentration and AVTMB 1 the lowest. As all mungbean lines were grown under the same environmental conditions, the differences in phenolic acid profiles between lines can be attributed to their genetic differences, leading to differential expression of key enzymes in phenolic synthesis pathways, primarily the shikimic acid pathway (Santos-Sánchez, Salas-Coronado, Hernández-Carlos, & Villanueva-Cañongo, 2019). Although no genetic analysis was performed in this study, similar observations of upregulated gene expression have previously been made for varieties of other grain crops displaying increased content of specific phenolic acids (Laddomada et al., 2017; Ma et al., 2016).

However, despite these differences in contents of individual phenolic acids and flavonoids, no significant differences were observed between lines for the sum of hydroxybenzoic acids or flavonoids. AVTMB 4 did show a significantly higher content of total hydroxycinnamic acids compared to Jade-AU, but not to any other line.

The vitexin and isovitexin concentrations found here (115–149 and 132–174 μg/g, respectively) were comparable to or slightly higher than that found by Zhang et al. (2013) in methanol extracts from 10 lines of commercial mungbean in China (44–144 and 37–112 μg/g, respectively). The vitexin and isovitexin concentrations were highly correlated ($R^2 = 0.969$), with the isovitexin content around 20% higher than vitexin content (Figure S3). This strong correlation is expected, as vitexin and isovitexin are structural isomers of apigenin glucoside. Vitexin is apigenin-8-C-glucoside, while isovitexin is apigenin-6-C-glucoside (Figure 2); hence, both would be expected to form via a similar metabolic pathway (Abdullah, Chua, & Rahmat, 2017). However, despite the structural similarity between these compounds, they differ in their biological activities, with vitexin displaying greater spasmyloytic activity and inhibition of $\alpha$-glucosidase compared to isovitexin (Choo, Sulong, Man, & Wong, 2012; Ragone, Sella, Conforti, Volonté, & Consolini, 2007). However, isovitexin shows a higher antioxidant activity in many bioassay methods (He et al., 2016).

Notably, the free apigenin content was below the limit of quantification (<0.1 μg/g; data not shown), indicating that virtually all apigenin present in mungbean is in a glycosylated form, either as vitexin or isovitexin. Previous work has shown that vitexin and isovitexin contents decrease to below detection limits when mungbean is sprouted (Yao, Cheng, & Ren, 2011), suggesting that these
compounds may be hydrolysed into apigenin during germination. This agrees with other research that found apigenin concentrations increase during sprouting (Pająk, Socha, Galkowska, Roźnowski, & Fortuna, 2014). However, magnitude of reported increase in apigenin content (increasing from 0 to 1.9 μg/g) does not correspond directly to the decrease in combined vitexin/isovitexin content (decreasing from ~300 to 0 μg/g), suggesting that some intermediate form or side reaction may be at play during the germination process.

A number of other phenolic acids and flavonoids showed moderate to strong correlations with one another (Figure 4), particularly between caffeic and coumaric acids, vanillic and coumaric acids, and vanillic and syringic acids. In contrast, the levels of gallic, protocatechuic or gentisic acid were not strongly correlated with any other compounds. Such correlations between individual phenolic and flavonoid constituents are likely related to their similar synthesis pathways (Santos-Sánchez, Salas-Coronado, Hernández-Carlos, & Villanueva-Cañongo, 2019) or upregulation of regulatory genes increasing expression of multiple unrelated synthesis pathways.

The TPC and antioxidant capacity were positively correlated with one another ($r = 0.47–0.57$; $P < 0.05$), as observed in previous research (Johnson et al., 2020b; Xiang, Li, Ndolo, & Beta, 2019), although the antioxidant capacity was not correlated with the total monomeric anthocyanin content (Table S1). However, the TPC was negatively correlated with TMAC ($r = –0.41$, $P < 0.05$). The sinapic acid content was also positively correlated with the FRAP and TPC ($r = 0.40$ and $r = 0.53$, respectively; $P < 0.05$), although no other individual phenolic acids showed any significant correlation (Table S1).

## CONCLUSION

Overall, few significant differences were found between the yield, physical seed characteristic or nutritional quality of the four new mungbean lines and that of the commercial line Jade-AU. Three new mungbean lines (AVTMB 1, 3 and 4) developed by AgriVentis recorded a slightly higher yield (14%) compared to the commercial line Jade-AU. No significant difference was found in the overall phytochemical composition, although a number of differences were found for specific phenolic acids and vitexin. The results suggest that new mungbean lines tested for adaptation to warmer northern Australia environment performed equal to or better than a currently established commercial line. Multilocation trials over multiple seasons are suggested for detailed evaluation for regional adaptions of these new lines for stability of yield and quality parameters under the warmer north Australian production environment for mungbean.

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## CONFLICT OF INTEREST

Surya Bhattarai and Kerry Walsh are board advisors to AgriVentis Technologies. The authors declare no other competing interests.

## ETHICS STATEMENT

This work did not involve research on human or animal participants; hence ethics approval is not required.

## AUTHOR CONTRIBUTION

Conceptualisation: JJ, MN and SB; Resources: MN; Data Curation: JJ; Formal Analysis: JJ and JM; Supervision: MN and KW; Funding Acquisition: MN and SB; Investigation: JJ and JM; Visualisation: JJ; Methodology: JJ and MN; Writing - original draft: JJ; Writing - review & editing: JJ, JM, DS, KB, SB and MN.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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