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Milling Performance and Other Quality Traits Affected by Seed Shape in Isogenic Lines of Desi Chickpea (*Cicer arietinum* L.)

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Abstract

Milling of pulses generally involves removal of the seed coat and splitting of the cotyledons to produce splits or 'dhal'. The aim of this research was to investigate whether seed shape affected seed quality using two sets of near-isogenic lines differing by a single allele at the seed shape locus (*Rd/rd*). Seed shape had a profound effect on milling quality: rounded seeds produced 7% more dhal than their angular counterparts. There were also significant effects on other quality parameters attributable to seed shape: rounded seeds had less seed coat content because it was thinner, a more intense seed coat colour, faster water absorption but lower hydration capacity and a slightly longer dhal cooking time. This study shows that seed shape is more important than seed coat thickness from a milling perspective and suggests that breeding programs should select for rounder shaped desi chickpeas to maximise dhal yields and profitability for the splitting industry.

Keywords: grain legume, *Cicer arietinum*, gene, seed shape, testa, milling, dehulling, splitting

1. Introduction

Desi chickpeas are commonly milled prior to further processing for human consumption. Milling involves removal of the seed coat and splitting the two cotyledons to produce 'dhal'. Chickpea dhal is a staple food of the Indian sub-continent (ISC) where it is prepared for consumption by various methods including boiling, puffing, frying and grinding to a flour (called 'besan'). It is important that the dhal yield is maximised for profits and to reduce the amount of waste by-products (seed coat, kibble and fines) that are produced by the milling process. The dhal quality is also important: the seed coat must be removed completely and the dhal produced should not be broken, chipped or abraded. Such imperfections in quality lead to price penalties in the marketplace.

Milling performance of pulses can be influenced by a number of factors such as seed quality, storage and handling, processing equipment and the methods used which include seed pre-treatment and dhal-polishing (Kulkarni, 1993; Kurien, 1981; Matanhelia, 1981; Siegel & Fawcett, 1976; Wood & Malcolmson, 2011). Kurien (1984) reported that commercial yields on the ISC range from 73-83 %, depending on equipment and method; the higher figure approaches the theoretical maximum dhal yield of approximately 88%, which is based on seed coat content.

Genotype has a significant effect on dhal recovery. A study of the milling performance of 8 cultivars and breeding lines grown over multiple sites and seasons revealed that dhal yield (SY) varied up to 16.6% between genotypes (Wood, Knights, & Harden, 2008). There is an inverse relationship between seed size and seed coat content; moreover factors independent of seed size could account for differences of 3.9% in seed coat content (Knights, 1989). Seed shape, which ranges from angular (protruding beak, large length/width ratio and prominent ridging and indentation) to a more rounded form (much less pronounced beak, ridging and indentation and smaller length/width ratio) has also been implicated in differences in milling quality. Feedback from Australian millers indicates that rounded seed types are preferred (Wood & Knights, 2003). Dhal with obvious dimpling is believed to be preferred in the ISC (to differentiate them from the cheaper split field peas) and this will more likely result from milling the more angular seed types. These market signals are confusing and, at times, conflicting. Hence, this study aims to clarify the effect of seed shape on seed quality attributes and its marketing implications.

Two categories of seed shape are identifiable within desi chickpeas: rounded and angular. The difference between the two shapes is controlled by a single gene (Knights, Wood, & Harden, 2010). In this study, a set of near isogenic lines was used to study the effect of two contrasting desi seed shapes on milling performance and the important quality attributes of the resulting dhal.

2. Material Studied

The source material was the $F_{5/6}$ breeding line 9113-13N-2, derived from the cross 8507-28H/946-31. During the production of 'Pedigree Seed' from the F_{13} generation it was observed that single plant progeny of this line displayed one of two distinct seed shapes: 'angular' and 'rounded'. Ten angular and ten rounded near-isogenic lines differing by a single allele at the seed shape locus (*Rd/rd*) were randomly selected from the 340 single plant progenies comprising Pedigree Seed of 9113-13N-2.

3. Area Description

They were sown in a randomised trial (2 replications) at Tamworth Agricultural Institute on 17 July, 2003 and harvested with an experimental plot harvester on 11 December. Observations on plant height, time to flower and leaflet number were recorded during the trial.

4. Methods

4.1 Sample Preparation

Harvested seed was stored in an air conditioned room prior to testing to facilitate equilibrium of moisture contents to approximately 10%. No significant difference was found between the moisture contents of angular and rounded lines as measured by the American Association of Cereal Chemists [AACC] International Approved Method 44-17.01 (AACC, 2010a). Seed from every plot was assessed for seed quality.

4.2 Whole Seed Characterisation

The weight of 100 seeds (unsized) was recorded and whole seed colour was characterised by the CIELAB parameters L*, a* and b* using a Minolta chroma meter CR-310 (Minolta Camera Co., Osaka, Japan). L* indicates brightness (0 = black → +100 = white), a* indicates redness (-60 = green → +60 = red), b* indicates yellowness (-60 = blue → +60 = yellow) and all three axis intersect at their mid points to form a sphere from which any colour can be plotted.

The seed was then sieved and size distribution calculated according to method APQ-103: Seed Size Distribution of Pulse Seeds (Burridge, Hensing, & Petterson, 2001), in duplicate. The size in majority (67.3% for angular, 62.3% for rounded) was found to be 7.0 – 8.0 mm and this size was subsequently used for all seed quality analyses. In addition to this size, milling was also conducted on the 6.0 – 7.0 mm fraction which was second in abundance.

The weight and dimensions (length, width and depth) of 10 randomly selected seeds from each plot were measured with vernier callipers.

The sphericity of 10 randomly selected individual seeds was calculated using the method of Wood and Keir (2008) as follows:

$$\phi = \frac{d_i}{d_c} \times 100 \quad (1)$$

where d_i was the diameter of the greatest inscribed circle and d_c was the diameter of the smallest circumscribed circle drawn on a ventral view of the chickpea seed (Figure 1). The higher the sphericity value, the more spherical is the seed, where a sphere has a sphericity value of 100.

Seed coat content was determined by peeling the seed coat off seeds soaked in hot water for 5 minutes and drying (50°C oven for 48 h, cooled to room temperature in a desiccator for 30 min). The result was expressed as a percentage of the dry seed weight.

Seed coat thickness was determined from a randomly selected plot of the rounded type and a randomly selected plot of the angular type. Seed coat pieces remaining after the milling process (refer to section 2.3) were retained and the thickness measured using vernier callipers (mm). Forty measurements were taken for each sample, taking care that the seed coat pieces measured were flat and without ridges or raised veins.

4.3 Milling Performance

Unconditioned seed (50.0 g) was milled using the 'Sheller' component (attrition-style) of an SK Engineering Mill (S.K. Engineering and Allied Works, Bahraich, India), in duplicate. The sheller 'gap' (distance between the

two stones) was standardised to ensure optimum splitting results for each of the 6.0 – 7.0 mm and 7.0 – 8.0 mm seed sizes. Dehulling Efficiency (DE) and Splitting Yield (SY) were calculated according to APQ-104.2: Rapid Dehulling of Field Peas and Desi Chickpeas (Burridge et al., 2001); where DE_7 and SY_7 used seed of 7.0 – 8.0 mm size and DE_6 and SY_6 used seed of 6.0 – 7.0 mm size.

$$DE(\%) = \frac{Dhal + DehulledWholeSeed}{OriginalSeedWeight} \times 100 \quad (2)$$

$$SY(\%) = \frac{Dhal}{OriginalSeedWeight} \times 100 \quad (3)$$

The resulting dhal dimensions and colour were measured as described for whole seeds (in section 2.2).

4.4 Water Absorption Characteristics

Hydration and swelling properties and density were determined by measuring the weight and volume of 100 seeds at 0, 2, 4.5, 7 and 24 hours after immersion in distilled water. The initial density (g/ml) was calculated from the initial weight and volume.

A two parameter model was used to characterise the hydration data (Wood & Harden, 2006):

$$y = \alpha\ (1-\beta^x) \quad (4)$$

where y = weight increase during soaking, α = maximum weight increase, 1-β = rate of imbibition, and x = hours soaking.

The asymptote of the curve, α, is a reliable estimator of maximum hydration (Hydration Capacity) since there is negligible weight increase after 24 hours of soaking (designated H_{max}). The shape parameter, β, determines how quickly the curve nears the asymptote (rate of imbibition), with 1-β being the proportion of the total weight gain achieved after one hour (designated H_{rate}).

4.5 Dhal Cooking

Cooking time is difficult to measure because assessment of the moment when the chickpeas are optimally cooked is highly subjective. The cooking time of dhal was therefore measured indirectly using the Australian Pulse Quality Laboratory texture analysis method APQ-102.2: Cooking Quality of Boiled Pulse Seeds and Dhal (Burridge et al., 2001), in triplicate. Briefly, 35 g dhal is placed in demineralised boiling water and cooked for 25 min. The dhal is drained and immediately placed into a sample cup (5 cm diameter, 1 cm depth), levelled at the top of the cup (excess removed) and squashed to 75% deformation using a TA-XT2 texture analyser (Stable Microsystems Ltd, Godalming, UK) fitted with a 4.5 cm back-extrusion/compression probe travelling at 2.0 mm/s. The maximum force (in Newtons) was recorded and is positively correlated with cooking time.

4.6 Amylose Content

The amylose content was measured on dhal from seeds from a randomly selected plot of the rounded type and a randomly selected plot of the angular type. The dhal samples were ground to flour by passing through a 0.5 mm screen (Cyclotec 1093 Sample Mill, Tecator, Hoganas, Sweden). Amylose was determined by the AACC Method 61-03.01 (AACC, 2010b) for amylose content in rice, based on the method of Juliano (1971). Briefly, flour (100 mg) was wetted with ethanol (95%, 1 ml), followed by the addition of a NaOH solution (1M, 9 ml) and refluxed (180°C, >5 min) to evaporate the ethanol and gelatinise the starch. The volume was increased to 100 ml with distilled water and duplicate aliquots pipetted for each sample. Citric acid (0.1N, 2 ml), iodine solution [1 ml containing I_2 (3.2 mM) and KI (48.2 mM)], and distilled water (16 ml) were added to each. Solutions were vortexed (5 sec), left to stand (20 min) and then vortexed again (5 sec). Absorbance was measured at 620 nm using a UV-visible spectrophotometer. Amylose content was calculated from a standard curve derived from potato amylose.

4.7 Statistical Analysis

Data from the field trial was analysed using ASREML (Gilmour, Cullis, Gogel, Welham, & Thompson, 2002) by fitting a linear mixed model with seed shape as a fixed factor and with replicate and single plant progeny as

random factors. Field trials can often have local trends caused by variations in soil or other factors which cause neighbouring plots to be more alike than ones further away. This local trend was modelled using a first-order separable autoregressive process (AR1) in both the row and column directions as described by Gilmour, Cullis and Verbyla (1997).

Seed of ten single plant progenies from each of the two field replicates was analysed in the laboratory. Three laboratory replicates were tested in a design balanced for field replicates. The same linear mixed model was fitted to the laboratory data with laboratory replicate as an additional random factor. The resulting predicted values of the quality parameters for both rounded and angular seed shape are shown in Tables 1 and 2. The least significant difference (LSD) is shown when seed shape was significant ($P < 0.05$).

The amount of variation attributable to differences amongst single plant progeny within a seed shape group was also examined. The significance of single plant progeny, a random term, was tested by dropping it from the model and comparing twice the change in the log likelihood (d) to a χ_1^2 statistic. A large value ($d > 3.84$) indicates significant variation between progenies within each seed shape group (data not shown but is discussed).

5. Results

Analysis of plant height, time to flower and leaflet number showed no or minor differences between the two sets of lines, consistent with a population that had segregated for a single gene determining seed shape.

Seed shape was associated with significant differences in seed weight, dimensions and sphericity, and seed coat content and colour (Table 1). Rounded seeds were 6.3% heavier than angular seeds but were similar in density. For the 7.0 – 8.0 mm size, rounded seeds were shorter (5.0%), wider (2.8%) and deeper (4.2%); these differences were reflected in a higher sphericity value for the rounded type (Figure 1).

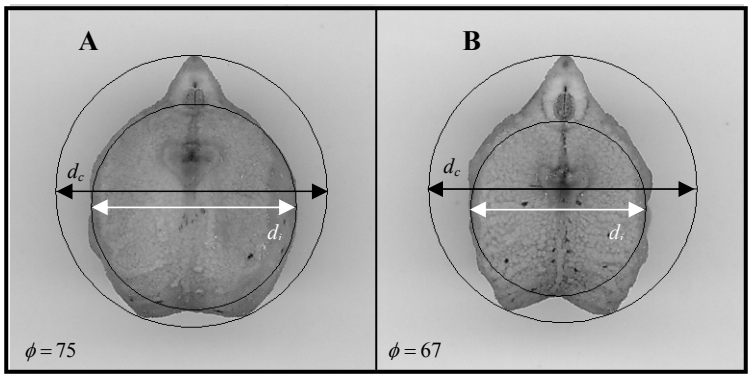

Figure 1. Determination of sphericity values (Φ) for contrasting seed shapes of desi breeding line 9113-13N-2, viewed from the base: (A) 'rounded', (B) 'angular'

The influence of shape was most apparent in seed coat content: the seed coat of angular seeds accounted for 3.3% more of the total seed weight than for rounded seeds. The seed coat was also slightly thicker in the angular seeds. The seed types differed significantly for the colour parameters (a* and b*) but not for brightness (L*). Rounded seeds were redder (a*) and yellower (b*) than their angular counterparts.

Both hydration parameters were affected significantly ($P < 0.05$) by seed shape. The maximum weight increase on soaking (H_{max}) was 3.6% higher for angular types. Conversely, the rate of imbibition (H_{rate}) was higher in rounded seeds.

Milling performance generally exhibited a large seed shape effect (Table 1). DE_6 was slightly but significantly higher for rounded seeds of 6.0 – 7.0 mm size (although DE_7 did not differ significantly between seed shapes for 7.0 – 8.0 mm sized seeds). However, there was a much more pronounced (and significant) effect of seed shape on the recovery of split cotyledons, as reflected in SY values. For the 6.0 – 7.0 mm fraction (SY_6), rounded seeds yielded 6.9% more dhal than angular seeds (54.3% and 47.4% respectively), while for the 7.0 – 8.0 mm fraction (SY_7), rounded seeds yielded 7.4% more dhal than angular seeds (30.4% and 23.0% respectively).

Table 1. Seed quality attributes (7.0 - 8.0 mm) for rounded and angular seeds

Quality Parameter	Angular group	Rounded group	*LSD* [a] (0.05)
Unsized Weight (g/100 seeds)	18.95	20.18	0.51
Weight per seed (g)	0.207	0.220	0.006
Length (mm)	8.6	8.2	0.22
Width (mm)	6.4	6.6	0.05
Depth (mm)	6.2	6.5	0.08
Density (g/ml)	1.27	1.29	NS
Sphericity (%)	67.2	75.4	0.01
Colour - L*	43.21	42.95	NS
Colour - a*	9.50	11.14	0.23
Colour - b*	16.34	16.64	0.24
Seed coat content (% w/w)	16.33	13.63	0.36
Seed coat thickness (mm)[b]	0.18	0.16	0.001
Maximum Hydration, H_{max} (%)	112.3	108.7	1.33
Rate of Hydration, H_{rate}	0.169	0.187	0.015
Milling, DE_6 (%)	64.6	66.5	0.72
Milling, SY_6 (%)	47.4	54.3	1.44
Milling, DE_7 (%)	49.7	49.1	NS
Milling, SY_7 (%)	23.0	30.4	1.38

[a] LSD (Least Significant Difference) is provided where there was a significant difference between Angular and Rounded lines ($P < 0.05$); NS denotes no significant difference

[b] only a single plot of each isogenic line was analysed for seed coat thickness; the LSD denotes a significant difference between single plots of each group ($P < 0.05$)

Seed shape significantly affected dhal weight, shape and cooking quality, but not colour or amylose content (Table 2).

Table 2. Dhal quality attributes (7.0 - 8.0 mm) from rounded and angular seeds

Quality Parameter	Angular group	Rounded group	*LSD* [a] (0.05)
Dhal weight per seed (g)	0.171	0.190	0.005
Length (mm)	6.57	6.60	NS
Width (mm)	5.57	6.02	0.06
Depth (mm)	3.10	3.27	0.03
Colour - L*	67.51	67.47	NS
Colour - a*	6.45	6.49	NS
Colour - b*	33.86	33.77	NS
Dhal Cooking Force (N)	266	276	8.78
Amylose content[b] (%)	19.76	18.73	NS

[a] LSD (Least Significant Difference) is provided where there was a significant difference between Angular and Rounded lines ($P < 0.05$); NS denotes no significant difference

[b] only a single plot of each isogenic line was analysed for amylose content; the LSD denotes a significant difference between single plots of each group ($P < 0.05$)

While the intact seeds from the rounded lines were only 6% heavier than the angular seeds, individual cotyledons milled from rounded seeds were 11% heavier, 8% wider and 5% deeper than those milled from angular seeds. There was no significant difference in dhal length. Cooked dhal from rounded seeds required a 4% greater force to achieve a 75% deformation than those from angular seeds, indicating a slightly longer cooking time. The CIELAB colour scores for dhal milled from rounded seeds did not differ significantly from dhal milled from angular seeds.

Most of the whole seed quality parameters and all of the dhal quality parameters did not show significant variation among single plant progeny within the shape groups (data not shown). Hence, any significant differences for these quality parameters between shape groups are likely attributed to the single gene difference at the *Rd/rd* locus. On the other hand, significant variation among single plant progeny within both shape groups was evident for unsized weight, SY_6 and the seed coat colour parameters. Single plant progenies within a shape group may differ slightly due to heterozygosity at a small percentage of loci (other than the *Rd/rd* locus) in the original plant.

6. Discussion

The visual differences in shape due to the roundness gene were confirmed by dimensional and sphericity measurements. Rounded seeds were less 'pinched', heavier and occupied a greater volume (greater width and depth) than their angular counterparts (Figure 1). However differences conferred by the *Rd/rd* locus extended beyond seed shape: rounded and angular seeds were also differentiated on the basis of seed coat content, thickness and colour; the rounded isolines contained less seed coat which was thinner and coloured more intensely.

Léon-Kloosterziel, Keijzer and Koornnef (1994) identified an *Arabidopsis* mutant exhibiting heart-shaped seeds as opposed to the normal oval shape and named it *aberrant seed shape* (*ats*). The mutant was found to be maternally inherited and microscopy revealed that immature seed coats of the *ats* mutant (3 days after anthesis) consisted of only three cell layers compared to the five layers found normally. They concluded that the shape of *ats* seeds is determined by the seed coat during early seed development. This is less likely to be the case in chickpeas as *Arabidopsis* belongs to a different Family (Brassicaceae, as compared to Fabaceae) which have distinctly different flower and seed characteristics. In particular, the seed coat of *Arabidopsis* conveys dormancy (Debeaujon, Léon-Kloosterziel & Koornneef, 2000) and its pigmentation is located in the innermost seed coat layers (Bouman, 1975) whereas chickpea seeds have no dormancy and the pigmentation is derived from the outermost seed coat layers (Singh & Mathur, 2004). In addition, while both genes confer a more rounded eccentricity, the *ats* mutant of *Arabidopsis* was found to be single recessive (Léon-Kloosterziel et al., 1994) whereas the *Rd* allele of chickpea was single dominant (Knights et al., 2010). Léon-Kloosterziel et al. (1994) also demonstrated, by combining *ats* with other seed coat mutations, that the formation of seed coats is a complex process involving several genes influencing cell division planes and differentiation.

While seed coat content appears to be constant within each group of single plant progenies the basis of this apparent pleiotropic effect of the seed shape gene is unknown. On the other hand, there were significant differences between single plant progeny within both shape groups for seed coat colour, suggesting that colour is likely controlled by a number of genes.

The rounded seed type occupied a greater volume with a similar density, yet contained a smaller percentage of seed coat which was also thinner than the angular seed type. This lower seed coat content effectively increased the maximum theoretical DE and SY achievable compared to the angular seed type. The observed difference in SY (7%) is, however, greater than the 3% difference in seed coat content. Conversely, there was a much smaller (2%) or no significant difference in DE attributable to seed shape, which suggests that factors other than the seed coat contribute significantly to differences in milling quality. In particular, the angular lines had similar dehulling but the roundness gene improved the ease of cleaving cotyledons during the milling process.

A more rounded seed (more spherical) may facilitate milling by having a larger surface area in direct contact with the mill stones, compared to the angular seed type. This would allow greater grip and force to be applied to the rounded seed during dehulling/splitting. The angular type, being less spherical, is more likely to have its more prominent 'beak' broken off from contact with the stones during milling resulting in increased waste product and lower dhal yields.

The single gene difference between the near-isogenic lines significantly improved dhal yield (7%). Any improvement in dhal yield on a commercial scale would enhance profitability through reduced mechanical input and increased yields. According to an Australian chickpea miller (pers. com. Anonymous, 2010) a 10% increase

in chickpea dhal yield is equivalent to an extra AUD$70/tonne in Australian mills and possibly an additional $30/tonne from increased throughput.

For milling performance, the yields were much higher for the 6.0 – 7.0 mm size fraction compared to the 7.0 – 8.0 mm size fraction for both lines, in agreement with the trend demonstrated in other desi chickpea genotypes by Wood et al. (2008).

Differences in hydration parameters observed between the two seed shapes may be explained by differences in the seed coat, its microstructures and cotyledons. Pietrzak, Fregeau-Reid, Chatson, and Blackwell (2002) explained in soybean that water absorption as a 3-step process: (1) penetration through the seed coat via seed coat cracks, microstructures (lens/micropyle/hilum) or seed coat absorption; (2) movement between the cotyledons and underneath the seed coat; and (3) finally, absorption by the cotyledons and seed coat, with dissolution of the middle lamella (Heil, McCarthy, & Ozilgen, 1992). More recently, Kikuchi, Koizumi, Ishida and Kano (2006) showed that in beans, water uptake is controlled by: (1) penetration through the lens; (2) movement through the cotyledon/seed coat junction to the hilum/radicle; (3) movement through the adaxial epidermis of the cotyledons and seed coat absorption; followed by (4) absorption by the cotyledons. Chickpeas have a rougher, less shiny seed coat than beans, probably due to the absence of waxes which reduce the permeability of seed coats to water. Hence, in chickpea the lens may not be the only access point for initial water absorption with some absorption occurring directly through the seedcoat. The thinner seed coat of the rounded line absorbed water more quickly (as evinced by a larger H_{rate}). This may be attributable to either a quicker/shorter path of water through the seed coat, the presence of more numerous minute seed coat cracks as a legacy from harvest or the seed coat microstructures being more reactive. Brosio et al. (1992) found that the water absorption in cowpea was greater through the seed coat than through either the hilum or micropyle. In contrast, Pietrzak et al. (2002) used nuclear magnetic resonance imaging to show that the hilum and micropyle were the first points of water entry into soybean seeds and Kikuchi et al. (2006) found the lens to be the sole water channel in kidney and adzuki seeds.

Maximum hydration (H_{max}) is independent of the rate of water absorption (H_{rate}) (Wood & Harden, 2006). The chickpea seed coat is composed mainly of fibre (Williams & Singh, 1987) which is known to have a high affinity for water. McDonald, Vertucci and Roos (1988) showed that the seed coat initially retards water imbibition in soybean and then acts as a water storage organ for the embryo. A similar retardation and water holding process would occur in chickpea. Hence, the thicker seed coat of the angular seed type would slow the penetration of water (lowering H_{rate}) then the greater seed coat content would increase the final amount of water held by the soaked seed (increasing H_{max}) compared to the rounded line which has less seed coat which is thinner.

In pea (*Pisum sativum*), wrinkled seeds contain higher amylose content and less total starch than rounded (*RR*) seeds (Bhattacharyya, Martin & Smith, 1993). The higher sucrose concentration in the developing pea seeds of wrinkled lines is responsible for the wrinkled appearance of the mature seed due to greater water absorption during development and subsequent loss on maturity. A similar mechanism was posited to explain the differences between rounded and angular chickpea seeds (Knights et al., 2010), however this is not supported by the absence of a significant difference in the amylose contents of rounded and angular seeds.

The dhal produced from the two seed types differed in weight and dimensions, reflecting the differing shapes of the seed from which they were produced; rounded seed had wider/deeper dhal. There was no visible difference in dhal colour and little observable difference in the amount of abrasion due to milling. The cooking test showed that dhal from the rounder seed types required a greater force to compress the partially cooked dhal. This implies the dhal would require a slightly longer cooking time to reach the required softness for consumption. Dhal has no seed coat to impede water absorption, so although the rounder whole seeds had a faster water absorption, this is not necessarily true for the dhal. The longer cooking time of the dhal from rounder lines would reflect the greater distance that water must travel to reach the centre of the dhal in order for starch to gelatinise and the dhal to soften.

7. Conclusion

A single gene modifying seed shape in near-isogenic lines of desi chickpeas was shown to have a profound effect on milling quality and to significantly affect several other important quality parameters of chickpea. Compared to angular seeds, the near-isogenic rounded seeds yielded 7% more dhal, potentially increasing profitability by AUD$70/tonne profitability on a commercial scale. The only significant adverse consequences of rounded seeds were a small increase in dhal cooking time and a decreased maximum hydration. The gene for seed shape also appeared to have significant effects on some other seed quality parameters including seed coat content, thickness and colour, but not enough to affect marketability. This study is the first to show that seed

shape is more important than seed coat thickness from a milling perspective. Hence, breeding programs should aim for rounder shaped desi chickpeas to maximise dhal yields and profitability for the splitting industry, with little effect on other quality attributes.

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