Characterisation of seed marking types in chickpea (Cicer arietinum L.): Tiger stripe and other blemishes

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Abstract
Desi chickpea is a significant export crop for Australia; Australia being the largest exporter globally. Visual appearance of the seed is an economically significant measure of seed quality by the Indian subcontinent, the major importer of desi chickpea worldwide. Any visual blemish on the seed is considered undesirable, regardless of the cause (biotic or otherwise). Literature on biotic causes of seed blemishes, such as ascochyta blight, are available; however, little could be found on abiotic blemishes. Abiotic seed blemishes caused by physiological plant responses are more commonly known as seed markings. Despite the presence of seed markings being confirmed by several chickpea-producing countries during personal discussions (India, Canada), no scientific literature has been published. The aim of this study was to proactively seek out and characterise different types of seed marking patterns using a wide genetic pool of desi chickpea across a range of environments in Australia. Thirteen different seed marking patterns were identified in desi chickpea and three in kabuli chickpea, including several rare seed markings that were discovered, photographed, and described. Seed markings (blemishes thought to be caused by physiological plant stress) can be characterised as dark patterns on the testa (seed coat) that do not visually affect the underlying cotyledon. In contrast, other seed blemishes (caused by pests and disease, physical damage, or poor storage) were more likely to affect the cotyledons underlying the testa, but not always. This paper classifies and describes various types of seed markings and blemishes for future reference by the global chickpea industry.

KEYWORDS
Cicer arietinum, pulse grain classification, seed defect, seed marking, blotch/tiger stripe

1 | INTRODUCTION

Chickpea is an important crop globally and plays a significant role as both a whole food and an ingredient across the Indian subcontinent, Mediterranean, and westernised countries. There are two major types of chickpeas: the generally smaller desi chickpea with a yellow-brown testa (seed coat) and the kabuli-type, with a thin, white-cream testa, which is generally larger in size. (Singh & Diwakar, 1995; Wood, Knights, & Choct, 2011). Australia is a major producer and exporter of both desi and kabuli chickpeas, with the desi type dominating production (FAO, 2017). Visual appearance of chickpea seed is critical to meeting market specifications and attracting premium prices. Due to this emphasis on visual appearance, Australian chickpea growers may...
suffer from price penalties or rejection of harvested crops due to visual seed defects (Pulse Australia, 2005).

Visual seed defects include the presence of foreign material and obvious seed damage like sprouting, broken or chipped seeds, insect damage, shrivelled seeds, and green or sappy seeds. These defects are relatively easy to discern. Visual blemishes appearing as darkened areas of the seed can also be classified as defective, with the tolerance differing depending on the size of the blemish and the cause. Seed markings are one type of seed blemish, appearing as darkened patterns on the seed coat, not affecting the underlying cotyledons and presumably caused by physiological plant stress. Biotic causes of seed blemishes include ascochyta blight and moulds, and the market has a very low or “nil” tolerance for these types of blemishes. Other causes of poor colour and blemishes, including seed markings like “tiger stripe,” range in their tolerance allowable under the Standards (Pulse Australia, 2019). Tiger stripe is defined in the Australian Pulse Standards as “dark-coloured lines of striping on the seed coat or kernel” (Pulse Australia, 2019).

Most seed markings are not commonly observed or noticed in commercial crops. However, on grain delivery, routine evaluation of samples can lead to marked seeds being mistaken for disease. A potential misclassification of a seed marking, such as tiger stripe, into a nil tolerance disease category would unfairly penalise a chickpea grower.

Although no scientific literature on physiological seed markings in chickpea exists, tiger stripe markings have previously been seen in some breeding accessions of desi chickpea in Australia, Canada, and India (Pers. Comm.). As seed markings detract from the visual appearance of the seed, they are undesirable and accessions displaying obvious seed markings are removed from breeding programmes. The aim of this 5-year study was to proactively seek out and characterise different types of seed marking patterns from a diverse genetic pool of desi chickpea across a wide range of environments in Australia. This characterisation of seed marking types is now being used as a tool to correctly identify and differentiate different seed marking types for breeding and research purposes in Australia.

2 | MATERIALS AND METHODS

2.1 | Samples

Harvested chickpea seed samples were obtained from Australian grain growers (predominantly from Southern NSW to Central Queensland; 2014–2017 seasons) and research agronomy trials and breeding accessions (spanning all the major growing regions of Australia; 2013–2017 seasons). The grower seed samples comprised commercial cultivars (Almaz, Ambar, Flipper, Genesis Kalkee, Genesis Monarch, Genesis090, Genesis114, Genesis425, Jimbour, Kyabra, Neelam, PBA Boundary, PBA HatTrick, PBA Pistol, PBA Seamer, and PBA Slasher). The trial seed samples differed across the range of trials which contained diverse breeding germplasm and commercial varieties (including Almaz, Amethyst, Flipper, Genesis Kalkee, Genesis079, Genesis090, Genesis0509, Genesis0836, Gully, Howzat, Jimbour, Kyabra, Moti, PBA Boundary, PBA Drummond, PBA Drummond, PBA Maiden, PBA Monarch, PBA Pistol, PBA Seamer, PBA Slasher, PBA Striker, Rupali, Sonali, and Yorker). These trials were subject to a wide range of environmental conditions and management treatments. In total, 9,493 chickpea samples were evaluated over five seasons.

Seed sample quantities received ranged between 500 g and 3 kg, depending on seed availability and source. The entire sample was visually evaluated for the presence/absence of seed markings, through individual seed-by-seed examination. All seeds with markings were recorded, and photographs were taken for any new types of markings discovered.

2.2 | Microscopy

Mature seeds of desi chickpea identified to have seed markings were soaked overnight in distilled water to facilitate removal of the testa and improve pliability for mounting. The testa of the soaked seeds were removed from the cotyledons manually after incising with a scalpel. Tweezers were used to peel back the lower parenchyma layer of the testa which severs at the hourglass cells (Wood et al., 2011). Both the top layer (palisade layer) and lower layer (parenchyma layer) of the testa and the underlying cotyledons were examined for visual pigmentation and other visual changes.

For mature blotch/tiger-striped seeds of desi chickpea (cv. PBA Pistol), detached testa (as described above) were cut finely with a razor blade and cross sections directly mounted on microscopic slides under a coverslip. Marked and unmarked testa were studied without the need for artificial staining by phase-contrast microscopy (BHA model, Olympus) to ascertain more detailed information about the location of pigmentation in the testa responsible for the markings.

2.3 | Histochemical staining for proanthocyanidins

Mature kabuli and desi seed coats were stained using three different methods to visualise differences in proanthocyanidin accumulation, as follows:

- 4-dimethylaminocinnamaldehyde (DMACA)/Methanol/6M HCl method of Li, Tanner, and Larkin (1996),
- DMACA/Butanol method of Brilouet & Escoute, 2012; Cadot, Miñana Castelló, & Chevalier, 2006, and
- 1% Vanillin–6M HCl method of Aastrup, Outtrup, and Erdal (1984).

3 | RESULTS

3.1 | Seed markings of desi chickpea

3.1.1 | Visual characterisation

Previously known and named seed markings were observed during our detailed visual evaluation, including black, mosaic, speckled, and
# TABLE 1  Seed marking types observed and classified in desi chickpea (*Cicer arietinum* L.) material

| Seed marking name                  | Image | Description                                                                 |
|------------------------------------|-------|-----------------------------------------------------------------------------|
| Black                              | ![Image](image1.png) | Black testa. The black colour normally extends over the entire testa.     |
| Mosaic                             | ![Image](image2.png) | Testa features a darker brown or black mosaic pattern. This can affect the whole seed or only a portion of the seed. |
| Speckled                           | ![Image](image3.png) | Testa has speckles that are darker than the main testa colour.              |
| Blotch/tiger stripe (typical tiger stripe) | ![Image](image4.png) | Typical tiger stripe pattern of the testa found on the ventral side of the seed toward the base. There may be a single stripe on only one side of the seed or there may be a pair of stripes, one on each side of the postchalazal bundles. Stripes do not extend past the chalaza. |
| Blotch/tiger stripe (typical blotching) | ![Image](image5.png) | Blotch pattern of the testa is more variable than tiger stripe appearing as an extension or widening of the stripes. The marks can expand above the chalaza, under the base or onto (Continues) |

(Continues)
| Seed marking name | Image | Description |
|-------------------|-------|-------------|
| **the lateral side of the seed. May appear as an isolated blotch, often on the lateral side(s) of the seed. The blotch/stripes may spread to the dorsal side of the seed. The beak (embryonic axis) of the seed is not affected.** |
| **Slit** | One thin line on the testa that is darker than the main testa colour. Occurs on the dorsal and ventral sides of the seed and are commonly not visually obvious in a sample. |
| **Owl eyes** | One single, or pair of dark circles (one on each side), visible on the ventral side of the seed between the chalaza and the microphyle. Very rare. |
| **Critters** | One single, or pair of dark circles (one on each side), visible on the dorsal side of the seed behind the beak (embryonic axis). They may extend over the bridge of the beak or joined like a blindfold. Very rare. |
| **Black beak** | (Continues) |
| Seed marking name | Image | Description |
|-------------------|-------|-------------|
| Darkened testa around the beak (embryonic axis). Very rare. | ![Image](image1.png) | |
| Scroll | ![Image](image2.png) | Darker testa colour which follows the natural seed depressions and contours. Rare. |
| Veined | ![Image](image3.png) | Fine darker testa colour which follows the natural seed depressions and contours. A finer version of the scroll marking. Rare. |
| Ornate scroll | ![Image](image4.png) | Darker testa colour outlined by a finer lighter colour which follows the natural seed depressions and contours. Very rare. |
| Painted | ![Image](image5.png) | Darker testa colour which does not follow the natural seed depressions, instead cutting across the seed contours (only one seed observed). |
| Irregular markings | ![Image](image6.png) | Darker testa colour of no consistency. Can occur anywhere on the seed |

(Continues)
tiger stripe. We also named and described several newly discovered markings (Table 1). These seed markings normally only appear on a low percentage of seeds within any given sample.

Tiger stripe was the most commonly observed seed marking throughout the five seasons. A new marking, discovered in 2013, was named “blotch” (Wood, 2013). Initially, attempts were made to classify seeds as having either tiger stripe or blotch. However, it was technically difficult to achieve this differentiation because both marking types could be found in combination on individual seeds. It is noteworthy that when blotching occurred, there were always some seeds displaying the tiger stripe marking within the same sample. It became apparent that the blotch marking often appeared as the severity of the tiger stripe marking in samples increased (i.e., markings becoming larger, darker, and more obvious). We decided that the two markings were intrinsically linked and impossible to differentiate when individual seeds displayed both marking types, hence the two classifications were reassigned into one “blotch/tiger stripe” marking category from 2014 onwards (Figure 1).

We discovered additional new markings and named them as slit, owl eyes, critters, black beak, scroll, veined, ornate scroll, and painted. Table 1 shows images of all the types of seed markings and the classification names assigned. In addition, several non-uniformly located and irregularly shaped markings were also observed.

### 3.1.2 Microscopic characterisation

All seed markings characterised in Table 1 were found to be confined to the outer layers of the testa (Figure 2), known as the palisade layer. The inner testa layer, known as the parenchyma layer, was opaque and contained no pigments (Figure 2), and no pigmentation or visual changes were identified in the underlying cotyledon.

Microscopy showed that the markings involve an accumulation of amber-coloured pigment within the vacuoles of the macrosclerid cells below the light line of the testa (Figure 3). This is the same region where normal pigmentation occurs in the nonmarked portion of the testa and in regular desi chickpeas, as previously characterised by (Cubero, 1987). The amber-coloured deposits appear to be phenolic compounds, such as proanthocyanins.

### 3.2 Seed markings of kabuli chickpea

Of all the thousands of samples evaluated, kabuli chickpeas showed an almost total absence of seed markings, with only several individual marked seeds found once in only three genetically diverse breeding accessions, representing <1% of the kabuli samples evaluated (Table 2).

The reason for seed markings being extremely rare in chickpeas of the kabuli type may be due to their white-cream testa colour. Kabuli chickpeas are readily distinguished from desi plants as having white (nonpigmented) flowers and an absence of anthocyanin pigments in their vegetative tissues. Staining of testas for proanthocyanidin confirmed no proanthocyanidins in the kabuli testa, whereas desi chickpeas were abundant in proanthocyanidins as shown through staining green with DMACA and brown with Vanillin (Figure 4). In addition, a desi chickpea variety, gully, which is characterised by an absence of anthocyanin pigments in the vegetative tissues but produces a light pink flower and light salmon coloured

### Table 1 (Continued)

| Seed marking name | Image | Description |
|-------------------|-------|-------------|
| slit              |       | or be dispersed across the seed. Very rare. |
seed, was also stained and showed a medium level of staining by both methods at an intermediate level between the typical kabuli and desi staining (not shown), suggesting that it contained an intermediate level of proanthocyanidins in its testa.

3.3 Other blemishes of chickpea seeds

We observed other blemishes on seeds that we believe are not physiological seed markings during this study. These blemishes included bruised seeds, ascochyta blight-infected seeds, and several seeds with blemishes reminiscent of symptoms associated with biotic causes in other species (Table 3). These will be discussed in more detail in the discussion section.

4 DISCUSSION

Physiological seed markings are known to occur in desi chickpeas throughout breeding programmes of the major producing countries (Pers. Comm.), including India, Canada, and Australia. Moreover, seed markings are likely occurring in most, if not all, countries as chickpea domestication has led to a narrow genetic base globally (von Wettberg et al., 2018). Most chickpea-producing countries have seed evaluation within their breeding programmes that will evaluate and discard germplasm that is affected.

We identified numerous different patterns of seed markings in this study. All of the seed markings identified in Table 1 were confined to the testa (localised in the vacuoles of the macrosclerid cells below the light line) and had no effect on the underlying cotyledons. These markings are believed to be abiotic or genetic in origin and generally not caused by biotic factors. The majority of the markings were very distinct in their patterning and location on the seed. Genetics control the regular patterning of testa in other pulses, like lentils (Vandenberg & Slinkard, 1990) and common bean (Caldas & Blair, 2009). However seed markings that occur when physiologically triggered by environmental factors have not been reported in the literature previously. The frequency of seed marking incidence and the role of genetics and environment will be explored further for some of the more common seed markings in subsequent publications.

We observed and classified seed marking types on desi chickpea seeds as black, mosaic, speckled, blotch/tiger stripe, slit, owl eyes, critters, black beak, scroll, veined, ornate scroll, painted, and non-uniform irregular markings. Many of these seed markings are very rare and were only observed in one or two samples, and sometimes, only on a single seed. Light speckling is the most common marking observed in commercial desi chickpea crops. The next most commonly observed markings were blotch/tiger stripe and mosaic type markings. Although they are much less common in commercial crops of chickpea, when they do occur, it is normally at a rate of less than 3% of seeds affected. At these levels, the markings are often not visually discernible in a sample during normal screening processes.

Australia has very strict standards of grain quality for desi chickpea. The Australian Grain Receival Standards (Pulse Australia, 2019) describe speckled and tiger stripe seeds as sound (i.e., nondefective)
because desi chickpeas are most commonly dehulled prior to consumption and seed markings do not cause any damage to the underlying cotyledons of the seed. However, there is potential for the blotch/tiger stripe marking to be mistaken for disease (such as ascochyta blight, botrytis grey mould, or saprophytic fungi) or stained and weather damaged, for which the Standards state strict tolerances.

There was no literature evidence nor personal communications that seed markings could occur in kabuli chickpea. Seed markings were almost never found in chickpeas of the kabuli type, attributed to their white-cream coloured testas. Kabuli plants have white flowers and lack anthocyanin in vegetative tissues so the testas of the seeds have little to no proanthocyanins. We hypothesise that proanthocyanins are involved as precursors in the appearance of seed markings in desi types (i.e., chickpeas with coloured testas), and expression occurs when triggered by some sort of environmental plant stress. We did not assess the different environmental stresses.

**TABLE 2** Seed marking types observed and classified in kabuli chickpea (*Cicer arietinum* L.)

| Seed marking name | Image | Description |
|-------------------|-------|-------------|
| Lacework          | ![Image](image_url) | A pattern of light spots and slightly darker swirls extending over the entire testa. |
| Slash             | ![Image](image_url) | Testa features a brown slit-like marking, reminiscent of a cut but not a physical wound. Occur on the dorsal side toward the base. |
| Dark chalaza      | ![Image](image_url) | Dark chalaza, sometimes extending along the seed midline. |
seeds from desi-types contained approximately 2.3 mg/g, total phenolics and proanthocyanidin, respectively (Segev et al., 2010; Wood & Grusak, 2007). Due to the large natural diversity of phenolic chemical structures and the complexity of their biochemical interactions, the scientific understanding of colour chemistry in plant biology is still in its infancy. The specific compounds accumulating in the testa that create the visual appearance of seed markings will require further investigation to elucidate, as will the environmental triggers.

We observed seed bruising during our study. Pod restriction can cause seed bruising when there is limited pod expansion during development, causing seeds to be squashed too tightly against each other in the pod (Figure 5A). Seed bruising was also caused by hail directly hitting pods during the podding stage (Figure 5B). Both types of seed bruising led to cotyledon damage (Figure 5B, insert). Bruising can also be caused through mechanical damage sustained during the season or at harvest, particularly when the seeds contain higher than recommended moisture content.

Although bruised seeds may at first appear similar to the blotch/tiger stripe seed marking, bruising almost always caused some damage (necrosis) to the underlying cotyledon, whereas seed markings were always confined to the testa. Therefore, removal of the seed testa on inspection was the best way to identify damaged cotyledons sustained from bruising events.

Biotic causes also can result in seed blemishes, particularly when infection of plants occurs late in the season just prior to, or during, seed development. Ascochyta blight (Ascochyta rabiei) is a fungus that can cause large yield losses in chickpea, and late season infection can result in seed blemishes. Typical ascochyta blight symptoms of mature pods and seeds of desi chickpea are shown in Figure 6. Infected seeds show dark round or irregular cankers, sometimes with a grey centre and bearing pycnidia, whereas the underlying cotyledons of badly affected seeds will also have dark necrotic marks (Pande et al., 2005). We observed damaged cotyledons due to infection in this study (Figure 6). However, we observed also that many infected blemished seeds can have normal underlying cotyledons with no visual blemish or necrosis. Infected seeds may also be dark and shrivelled when infection occurred on pods and the seeds were still small and developing (Nene, 1982; Pande et al., 2005).

A substantial amount of research into ascochyta blight occurred due to its significant contribution to yield losses in Australia and other chickpea-producing countries. Ascochyta blemishes on seeds may not damage the underlying cotyledons, and they can appear somewhat similar to blotch markings. This can lead to confusion over classification of seed quality in relation to receiveable standards. Pods display typical concentric circles, caused by pycnidia (Figure 6). Pycnidia circles are the best characterising symptom to look for in seed samples that still contain some pod material. Knowledge of paddock history and crop infection will also assist in differentiating between blemishes caused by disease and seed markings caused by physiological effects; however, this information is not always available with harvested seed samples.

We observed a single seed from only one sample that displayed distinct concentric circles (Table 3; Figure 7A). These types of circles are often associated with disease and although ascochyta blight causes similar circular patterns on pods, it does not cause this appearance on seeds (Figure 6). No literature or images of similar markings could be found in searches conducted by the authors, except for an image of watermelon mosaic virus (Figure 7B). It is possible that this pattern could be caused by a viral infection; however, we found only one single seed with this pattern in one sample of the thousands of samples examined.

Similarly, one seed was found in a different sample with a marking that appeared similar to tiger stripe but with the addition of "wiggly lines" around the outer rim of the chickpea sides (Table 3; Figure 8A). We discovered this wiggly lines seed during temporal sampling and evaluation of seeds in a different experiment, and no other seeds on the same plant displayed this marking. The wiggly lines around the seed perimeter (Table 3; Figure 8A) did resemble the pea seed-borne mosaic virus (PStbMV; Genus Potyvirus, Family Potyviridae) symptoms observed on faba bean (Vicia faba L.) seeds shown in Figure 8B (GRDC, 2017). In field pea (Pisum sativum L.), PStbMV symptoms include necrotic rings and line patterns, most obvious on white-seeded pea varieties, often referred to as "tennis ball" patterns that...
TABLE 3  Other blemishes observed and classified in desi chickpea (*Cicer arietinum* L.)

| Seed marking name | Image | Description |
|-------------------|-------|-------------|
| Bruising          | ![Image](image1.png) | Bruised seeds. Darkened blemishes of irregular to round shapes. Inset: Cotyledon damage (necrosis) beneath the testa blemish. |
| Ascochyta         | ![Image](image2.png) | Infected seeds show dark round or irregular cankers, sometimes with a grey centre. Infected seeds can also be dark and shrivelled. Inset: Badly infected seeds exhibit dark necrotic marks on the cotyledon underlying the testa blemish. |
| Wiggly lines      | ![Image](image3.png) | Tiger stripe like marking plus wiggly lines around the outer rim of the chickpea lateral sides particularly near the ventral tiger stripe (only one seed observed). |
| Concentric circles| ![Image](image4.png) | Distinct concentric circles all over the seed surface (only one seed observed). |

FIGURE 5  Seed marks on desi chickpea (*Cicer arietinum* L.) due to bruising caused by (A) pod restriction and (B) hail (cotyledon damage from bruising; inset)
can be blistered and also can cause reduced seed size, splitting, and malformation (Figure 8C; Coutts, 2018).

There is little literature describing the effect of PSbMV on the seed of chickpea. Latham and Jones (2001) stated that necrotic ring and line pattern symptoms were observed in seed coats from a range of legume species, including chickpea; however, only images of field pea, faba bean, and Lathyrus ochrus seed symptoms were shown. They also tested six varieties of chickpea for PSbMV and classified all as resistant and seed transmission as low. Coutts, Prince, and Jones (2008) infected chickpeas with PSbMV and showed images of infected desi and kabuli chickpea seeds at maturity being shrivelled and darker in appearance (Figure 9); however, there were no obvious characteristic wiggly lines on those seeds to match this single seed (Figure 8A). Additionally, the tennis ball patterns of PSbMV-infected field pea (Figure 8C; Coutts, 2018) also somewhat resemble the ornate scroll markings (Table 1). We are unable to conclude why the green seed had a wiggly lines appearance. We also cannot conclude whether the wiggly lines or ornate scroll markings are symptoms of biotic infection, but due to the low transmission rate and resistant classification of Australian chickpeas to PSbMV, we believe that PSbMV is unlikely to be the cause. Further research in this area will be required to elucidate the answer.

Some images of kabuli seeds from phytoplasma-infected plants were uncovered during the literature search for chickpea seed blemishes caused by diseases (Brier, 2018).
were visually similar in appearance to the “veined” seed marking characterised on desi chickpea seeds in Table 1. The images are reproduced in Figure 10 for direct comparison. The biggest difference between these two images is the type of seed affected, desi compared with kabuli.

Phytoplasma is a specialised bacterial species that infects plants and, in northern Australia, a brown leafhopper (Orosius orientalis) is believed to be the likely insect vector (Brier, 2018). Phyllody is a recognised plant symptom of phytoplasma infection, whereby abnormal clumps of tiny leaves or secondary flowering structures grow out of the original flowers (Brier, 2018). Very little literature exists on phytoplasma in chickpea, and no specific mention of infection in desi types could be sourced. However, occasionally, we have observed phyllody in desi chickpea plants.

Phyllody typically reduces the number of normal plant reproductive organs and reduces seed yield. Hence, seeds displaying phytoplasma induced blemishes were most likely the result of late-season infection during plant podding. Therefore, it is possible that the veined pattern observed on desi chickpea could be caused by phytoplasma infection late in the season, similar to the images in Figure 10B reproduced from Brier (2018). Whether all phytoplasma plants infected late in the season produce these veined type of seed blemishes will need to be confirmed; similarly, it will be important to determine whether veined seed markings can be produced by plants that are not infected by phytoplasma. Then, this would provide evidence whether the veined appearance of desi chickpea was caused by phytoplasma, abiotic stress, or both. Furthermore, since the “scroll” and ornate scroll seed markings occur with a similar patterning to the veined marking (Table 1), there is a possibility that these markings also may be caused by phytoplasma.

It is noteworthy that the phytoplasma-infected kabuli seed blemished very heavily despite the testa lacking proanthocyanidins. Relatively, little is known about the polyphenol mechanism(s) responsible for changes in testa colour and darkening. However, oxidation of proanthocyanidins and resultant formation of complexes with other phenolic compounds were implicated in seed darkening of other species including bean (Phaseolus vulgaris L.), grape (Vitis vinifera L.), sorghum, and Arabidopsis, (Beninger et al., 2005; Beninger & Hosfield, 1999; Dean et al., 2011; Jorgensen, Marin, & Kennedy, 2004). Further research is necessary to elucidate the biochemical pathway that can cause nonpigmented seeds to display these dark blemishes after phytoplasma infection. Such research may assist in understanding potential polyphenol biochemical pathways and mechanisms of darkening of other seeds and produce.

5 | CONCLUSIONS

Thirteen different seed marking patterns of desi chickpea, and three of kabuli chickpea, that are believed to be physiological seed markings were identified and characterised. To achieve this, 9,493 samples collected over 5 years spanning a diverse range of genetics and
environments in Australia were examined. Each seed marking type was characterised and photographed, and the new types were assigned names. Similarities of several newly identified seed markings were compared with known disease blemishes and discussed. Sometimes, it can be difficult to differentiate the cause of these seed blemishes, especially when visual blemishes appear similar to disease blemishes. This publication will serve as a reference document for chickpea breeders, researchers, agronomists, and marketers in identifying seed markings and other blemishes of chickpea seeds. Further research is required to understand the genetic, environmental, and biotic factors influencing the expression of seed markings, as well as research on the underlying biosynthetic pathways and regulation within chickpea plants.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES

Aastrup, S., Outrup, H., & Erdal, K. (1984). Location of the proanthocyanidins in the barley grain. Carlsberg Research Communications, 49, 105–109. https://doi.org/10.1007/BF02913969

Beninger, C. W., & Hosfield, G. L. (1999). Flavonol glycosides from Montcalm dark red kidney bean: implications for the genetics of seed coat color in Phaseolus vulgaris L. Journal of Agricultural and Food Chemistry, 47, 4079–4082. https://doi.org/10.1021/jf990440d

Beninger, C. W., Gu, L., Prior, R. L., Junk, D. C., Vandenberg, A., & Bett, K. E. (2005). Changes in polyphenols of the seed coat during the after-darkening process in pinto beans (Phaseolus vulgaris L.). Journal of Agricultural and Food Chemistry, 53, 7777–7782. https://doi.org/10.1021/jf0500511

Brier, H. (2018). Keep a lookout for phyttoplasm in mungbeans and other summer legumes. The Beetsheet.

Brillouet, J. M., & Escoute, J. (2012). A new technique for visualizing proanthocyanidins by light microscopy. Biotechnic & Histochemistry, 87, 195–200. https://doi.org/10.3109/10520295.2011.603703

Cadot, Y., Miñana Castelló, M. T., & Chevalier, M. (2006). Flavan-3-ol compositional changes in grape berries (Vitis vinifera L. cv Cabernet Franc) before veraison, using two complementary analytical approaches, HPLC reversed phase and histochemistry. Analytica Chimica Acta, 563, 65–75. https://doi.org/10.1016/j.aca.2006.01.063

Caldas, G., & Blair, M. (2009). Inheritance of seed condensed tannins and their relationship with seed-coat color and pattern genes in common bean (Phaseolus vulgaris L.). Theoretical and Applied Genetics, 119, 131–142. https://doi.org/10.1007/s00122-009-1023-4

Cheynier, V., Comte, G., Davies, K. M., Lattanzio, V., & Martens, S. (2013). Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. Plant Physiology and Biochemistry, 72, 1–20. https://doi.org/10.1016/j.plaphy.2013.05.009

Coutts, B. (2018). Pea seed-borne mosaic virus in field peas. Retrieved from https://www.agric.wa.gov.au/field-peas/pea-seed-borne-mosaic-virus-field-peas

Coutts, B. A., Prince, R. T., & Jones, R. C. A. (2008). Further studies on pea seed-borne mosaic virus in cool-season crop legumes: responses to infection and seed quality defects. Australian Journal of Agricultural Research, 59, 1130–1145. https://doi.org/10.1071/AR08113

Cubero, J. I. (1987). Morphology of chickpea. In M. C. Saxena, & K. B. Singh (Eds.), Plant Physiology and Biochemistry of Legumes: susceptibility, sensitivity, and seed transmission. CABI.

Dean, G., Cao, Y., Xiang, D., Provart, N. J., Ramsay, L., Ahad, A., ... Haughn, G. (2011). Analysis of gene expression patterns during seed coat development in Arabidopsis. Molecular Plant, 4, 1074–1091. https://doi.org/10.1093/mp/ssr040

FAO. (2017). FAOSTAT database. Food and Agriculture Organization of the United Nations, Statistics Division, Rome. 2013. http://faostat.fao.org/faostat/beta/en/

GRDC (2017). Faba bean - Section 9 Diseases. In GRDC Grownotes (p. 64). Canberra: GRDC.

Harborne, J. B. (Ed.) (1994). The flavonoids: advances in research since 1986. Boca Raton, Florida: CRC Press.

Jorgensen, E. M., Marin, A. B., & Kennedy, J. A. (2004). Analysis of the oxidative degradation of proanthocyanidins under basic conditions. Journal of Agricultural and Food Chemistry, 52, 2292–2296. https://doi.org/10.1021/jf035311i

Latham, L. J., & Jones, R. A. C. (2001). Alfalfa mosaic and pea seed-borne mosaic viruses in cool season crop, annual pasture, and forage legumes: susceptibility, sensitivity, and seed transmission. Australian Journal of Agricultural Research, 52, 771–790. https://doi.org/10.1071/AR00165

Li, Y.-G., Tanner, G., & Larkin, P. (1996). The DMACA–HCl protocol and the threshold proanthocyanidin content for blat safety in forage legumes. Journal of the Science of Food and Agriculture, 70, 89–101. https://doi.org/10.1002/(sic)1097-0011(1996)70:1<89::AID-jfsa470>3.0.CO;2-n

Lorrain, B., Ky, I., Pechemat, L., & Teissedre, P.-L. (2013). Evolution of polyphenols from grapes, wines, and extracts. Molecules, 18, 1076–1100. https://doi.org/10.3390/molecules18011076
Marles, M. A. S., Warkentin, T. D., & Bett, K. E. (2013). Genotypic abundance of carotenoids and polyphenolics in the hull of field pea (Pisum sativum L.). *Journal of the Science of Food and Agriculture, 93*, 463–470. https://doi.org/10.1002/jsfa.5782

Muzquiz, M., & Wood, J. A. (2007). Antinutritional factors. In S. S. Yadav, R. Redden, W. Chen, & B. Sharma (Eds.), *Chickpea Breeding and Management* (pp. 143–166). Wallingford, UK: CAB International.

Nene, Y. L. (1982). A review of Ascochyta blight of chickpea. *Tropical Pest Management, 28*, 61–70. https://doi.org/10.1080/09670878209370675

Pande, S., Siddique, K., Kishore, G., Bayaa, B., Gaur, P., Gowda, C., ... Crouch, J. (2005). Ascochyta blight of chickpea (Cicer arietinum L.): A review of biology, pathogenicity, and disease management. *Australian Journal of Agricultural Research, 56*, 317–332. https://doi.org/10.1071/AR04143

Pulse Australia (2005). Australian Pulse Trading Standards 2005/2006. In: National Agricultural Commodities Standards Manual (12th Edition ed., Vol. Section 4, pp. 1-61). Sydney, Australia: National Agricultural Commodities Marketing Association (NACMA).

Pulse Australia. (2019). Australian Pulse Standards 2019/2020. http://www.pulseaus.com.au/marketing/receival-trading-standards

Segev, A., Badani, H., Kapulnik, Y., Shomer, I., Oren-Shamir, M., & Galili, S. (2010). Determination of polyphenols, flavonoids, and antioxidant capacity in colored chickpea (Cicer arietinum L.). *Journal of Food Science, 75*, S115–S119. https://doi.org/10.1111/j.1750-3841.2009.01477.x

Singh, F., & Diwakar, B. (1995). Chickpea botany and production practices. Skill development series no. 16. Patancheru, Andhra Pradesh, India: ICRIASAT (International Crops Research Institute for Semi-Arid Tropics).

Treuher, D. (2006). Significance of flavonoids in plant resistance: A review. *Environmental Chemistry Letters, 4*, 147–157. https://doi.org/10.1007/s10311-006-0668-8

Vandenberg, A., & Slinkard, A. E. (1990). Genetics of seed coat color and pattern in lentil. *Journal of Heredity, 81*, 484–488. https://doi.org/10.1093/oxfordjournals.jhered.a111030

von Wettberg, E. J. B., Chang, P. L., Başdemir, F., Carrasquilla-Garcia, N., Korbu, L. B., Moenga, S. M., ... Cook, D. R. (2018). Ecology and genomics of an important crop wild relative as a prelude to agricultural innovation. *Nature Communications, 9*, 649. https://doi.org/10.1038/s41467-018-02867-z

Wood, A. & Napier, T. (2009). Diseases of cucurbit vegetables. *NSW DPI Prime Fact No. 832*, 1-6.

Wood, J. A. (2013). Seed markings of desi chickpea. *Pulse Breeding Australia (PBA) Fact Sheet, September 2013*.

Wood, J. A., & Grusak, M. A. (2007). Nutritional value of chickpea. In S. S. Yadav, R. Redden, W. Chen, & B. Sharma (Eds.), *Chickpea Breeding and Management* (pp. 101–142). Wallingford, UK: CAB International.

Wood, J. A., Knights, E. J., & Choct, M. (2011). Morphology of chickpea seeds: Comparison between desi and kabuli types. *International Journal of Plant Sciences, 172*, 632–643. https://doi.org/10.1086/659456

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