Seed quality and the effect of introducing *Cicer echinospermum* to improve disease and pest resistance in desi chickpea

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**Abstract**

The utilisation and consumption of food crops for human nutrition demand acceptable seed quality traits to enable efficient processing into food products. Desi chickpea (*Cicer arietinum* L.) is a staple food of the Indian subcontinent, and domestication has led to cultivars with seeds that are an acceptable size, colour, and shape, easy to mill, and quick to hydrate and cook. Domestication has also severely restricted the gene pool, so breeders are looking to "wild" chickpea relatives as sources of novel genes that may provide agronomic benefits such as disease and pest resistance. *Cicer echinospermum* (a "wild" relative of cultivated chickpea with black, echinate seeds) was used in the breeding programme to introgress phytophthora root rot and root-lesion nematode resistance genes into adapted *C. arietinum* backgrounds. The resulting *C. echinospermum* derivative lines were compared with commercial desi cultivars to examine any effects on seed quality attributes. The *C. echinospermum* derivatives had similar visual seed characteristics to the *C. arietinum* cultivars with, on average, lower milling performance and quicker cooking times; however, a few individual derivative lines met or exceeded the average cultivar milling performance. This paper shows the quality variation within the *C. echinospermum* derivatives, compares them with commercial desi cultivars, and confirms their potential to improve disease resistance whilst retaining the basic seed quality traits important for commercialisation and exporting new cultivars.

**KEYWORDS**

chickpea, *Cicer echinospermum*, cooking, domestication, milling, seed quality traits, wild species

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**1 | INTRODUCTION**

Chickpea (*Cicer arietinum* L.) is the second largest pulse crop in the world and is particularly important in the Indian subcontinent, Central and West Asia, and North Africa as a source of protein, carbohydrate, vitamins, and minerals. The species can be divided into two main groups on the basis of seed type. Kabuli chickpeas will not be discussed in this paper. Desi chickpeas generally have a small, angular shaped seed with a thick, darker coloured testa (seed coat). Some desi chickpeas are cooked whole or roasted, but most are decorticated and split to produce dhal, and the dhal can be further processed into flour (besan).

Moisture content, uniformity, and visual appearance (seed size and colour) are the major quality considerations for chickpea export (Dusunceli, Wood, Gupta, Yadav, & Yadav, 2007). Other quality parameters of chickpea, such as milling performance (Ravi &
Harte, 2009; Wood & Malcolmson, 2011), hydration characteristics (Wood & Harden, 2006), and cooking time (Reyes-Moreno, Okamura-Esparza, Armentia-Rodelo, Gómez-Garza, & Milán-Carrillo, 2000), are also important, particularly to primary processors, food processors, and consumers (Agbola, Kelley, Bent, & Rao, 2002) who ultimately drive demand. Consumers pay premium prices to attain good quality, and the Australian chickpea breeding programme routinely screens genotypes to ensure that these standards are maintained.

Australia is the largest exporter of chickpea, producing and exporting 960,000 tonnes annually, averaged over 5 years: 2012–2016 (FAO, 2019). The industry is concentrated in the north-east region of Australia (northern New South Wales and Queensland) where production is almost exclusively of the desi type. The main biotic production constraints for chickpea in north-eastern Australia are ascochyta blight (Ascochyta rabiei) and phytophthora root rot (Phytophthora medicaginis). Both diseases are capable of up to 100% mortality under conducive conditions (Du, Zhao, Raju, Davies, & Trethowan, 2012; Knights, Southwell, Schwinhamer, & Harden, 2008). Moderate levels of resistance to ascochyta blight has now been incorporated in C. arietinum cultivars and breeding lines, although there is some evidence that the pathogen may be adapting to become a threat to this resistance (Mehmood et al., 2017). Only moderate resistance to phytophthora root rot (P. medicaginis) had been identified in the cultigen (Brinsmead, Rettke, Irwin, Ryley, & Langdon, 1985). The presence of root-lesion nematodes (Pratylenchus thornei and Pratylenchus neglectus) can also constrain the production of chickpea, and they are widely distributed across the Australian grain-growing regions (Hollaway, Vanstone, Nobbs, Smith, & Brown, 2008; Reen, Mumble, & Thompson, 2019; Riley & Kelly, 2002; Riley & Wouts, 2001; Thompson et al., 2010).

It is now widely recognised that domestication of chickpea over thousands of years, like other crops, has resulted in significant narrowing of genetic diversity (Muñoz, Liu, Kan, Li, & Lam, 2017; Singh et al., 2015; von Wettberg et al., 2018). "Wild" relatives of C. arietinum, such as Cicer echinospermum and Cicer reticulatum, show a wider range of behaviours in response to various biotic and abiotic stresses than domesticated chickpea (Amalraj et al., 2019; Croser, Ahmad, Clarke, & Siddique, 2003; Iruela, Rubio, Cubero, Gil, & Millan, 2002; Reen, Mumble, & Thompson, 2019; Singh et al., 2015; Singh, Rani, Malhotra, Katna, & Sarker, 2018) and are a valuable source of resistance genes (Muehlbauer, Kaiser, & Simon, 1993).

The seeds of these "wild" relatives are, however, very different to domesticated chickpeas visually, and their quality attributes are largely unknown. The seeds of C. echinospermum, for example, are dark brown to black with a thick and echinate testa that is resistant to water absorption (Robertson, Ocampo, & Singh, 1997). In comparison, domesticated C. arietinum chickpea cultivars have smooth, yellow-brown testa and are generally easily hydrated in water. Backcrossing of the "wild" relatives into C. arietinum backgrounds that have acceptable seed characteristics and are adapted to local conditions is therefore necessary to achieve cultivars that can be successfully grown and marketed.

The Australian chickpea breeding programme targeted several "wild" relatives of C. arietinum as alternative sources of improved phytophthora root rot and root-lesion nematode resistance. The C. echinospermum accessions generated showed superior resistance to phytophthora root rot (Knights et al., 2008) and root-lesion nematodes (Thompson et al., 2011); hence, a backcrossing programme was developed to introgress disease resistance genes into adapted Australian C. arietinum backgrounds. The resulting C. echinospermum derivative lines examined in this study have a theoretical 25% C. echinospermum in their genome. They have similar yields to C. arietinum cultivars and no agronomic penalties (Knights et al., 2008). This paper reports an investigation into the quality characteristics of these C. echinospermum derivatives compared with Australian C. arietinum desi cultivars.

# 2 | MATERIALS AND METHODS

## 2.1 | Samples

The C. echinospermum accession L204 was previously identified as resistant to phytophthora (Schwinhamer, Moore, Knights, Welsby, & Murison, 1993). L204 was crossed to three C. arietinum parents (cvs Amethyst and Barwon, and the breeding line 946-512). F₂ plants, selected for their domesticated phenotype (i.e., did not show any of the primitive features of C. echinospermum: prostrate growth, indeterminate flowering, dehiscent pods, and spiny testa), were subsequently crossed with one of the same three C. arietinum parents. BC₁F₃ or BC₁F₄ lines were selected and subsequently progressed within the breeding programme. A subset of lines (C. echinospermum derivatives) exhibiting phytophthora resistance equal or superior to resistant C. arietinum parents, with high yield and acceptable seed size, shape, and colour, were eventually advanced to Stage 3 and Stage 4 trials. These C. echinospermum derivatives are presumed to have 25% of their genome as C. echinospermum. Seed quality was assessed for 31 desi genotypes (21 C. echinospermum derivative lines and 10 C. arietinum released cultivars) tested in some or all of 25 Stage 3 and Stage 4 trials at seven sites in northern New South Wales and six sites in southern Queensland in 1998–2000. The commercial cultivars examined were Amethyst, Barwon, Gully, Heera, Howzat, Jimbour, Norwin, Sona, Tyson, and Lasseter. Depending on seed availability, quality parameters were determined on samples from one to three replicates. Seed was allowed to dry naturally to approximately 10% moisture in a room with humidity and temperature control prior to testing. No significant difference was found between moisture contents as measured by the AACC International Approved Method 44-17.01 (AACC International, 2015). Testing was performed within 6 months of harvest.
2.2 | Visual quality

Whole seed colour was characterised by the parameters CIE L*, a*, and b* using a Minolta chroma metre CR-310 (Minolta Camera Co., Osaka, Japan). L* indicates brightness (0 = black → +100 = white), a* indicates redness (−60 = green → +60 = red), b* indicates yellownessness (−60 = blue → +60 = yellow), and all three axes intersect at their mid-points to form a sphere from which any colour can be plotted.

The weight of 100 unsized seeds was measured and recorded. Seed was then sieved into sizes according to APQ-103 Australian Pulse Quality Laboratory Manual (Burridge, Hensing, & Petterson, 2001). Seed weight is indicative of seed size. The size in majority (either 6 – 7 mm or 7 – 8 mm) was subsequently used for all seed quality analyses.

Seed coat content was determined by peeling the testa off seeds soaked overnight, drying both the testa and cotyledons (50°C oven until no weight change is recorded), and the result expressed as a percentage of the total dry seed weight.

2.3 | Water absorption measurements

Hydration properties were determined according to Wood and Harden (2006). Briefly, the weight and volume of seeds were recorded 0.2, 4.5, 7, and 24 hr after immersion in distilled water. Unhydratable (hard) seeds are those seeds that do not imbibe water and were recorded as a percentage (by weight of the initial dry sample weight).

Plots of seed weight versus soaking time were used with a three-parameter model \( y = I + \alpha (1 - e^{- \beta x}) \), where \( y \) = weight during soaking; \( I = \) initial weight, \( \alpha = \) maximum weight increase; \( 1 - \beta = \) rate of imbibition; and \( x = \) hours soaking. The asymptote of the curve, \( I + \alpha \), is a reliable estimator of maximum hydration, and \( \alpha \) is designated as \( H_{\text{max}} \). The shape of the curve, \( \beta \), determines how quickly the curve nears the asymptote (rate of imbibition), with \( 1 - \beta \) being the proportion of the total weight gain achieved after 1 hr (designated as \( H_{\text{ratio}} \)).

2.4 | Milling quality and dhal colour

Unconditioned seed from the 6 – 7 mm and 7 – 8 mm of size classes was separately milled using the Sheller component of an SK Engineering Mill. The sheller gap was set to the size of seed to ensure optimum splitting results (Wood & Malcolmson, 2011). Dehulling efficiency (DE) and splitting yield (SY) for seed of \( x \)-mm size class were calculated according to Wood, Knights, & Harden (2008), where

\[ \text{DE}_x(\%) = \frac{\text{Dhal} - \text{Dehulled Whole Seed}}{\text{Original Seed Weight}} \times 100 \]

calculates the ease of dehulling, and

\[ \text{SY}_x(\%) = \frac{\text{Dhal}}{\text{Original Seed Weight}} \times 100 \]

calculates the ease of splitting cotyledons.

The resulting dhal colour was measured as described for whole seeds (above).

2.5 | Cooking

Cooking time of whole seeds, soaked overnight in distilled water, was measured by the texture analysis method APQ-102.2 Australian Pulse Quality Laboratory Manual (Burridge, Hensing, & Petterson, 2001), modified slightly by squashing nine desi seeds rather than an approximate 5 g of seed to enhance direct comparisons between samples. Cooking time of dhal was also measured by this method; however, the dhal was not soaked before cooking, and 25 g of cooked dhal was placed in a custom-built Perspex dish of 50 mm inner diameter and 10 mm high side walls for the test. For both methods, the texture analyser (TA-XT², Perten) cross-head was fitted with a 40 mm diameter Perspex disc, descending at a test speed of 2.0 mm s⁻¹ with a load cell of 25 kg. The force (in Newtons) required to squash the seed or dhal sample to 75% deformation was recorded and is an indication of the time the sample would require, with quicker cooking samples having a lower force.

2.6 | Statistical analysis

A linear mixed model was fitted using the ASREML program (Butler, Cullis, Gilmour, Gogel, & Thompson, 2018) to provide predicted values for the quality parameters for each genotype and each group (domesticated C. arietinum cultivars and C. echinospermum derivative lines). The significance of fixed effects (Year, Site, Group, and their interactions) was determined using the Wald statistic (an approximate F-test), whereas the significance of the random term (Genotype) was examined by testing twice the change in the log-likelihood (d) as a \( x_1^2 \) statistic. For the few quality parameters where sample number was limited, as indicated in the tables, several fixed terms were omitted from the model: Year and Site were omitted for testa content, and the Site x Year interaction was omitted for milling quality of 7 – 8 mm sized seeds (DE₇₈ and SY₇₈). Correlations between all quality traits were also investigated.

3 | RESULTS AND DISCUSSION

3.1 | Comparison between the conventional and C. echinospermum derivative groups

The C. echinospermum parent had black seeds with a thick and echinate testa, yet it was possible to recover seed of derivative lines that had testa similar in visual smoothness, colour, and thickness to the C. arietinum cultivars and did not show any of the “primitive” features of their C. echinospermum parent (Figure 1). From a visual perspective, the seeds of these derivative lines would be acceptable for export and human consumption markets.

The testa of the C. echinospermum parent is also known to be resistant to water absorption (i.e., hard seeded; strong seed dormancy). The C. echinospermum derivatives had no hard seeds in most trials and
years; however, three of the 21 derivative lines showed between 2% and 21% unhydratable (hard) seeds at two of the 31 sites in 1998 (data not shown). This shows that the majority of C. echinospermum derivatives have no propensity to develop unhydratable seeds, even under varied environmental conditions. Nevertheless, three of the C. echinospermum derivatives displayed medium levels of unhydratable seeds under several environments, and if observed within a breeding programme, these genotypes should be discarded.

Testing for the significance of the random term genotype showed that genotype was significant for all the quality parameters indicating genotypic variation within the two groups. As a whole, the C. echinospermum derivatives did not differ significantly (5% probability level) from the C. arietinum cultivars for many of the quality parameters (Tables 1 and 2). There was, on average, no significant difference in the groups predicted means for seed size, testa content, or colour, dhal colour, or parameters related to water imbibition ($H_{\text{max}}$ or $H_{\text{rate}}$). However, the C. echinospermum derivative group did produce seed that was, on average, significantly more difficult to dehull (DE) and split (SY) than the C. arietinum cultivars (a disadvantage), and the resulting dhal had a significantly shorter cooking time requirement (an advantage).

Table 3 shows the range in genotype predicted means within the C. echinospermum derivative group and C. arietinum cultivar group for each quality parameter. There was little difference in the ranges between the groups for rate of imbibition ($H_{\text{rate}}$), seed colour (brightness $L^*$ and yellowness $b^*$), dhal colour (brightness $L^*$ and redness $a^*$), and dhal cooking time. The derivative group showed a tightening of the range compared with the cultivar group for 100 seed weight and dehulling efficiency, suggesting that the C. echinospermum parentage is reducing the variability of these traits. In comparison, the derivative group showed an expansion in predicted value ranges for testa content, seed colour (yellowness $b^*$), splitting yield (for the 6-mm size class), maximum hydration ($H_{\text{max}}$), and the cooking time of whole seeds, suggesting that the C. echinospermum parentage is contributing to a broadening of variability in these traits.

Both groups showed a lower splitting yield for the 7–8 mm size class compared with the smaller 6–7 mm size class (Table 1), in agreement with previous reports that, of the two most abundant seed size classes of a genotype, the smaller seeds are generally easier to split (Agrawal & Singh, 2003; Wood, Knights, & Harden, 2008).

### Table 1: Predicted mean values and significance of seed quality parameters for Cicer echinospermum derivative group and Cicer arietinum variety group from trials in northern New South Wales and southern Queensland, 1998–2000

| Variable                  | $P$ value of $F$ statistic | Predicted means |               |               |
|---------------------------|----------------------------|-----------------|---------------|---------------|
|                           | Group | Year × Group | Site × Group   | C. echinospermum derivative group | C. arietinum cultivar group |
| Size index                | .603  | .386        | .983          | 6.28          | 6.28          |
| 100 seed weight (g)       | .178  | .793        | .031*         | 17.17         | 19.37         |
| Testa (%)                 | .978  | NA          | NA            | 15.74         | 15.75         |
| Testa colour, $L^*$       | .732  | .879        | 1.000         | 42.80         | 42.85         |
| Testa colour, $a^*$       | .645  | .995        | .850          | 8.86          | 8.72          |
| Testa colour, $b^*$       | .620  | .234        | .992          | 16.18         | 16.34         |
| Dehulling, DE$_{6.7}$     | .024* | .581        | .000**        | 65.8*         | 68.9*         |
| Dehulling, DE$_{6.7}$     | .008* | .134        | .957          | 67.5*         | 68.3*         |
| Splitting, SY$_{6.7}$     | .013* | .313        | .055          | 43.0*         | 50.8*         |
| Splitting, SY$_{6.7}$     | .014* | .082        | .745          | 58.1*         | 60.2*         |
| Seed cooking (N)          | .941  | .106        | .744          | 210           | 227           |
| $H_{\text{max}}$, $a$     | .341  | .000**      | .189          | 1.02          | 1.03          |
| $H_{\text{rate}}$, $1 - \beta$ | .114  | .011*       | .000**        | 0.63          | 0.62          |

*Significant at $P < .05$.
**Significant at $P < .01$. 

FIGURE 1 Visual differences between the seed of the Cicer echinospermum parent (left) and the seed of two C. echinospermum derivative lines (middle and right) that appear visually similar to cultivated desi chickpea varieties.
Although the derivative group, on average, had poorer milling performance than the *C. arietinum* cultivars, there was a significant amount of variation, and a few of the individual *C. echinospermum* derivatives met or exceeded the average *C. arietinum* cultivar dehulling efficiency and/or splitting yield (Figure 2). An easy-to-mill *C. echinospermum* derivative was identified and studied in relation to this trait (Wood et al., 2008; Wood, Knights, Campbell, & Choct, 2014a, 2014b, 2014c); however, this is the first time a wider group of *C. echinospermum* derivative lines has been examined more broadly over numerous years and sites.

The *C. echinospermum* derivative group predicted means show reduced milling yields (both DE and SY) compared with the desi cultivar group (Figure 2). There was also a large amount of variation in softening of cooked seeds and dhal from individual *C. echinospermum* derivative lines. Dhal from all the individual *C. echinospermum* derivatives, except one, had predicted means that were softer after cooking (i.e., quicker cooking) than the respective *C. arietinum* cultivar mean (Figure 3). Figure 4 shows both the raw data and the predicted means for each genotype within each group for the three quality parameters where the *C. echinospermum* derivative group and the *C. arietinum* cultivar group means were significantly different. Predicted means for each genotype, averaged over environment, clearly reduce the variability compared with the raw data. However, it is apparent that both the actual data and the predicted means of genotypes within each group show significant differences between the groups for milling performance (DE and SY) and dhal cooking.

It is unclear why the derivative group was unable to be dehulled and split as easily as the cultivar group. The lower milling quality of

| Variable | *P* value of F statistic | Predicted means |
|----------|--------------------------|------------------|
|          | Group Year × Group Site × Group | *C. echinospermum* derivative group | *C. arietinum* cultivar group |
| Dhal colour, L* | .459 .351 .170 | 66.55 | 65.97 |
| Dhal colour, a* | .149 .020* .563 | 8.10 | 7.97 |
| Dhal colour, b* | .234 .826 .942 | 37.86 | 37.79 |
| Dhal cooking (N) | .002** .708 .863 | 223** | 257** |

*Significant at *P* < .05.
**Significant at *P* < .01.

| Variable | *C. echinospermum* derivative group | Range | *C. arietinum* cultivar group | Range |
|----------|-------------------------------------|-------|-------------------------------|-------|
| 100 seed weight (g) | 14.32–20.42 | 6.11 | 14.34–23.71 | 9.38 |
| Testa (%) | 14.01–16.83(9)* | 2.82 | 15.01–16.82 (8)* | 1.81 |
| Testa brightness, L* | 39.17–49.07 | 9.91 | 40.74–48.28 | 7.54 |
| Testa redness, a* | 7.76–10.19 | 2.43 | 7.88–9.43 | 1.55 |
| Testa yellowness, b* | 14.73–18.52 | 3.80 | 15.44–18.11 | 2.67 |
| Dehulling, DE7–8 | 64.64–67.00 (12)* | 2.37 | 66.17–70.71 (27)* | 4.54 |
| Dehulling, DE6–7 | 65.89–69.16 | 3.27 | 66.45–70.51 | 4.06 |
| Splitting, SY7–8 | 40.01–46.88 (12)* | 6.86 | 47.49–56.54 (27)* | 9.04 |
| Splitting, SY6–7 | 52.13–62.97 | 10.84 | 56.09–64.11 | 8.02 |
| Seed cooking (N) | 176–249 | 72 | 209–243 | 35 |
| H max, α | 0.95–1.10 | 0.15 | 1.00–1.07 | 0.07 |
| H rate, 1−β | 0.55–0.72 | 0.16 | 0.56–0.69 | 0.13 |

*Indicates where the number of samples tested was limited (n).
the derivatives could not be attributed to a thicker testa, inherited from the *C. echinospermum* parent (Murray, 1984), because there was no significant difference between the average testa content of the *C. echinospermum* derivatives and the *C. arietinum* cultivars (Table 1). It may be due to differences in the testa (composition or morphology) because the testa of the parent *C. echinospermum* is very different, at least in appearance, to cultivated desi chickpeas. Wood et al. (2014a) examined the broad chemical composition of the testa of three desi genotypes, one being a *C. echinospermum* derivative, and although this line had some significant differences in composition, it was often within the values of the other two desi genotypes. However, its testa did contain significantly less insoluble nonstarch polysaccharides (NSPs) and more soluble NSPs (Wood et al., 2014a), as well as some differences in the testa NSP composition (Wood et al., 2014c). Assuming that this derivative line is representative of the *C. echinospermum* derivative group, a reduced level of insoluble NSPs may indicate that the testa is less brittle and more pliable; these characteristics would reduce the cracking and brittleness of the testa and therefore make its removal more difficult. Deeper investigation of the minerals in the testa also revealed a very different composition for the derivative line (Wood et al., 2014b). It was significantly higher in total ash, iron (Fe), potassium (K), and manganese (Mn) and significantly lower in boron (B), calcium (Ca), magnesium (Mg), sodium (Na), and zinc (Zn) than the other two desi genotypes examined. It remains unclear, however,
whether or how these potential differences in testa composition may contribute to a reduced milling performance.

Another possible reason for the differences in milling performance, particularly SY, is tighter adhesion between the two cotyledons in *C. echinospermum* derivatives so that splitting them cleanly becomes more difficult. A *C. echinospermum* derivative line was previously found to contain significantly more soluble arabinose in the intermediate fractions abraded from the surface of dhal that appears to be associated with more difficult-to-mill samples via cell wall adherence and more boron potentially acting as bridging agents for cell wall stabilisation between dhal (Wood et al., 2014b, 2014c). In this case, preconditioning might improve the milling performance of the *C. echinospermum* derivatives in a commercial milling plant and the dhal quality by reducing abrasion. If not, the lower dhal extraction of *C. echinospermum* derivatives would have adverse economic implications for millers, although the observed differences in milling quality were small relative to the genotypic variation existing within the cultigen. Conversely, the anticipated reduction in seed "shatter" (splitting) caused by weather damage and/or low moisture contents would be advantageous to farmers yield and profitability.

Although there was no difference between the groups for whole seed cooking, the shorter cooking times required for *C. echinospermum* derivative dhal would be an advantage over current *C. arietinum* cultivars. Poorer milling performance leads to an increase in abrasion of cotyledons during the milling process, which might facilitate more rapid water absorption and speeding up the cooking process. An alternative explanation for the quicker cooking is potential differences in the cotyledon carbohydrate and/or protein chemistry of the *C. echinospermum* derivatives. Wood et al. (2014a, 2014b, 2014c) examined the composition of one *C. echinospermum* derivative and two desi chickpea genotypes and found that cotyledons of the derivative line contained similar protein content (although higher amounts of several of the most abundant amino acids: glutamic acid, leucine, and lysine), similar starch content, and significantly higher amounts of total NSP content, particularly arabinose (in both soluble and insoluble fractions). Arabinose (2,3,5-Ara residues, likely derived from arabinan) were found to be present in higher levels in dhal of a fast-cooking desi cultivar compared with a slow-cooking cultivar (Wood et al., 2018). More research is required to confirm whether these isolated associations are representative of a wider relationship (i.e., whether *C. echinospermum* derivatives have higher dhal arabinose content leading to quicker cooking).

### 3.2 Quality attribute relationships

Plots of paired data for the quality attributes were also examined and confirmed several expected relationships: (a) Testa content decreased with increasing weight (i.e., seed size), although there was variation of ±2% testa ($R = −.70$); (b) whole seed cooking, as measured by area under the force deformation curve, increased with increasing weight ($R = .74$), suggesting that larger seeds take longer to cook, again with considerable variation; and (c) dehulling efficiency and splitting yields were positively correlated ($R = .68$), again with variation.

There were also some expected correlations that did not eventuate. First, the parameters estimating cooking times for whole seeds and dhal showed little relationship to each other ($R = .12$ to .23; Figure 5a). This could be due to the confounding influence of seed size on the cooking time parameter for whole seeds as well as the involvement of the testa, whereas the dhal cooking time parameter involved only the cotyledon softening and without bias from size. Second, the maximum hydration was not related to the parameters estimating cooking times ($R = −.05$ and $R = .20$ for seeds and dhal, respectively; Figure 5b). Many researchers have previously found an association with hydration capacity ($H_{\text{max}}$) and whole seed cooking time in chickpea (Badshah, Ahmad, Aurangzeb, Mohammad, & Khan, 1987; Kaur, Singh, & Soda, 2005), yellow pea (Wang, Daun, & Malcolmson, 2003), dry bean (Ercan, Atli, Koelsel, & Dag, 1994), and mungbean (Antu, Sudesh, & Yadav, 2006). For this reason, many breeding programmes use hydration capacity as a proxy quick test to screen genotypes for cooking performance. However, our results agree with those of Tripathi et al. (2012) that no such correlation exists and therefore hydration capacity should not be used to estimate cooking times.
Finally, several unexpected relationships were discovered involving colour: (a) $L^*$ and $b^*$ colour parameters for brightness and yellowness, respectively, were positively correlated for seed coat colour ($R = .75$) but with a weak negative association in dhal colour ($R = -.57$). Moreover, $b^*$ of the seed coat and $b^*$ of the dhal were also positively associated ($R = .74$); (b) the rate of hydration was weakly associated with seed coat colour, $L^*$ and $b^*$ ($R = .51$ and $R = -.52$, respectively); and (c) the hardness of cooked seeds was negatively associated with seed coat redness, $a^*$ ($R = -.64$), suggesting that seeds with redder coloured testa would take less time to cook. Colour results from particular chemical compounds or pigments found in seeds; these are primarily carotenoids (yellow-orange hues), anthocyanins (red-purple-blue hues), and proanthocyanidins (brown-black hues) in desi chickpea (Egan & Wood, 2016; Magalhães et al., 2017; Quintero-Soto et al., 2018).

There have been no robust studies into the potential effects of testa and cotyledon colours on other physiochemical quality traits of desi chickpeas as the colour differences are commonly subtle ranging from lighter yellow-browns to darker browns. Hamid, Muzaffar, Wani, Masoodi, & Bhat (2016) found that red cowpeas took longer to cook than black cowpeas, in agreement with our relationship for desi chickpeas; however, their red cowpeas were also significantly larger than the black, which was likely the main contributing factor to their longer cooking times. A more detailed investigation into the potential role of seed pigments in the seed coat with water imbibition and cooking times may be warranted.

### 3.3 Effect of the environment

The environment was also shown to have an impact on the group means for some of the quality attributes (Table 1). The Year × Group interaction was significant for dhal redness ($a^*$), seed density, swelling capacity, and $H_{\text{max}}$ whereas a Site × Group interaction was observed for dehulling efficiency (DE) of the 6 to 7 mm size class and imbibition (both $H_{\text{max}}$ and $H_{\text{imb}}$). This demonstrates that for the dataset tested, environment can affect group performance for dhal colour, imbibition, and dehulling. In contrast, seed weight, testa colour, splitting, and cooking (of both whole seeds and dhal) of groups were largely unaffected by the environment.

### 4 CONCLUSION

Chickpea breeding programmes are increasingly seeking to reintroduce wild genetic diversity into domesticated crops to improve tolerance to pests and diseases. It is important to understand the potential ramifications of this on seed quality traits. It was possible to introgress C. echinospermum genetics into adapted C. arietinum backgrounds and retain the visual characteristics of desi chickpea seeds without unhydratable hard seeds in most trials and years, although three derivatives showed hard seeds at two sites.

In general, the seed quality attributes of the C. echinospermum derivatives investigated were comparable with C. arietinum released cultivars, although considerable variation was evident within both groups across environments for many traits. The C. echinospermum derivative group was, on average, more difficult to mill (dehulling and splitting), whereas the resulting dhal had a significantly shorter cooking time requirement than the commercial cultivar group. Despite poorer milling performance, several of the derivative lines met or exceeded the cultivar average for DE and SY, so it is possible to select better performing derivative lines from the group. Inclusion of C. echinospermum genetics lead to a broadening in variability for testa content and yellowness, SY, $H_{\text{max}}$ and whole seed cooking and a contraction in variability for seed weight and DE. Hydration capacity ($H_{\text{max}}$) was not correlated with whole seed cooking and should not be used as a predictor trait in breeding programmes. The potential of C. echinospermum derivative lines to introduce phytophthora root rot and root-lesion nematode resistance into domesticated chickpea whilst maintaining seed quality has now been confirmed. Individual lines of C. echinospermum possess not only a wide diversity of resistance to pests and diseases but also a wide diversity in seed quality attributes. Continued routine screening for quality traits within the breeding programme will be able to identify those derivates with suitable qualities for export and primary processing for progression to cultivar release.

FIGURE 5 Scatter plots of raw data showing no associations between (a) cooking of whole seeds and dhal, and (b) maximum hydration and whole seed cooking.
Chemical composition and sensory analysis now need to be undertaken on a wider range of C. echinospermum derivative lines to confirm food processing, nutritional, and consumer acceptance.

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DATA AVAILABILITY STATEMENT

Data subject to third party restrictions.

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REFERENCES

AACC International (2015). Approved methods of analysis, 11th Ed. Method 44-17.01 Moisture - Air Oven Method (Pulses), approved October 1, 2003 St Paul, Minnesota, USA: AACC International.

Agbola, F.W., Kelley, T.G., Bent, M.J., & Rao, P.P. (2002). Eliciting and valuing disease resistance in chickpea genotypes. *Euphytica*, 124, 275–280. https://doi.org/10.1023/A:1015931019085

Agrawal, K., & Singh, G. (2003). Physico-chemical and milling quality of some improved varieties of chickpea (*Cicer arietinum*). *Journal of Food Science and Technology*, 40, 439–442.

Amiraj, A., Taylor, J., Bithell, S., Li, Y., Moore, K., Hobson, K., & Sutton, T. (2019). Mapping resistance to Phytophthora root rot identifies independent loci from cultivated (*Cicer arietinum*) and wild (*Cicer echinospermum* P.H.Davis) chickpea. *Theoretical and Applied Genetics*, 132, 1017–1033. https://doi.org/10.1007/s00122-018-3256-6

Antu, G., Sudesh, J., & Yadav, R.K. (2006). Variability in physico-chemical properties and chemical composition of newly released green gram cultivars. *Haryana Agricultural University Journal of Research*, 36, 65–70.

Badshah, A., Ahmad, M., Aurangzeb, B., Mohammad, N., & Khan, I. (1987). Relationship between physicochemical properties and cooking time in chickpeas (*Cicer arietinum*). *Pakistan Journal of Scientific and Industrial Research*, 30, 795–798.

Birnneid, R.B., Retke, M.L., Irwin, J.A.G., Ryley, M.J., & Langdon, P.W. (1985). Resistance in chickpea to *Phytophthora megasperma* f.sp. *Medicaginis*. *Plant Disease*, 69, 504–506. https://doi.org/10.1094/PD-69-504

Birridge, P., Hensing, A., & Petterson, D. (Eds.) (2001). *Australian Pulse Quality Labortory Manual*. Urbran, SA: SARDI Grain Laboratory for GRDC.

Butler, D., Cullis, B. R., Gilmore, A. R., Gogel, B. J., & Thompson, R. (2018). *ASReml-R Reference Manual Version 4*. Hemel Hempstead, UK: VSN International Ltd. https://asreml.kb.vsni.co.uk

Crosier, J., Ahmad, F., Clarke, H.J., & Siddique, K. (2003). Utilisation of wild Cicer in chickpea improvement—Progress, constraints, and prospects. *Australian Journal of Agricultural Research*, 54, 429–444. https://doi.org/10.1071/AR02157

Du, W., Zhao, X., Raju, T., Davies, P., & Trehovan, R. (2012). Identification of *Ascochyta rabiei* disease resistance in chickpea genotypes. *Euphytica*, 186, 697–704. https://doi.org/10.1007/s10681-011-0554-3

Dusunceli, F., Wood, J.A., Gupta, A., Yadav, M., & Yadav, S.S. (2007). International trade. In S.S. Yadav, R. Redden, W. Chen, & B. Sharma (Eds.), *Nutritional value of chickpea* (pp.562–582). Wallingford, UK: CAB International.

Egan, N., & Wood, J.A. (2016). Investigating chickpea seed coat phenolics (p.68). Tamworth, Australia, Sept 14-16. Abstract, p. 66th Australasian Grain Science Conference. https://doi.org/10.13140/RG.2.2.24960.02562

Ercan, R., Atli, A., Koeksel, H., & Dag, A. (1994). Cooking quality and composition of dry beans grown in Turkey. *Gida*, 19, 313–316.

FAO. (2019). *FAOSTAT database*. Retrieved from http://faostat.fao.org/

Hamid, S., Muzaffar, S., Wani, I.A., Masoodi, F.A., & Bhat, M.M. (2016). Physical and chemical characteristics of two cowpea cultivars grown in temperate Indian climate. *Journal of the Saudi Society of Agricultural Sciences*, 15, 127–134. https://doi.org/10.1016/j.jssas.2014.08

Hollaway, G.J., Vanstone, V.A., Nobbs, J., Smith, J.G., & Brown, J.S. (2008). Pathogenic nematodes of cereal crops in south-west Victoria, Australia. *Australasian Plant Pathology*, 37, 505–510. https://doi.org/10.1071/AP08048

Irueña, M., Rubio, J., Cubero, J.J., Gil, J., & Millan, T. (2002). Phylogenetic analysis in the genus Cicer and cultivated chickpea using RAPD and ISSR markers. *Theoretical and Applied Genetics*, 104, 643–651. https://doi.org/10.1007/s001220100751

Kaur, M., Singh, N., & Sodhi, N.S. (2005). Physicochemical, cooking, textural and roasting characteristics of chickpea (*Cicer arietinum L*) cultivars. *Journal of Food Engineering*, 69, 511–517. https://doi.org/10.1016/j.jfoodeng.2004.09.002

Kothe, E.L., Southwell, R.J., Schwingamer, M.W., & Harden, S. (2008). Resistance to *Phytophthora medicaginis* Hansen and Maxwell in wild *Cicer* species and its use in breeding root rot resistant chickpea (*Cicer arietinum L*). *Australian Journal of Agricultural Research*, 59, 383–387. https://doi.org/10.1071/AR07175

Magalhães, S.C.Q., Taveira, M., Cabrita, A.R.J., Fonseca, A.J.M., Valenba, P., & Andrade, P.B. (2017). European marketable grain legume seeds: Further insight into phenolic compounds profiles. *Food Chemistry*, 215, 177–184. https://doi.org/10.1016/j.foodchem.2016.07.152

Mehmood, Y., Sambasivam, P., Kaur, S., Davidson, J., Lee, A.E., Hobson, K.,..., Ford, R. (2017). Evidence and consequence of a highly adapted clonal haplotype within the Australian *Ascochyta rabiei* population. *Frontiers in Plant Science*, 8, 1029–1029. https://doi.org/10.3389/fpls.2017.01029

Muehlbauer, F.J., Kaiser, W.J., & Simon, C.J. (1993). Potential for wild species in cool season food legume breeding. *Euphytica*, 73, 109–114. https://doi.org/10.1007/BF00027187

Muñoz, N., Liu, A., Kan, L., Li, M.-W., & Lam, H.-M. (2017). Potential uses of wild germplasm of grain legumes for crop improvement. *International Journal of Molecular Sciences*, 18, 328–355. https://doi.org/10.3390/ijms18020328

Murray, D.R. (Ed.) (1984). Seed physiology. In *Vol. Volume 1—Development*. North Ryde: Academic press Australia.

Quintero-Soto, M.F., Saracho-Peña, A.G., Chavez-Ontiveros, J., Garzon-Tiznado, J.A., Pineda-Hidalgo, K.V., Delgado-Vargas, F., & Lopez-Valezuela, J.A. (2018). Phenolic profiles and their contribution to the antioxidant activity of selected chickpea genotypes from Mexico and ICRISAT collections. *Plants for Human Nutrition*, 73, 122–129. https://doi.org/10.1071/nn18020328

Ravi, R., & Harte, J.B. (2009). Milling and physicochemical properties of chickpea (*Cicer arietinum L*) varieties. *Journal of the Science of Food and Agriculture*, 89, 258–266. https://doi.org/10.1002/psa.3435

Reen, R.A., Mumford, M.H., & Thompson, J.P. (2019). Novel sources of resistance to root-lesion nematode (*Pratylenchus thornei*) in a new collection of wild *Cicer* species (*C. reticulatum* and *C. echinospermum*) to
improve resistance in cultivated chickpea C. arietinum. Phytopathology, 109, 1270–1279. https://doi.org/10.1094/PHYTO-02-19-0047-R

Reyes-Moreno, C., Okamura-Esparza, J., Armienta-Rodelo, E., Gómez-Garza, R.M., & Milán-Carrillo, J. (2000). Hard-to-cook phenomenon in chickpeas (Cicer arietinum L): Effect of accelerated storage on quality. Plant Foods for Human Nutrition, 55, 229–241. https://doi.org/10.1023/A:1008106229189

Riley, I.T., & Kelly, S.J. (2002). Endoparasitic nematodes in cropping soils of Western Australia. Australian Journal of Experimental Agriculture, 42, 49–56. https://doi.org/10.1071/EA01054

Riley, I.T., & Wouts, W.M. (2001). Pratylenchus and Radopholus species in agricultural soils and native vegetation in Southern Australia. Transactions of the Royal Society of South Australia, 125, 147–153. https://doi.org/10.1071/DS09038

Robertson, L.D., Ocampo, B., & Singh, K.B. (1997). Morphological variationin wild annual Cicer species in comparison to the cultigens. Euphytica, 95, 309–319. https://doi.org/10.1023/A:1003004516921

Schwinghamer, M.W., Moore, K.J., Knights, E.J., Welsby, S., & Murison, R.D. (1993). Enhancing the resistance of chickpea to Phytophthora root rot. Final Report, GRDC Project DAN10C (pp. 1–36).

Singh, M., Kumar, K., Bishi, I.S., Dutta, M., Rana, M.K., Rana, J.C., ..., Sarker, A. (2015). Exploitation of wild annual Cicer species for widening the gene pool of chickpea cultivars. Plant Breeding, 134, 186–192. https://doi.org/10.1111/pbr.12254

Singh, M., Rani, S., Malhotra, N., Katna, G., & Sarker, A. (2018). Transgressive segregations for agronomic improvement using interspecific crosses between C. arietinum L x C. reticulatum Ladiz. and C. arietinum L x C. echinospermum Davis species. PLoS ONE, 13, e0203082. https://doi.org/10.1371/journal.pone.0203082

Thompson, J., Reen, R., Clewett, T., Sheedy, J., Kelly, A., Gogol, B., & Knights, E. (2011). Hybridisation of Australian chickpea cultivars with wild Cicer spp. increases resistance to root-lesion nematodes (Pratylenchus thornei and P. neglectus). Australasian Plant Pathology, 40, 601–611. https://doi.org/10.1007/s13313-011-0089-z

Thompson, J.P., Clewett, T.G., Sheedy, J.G., Reen, R.A., O’Reilly, M.M., & Bell, K.L. (2010). Occurrence of root-lesion nematodes (Pratylenchus thornei and P. neglectus) and stunt nematode (Merlinius brevidens) in the northern grain region of Australia. Australasian Plant Pathology, 39, 254–264. https://doi.org/10.1071/AP09094

Tripathi, S., Sridhar, V., Jukanti, A.K., Suresh, K., Rao, B.V., Gowda, C.L.L., & Gaur, P.M. (2012). Genetic variability and interrelationships of physiological, physicochemical and cooking quality traits in chickpea. Plant Genetic Resources, 10, 194–201. https://doi.org/10.1017/S1479262110000251

von Wetthberg, E.J., Chang, P.L., Bademir, F., Carrasquilla-Garcia, N., Korbu, L.B., Moenga, S.M., ..., Cordeiro, M.A. (2018). Ecology and genomics of an important crop wild relative as a prelude to agricultural innovation. Nature Communications, 9, 649–661. https://doi.org/10.1038/s41467-018-02867-z

Wang, N., Daun, J.K., & Malcolmson, L.J. (2003). Relationship between physicochemical and cooking properties, and effects of cooking on anti-nutrients, of yellow field peas (Pisum sativum). Journal of the Science of Food and Agriculture, 83, 1228–1237.

Wood, J.A., & Harden, S. (2006). A method to estimate the hydration and swelling properties of chickpeas (Cicer arietinum L.). Journal of Food Science, 71, E190–E195. https://doi.org/10.1111/j.1750-3841.2006.00009.x

Wood, J.A., Knights, E.J., Campbell, G.M., & Chotc, M. (2014a). Differences between easy- and difficult-to-mill chickpea (Cicer arietinum L.) genotypes. Part I: Broad chemical composition. Journal of the Science of Food and Agriculture, 94, 1437–1445. https://doi.org/10.1002/jsfa.6437

Wood, J.A., Knights, E.J., Campbell, G.M., & Chotc, M. (2014b). Differences between easy- and difficult-to-mill chickpea (Cicer arietinum L.) genotypes. Part II: Protein, lipid and mineral composition. Journal of the Science of Food and Agriculture, 94, 1446–1453. https://doi.org/10.1002/jsfa.6445

Wood, J.A., Knights, E.J., & Harden, S. (2008). Milling performance in desi-type chickpea (Cicer arietinum L.): Effects of genotype, environment and seed size. Journal of the Science of Food and Agriculture, 88, 108–115. https://doi.org/10.1002/jsfa.3053

Wood, J.A., & Malcolmson, L. (2011). Milling technologies (Chapter 8). In B. Tiwari, A. Gowen, & B. McKenna (Eds.), Pulse foods: Processing, quality and nutraceutical application (pp.193–222). Maryland Heights, MO, USA: Elsevier.

Wood, J.A., Tan, H.T., Collins, H.M., Yap, K., Khor, S.F., Lim, W.L., ..., Tucker, M. R. (2018). Genetic and environmental factors contribute to variation in cell wall composition in mature desi chickpea (Cicer arietinum L.) cotyledons. Plant, Cell & Environment, 1–14. https://doi.org/10.1111/pce.13196

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