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Inheritance of seed size in chickpea (*Cicer arietinum* L.) and identification of QTL based on 100-seed weight and seed size index

S. Hossain[1*], R. Ford[2], D. McNeil[3], C. Pittock[1], J. F. Panozzo[1]

[1] Department of Primary Industries, Private Bag 260, Horsham, Victoria 3401, Australia
[2]BioMarka, Melbourne School of Land and Environment, The University of Melbourne, Victoria 3010, Australia
[3] University of Tasmania, Private Bag 54, Tasmania, Hobart 7001, Australia

***Corresponding author: Shanoor.hossain@dpi.vic.gov.au**

Abstract

Chickpea is valued for its nutritive seed composition which is high in protein content and used increasingly as a substitute for animal protein. High quality seed has the potential to attract premium prices. Hence, the breeding of desirable quality traits, including seed size for desi and kabuli-types, is of major importance. For this, two RIL populations derived from intraspecific crosses of a kabuli-type (S95362; light cream colour) crossed to two desi-types (Howzat and ICC3996; medium tan and dark tan colour, respectively) were assessed across two environments. Fitting of seed size group ratios to inheritance models indicated that seed size is governed by two major complementary genes, where small size is dominant. The low genotype x environment interaction (<6.0% of the total variation for either population) suggests limited environmental influence on this trait. Subsequently, two major quantitative trait loci (QTL) were identified, one on LG 4 (QTL_1) and one on LG 1 (QTL_2), that together accounted for 20% of the seed size trait and may be targeted for future fine mapping and associated selectable marker development. These same loci also accounted for 37% of the phenotypic variance for 100-seed weight across the two environments, indicating the close genetic relationship between seed size and weight.

Key words: Chickpea, seed size, seed weight, seed size index, heritability, marker, QTL analysis.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the earliest annual pulse crops to be cultivated by man and consumed as a source of vegetable protein. Grown over a vast geographical range including tropical, sub-tropical and temperate regions, in recent years it has become popular in developed countries particularly Australia and Canada (Siddique and Sykes 1997). Australia has been a producer and major exporter in the international marketplace of both desi and kabuli types since the late 1980s and is currently one of the largest exporters (FAOSTAT 2005). Appearance of chickpea seed is a key market factor and acceptability varies with cultural preference. In particular, larger seed size coupled with other desirable traits (eg light colour) commands price premiums in a market-dependant manner (Graham et al. 2001; Anonymous 2005/2006). Export markets require uniform seed size, which may influences a range of processing properties including splitting, hydration and the quality of the final product, as it has for other food legumes (Poysa et al. 2002). In chickpea, seed size is considered as an important factor for subsequent plant growth parameters including germination, seedling vigor and seedling mass (Narayanan et al. 1981; Dahiya et al. 1985). Vadivelu and Ramakrishnan (1983) also proposed seed size to affect seed yield. Machine vision systems have been employed to investigate quantitative measures of seed size on various grains such as wheat (Shouche et al. 2001), oats (Doehlert et al. 2004), rice (Sakai et al. 1996), linseed (Keefe 1999), corn (Steenhoek and Precetti 2000) and lentil Chickpea has also previously been size- separated using image analysis (Shahin and Symons 2005). An alternative method is screen sieving, widely used as a standard method to determine seed size distributions, whereby a set of screens with different sized holes are used to classify seeds into size categories. Importantly, seed weight was also proposed as an accurate measure of chickpea seed size (Upadhyaya et al. 2006). Therefore, to produce seed of a specific size, and to meet a specific market demand through targeted breeding, knowledge of the genetics that determine seed size is required. Indeed, a better understanding of the inheritance pattern and gene interactions that govern seed size is required along with an understanding of potential environmental influence. A large seed size variation exists within and between chickpea types, with some desi types as large as kabuli types and some kabuli types as

Table 1. Population size (n), mean and range for 100-seed weight of two different chickpea crosses

	ICC3996 x S95362 (P445)				Howzat x S95362 (P453)			
Site	Genotype	n	Mean± S.E.	Range (g)	Genotype	n	Mean± S.E.	Range (g)
	P_1	12	14.01± 0.88	12.79-16.12	P_1	26	19.88± 0.89	17.70-22.50
Horsham	P_2	5	28.79± 2.04	26.40-31.14	P_2	5	28.79± 2.05	26.40-31.14
	RILs	113	18.54± 3.58	12.53-26.55	RILs	128	22.96± 4.19	14.25-35.82
Mid-parent value			21.40		Mid-parent value		24.33	
	P_1	12	15.68± 0.80	13.53-16.39	P_1	26	23.58± 0.77	22.03-24.88
Warne	P_2	4	31.88± 1.41	30.19-33.59	P_2	4	31.88± 1.41	30.19-33.59
	RILs	113	21.76± 4.03	13.12-29.97	RILs	128	27.70± 5.01	17.20-38.95
Mid-parent value			23.78		Mid-parent value		27.73	

small as desi types (Kumar and Singh 1995). Although, Argikar (1956) proposed seed size to be controlled by a single recessive gene and Ghatge (1993) proposed control by two genes, it has also been reported to be under oligogenic (Patil and D'Cruz 1964) and polygenic (Kumar and Singh 1995; Malhotra et al. 1997) control. Upadhyaya et al. (2006) determined chickpea seed size to be controlled by two genes with dominant epistasis in F_2 and backcrossed populations. Most of the previous investigations have relied on F_2 and backcross populations for seed size segregation analysis and used 100-seed weight data for QTL analysis in chickpea. Thus, in this present study, recombinant inbred line (RIL) populations were used to determine the Mendelian inheritance mechanism for seed size and two different measures of seed size data were employed to identify QTL for seed size in chickpea. The objectives of this study were to use two RIL populations to: 1) determine the genetic inheritance of seed size measured using seed-weights; 2) determine the chromosomal locations of the major gene effects governing size on a chickpea genome framework map and determine the environmental influence; and 3) identify molecular markers closely associated and/or flanking seed size QTL that may be targeted for future marker-assisted breeding strategies.

Materials and methods

Plant materials and design of field experiments

Two F_5 derived F_7 ($F_{5:7}$) RIL populations were produced at the Victorian Department of Primary Industries, Horsham, Australia from parents with differential seed sizes. These were derived from intraspecific crosses of a kabuli-type (S95362) with two desi-types (*cvs*. Howzat and ICC3996). The populations were labeled P445 (ICC3996 x S95362) and P453 (S95362 x *cv*. Howzat) comprising 113 and 128 lines, respectively. RIL from each population, together with parental genotypes, were grown as duplicated and randomized single line plots (35 plants per plot). The trial was conducted in 2005 and 2006 in two environments with contrasting rainfall, soil type and temperature. Site 1 was located in a dryland paddock at the Plant Breeding Centre, Horsham, Victoria, Australia (Horsham, longitude 142.16º E, latitude 36.71º S, 143 m elevation) and Site 2 was located at Warne, Victoria, Australia (longitude 143.03º E, latitude 35.79º S, 98 m elevation).

Seed size evaluations

After harvest, seed size data were collected in two ways; by 100-seed weight and with nested round-holed sieves.

100 seed weight (g)

Replicated samples of clean seed (broken grain and foreign material removed) were sampled randomly and 100-seed were counted and weighed.

Sieve method to calculate seed size index (SSI)

The standard method for measurement of seed size employs a nested set of seven sieves with decreasing circular hole diameters from 4 mm to 10 mm (method No. APQ-103, Australian Pulse Quality Manual, updated 31.09.2000). Replicated clean seed samples of approximately 250g were sequentially passed through the sieve stack using a flatbed shaker set to 1,425 min^{-1} at 50 Hz. The seed samples retained on each of the sieves were weighed. The seed size index (SSI) for round holed sieves which represents seed size distribution was calculated from the weighed mean of seed retained on each sieve. This method categorizes seed size based solely on the longest dimension of the seed cross section through the axis.
Seed size index (SSI) was calculated as:
% on sieve = wt (g) on the sieve/ wt (g) of sample X 100
SSI = SUM (% of each fraction X sieve hole diameter) / 100

Genetic inheritance model for seed size

To investigate the mode of simple Mendelian inheritance for seed size, the 100-seed weight data was used. Analyses of variance (ANOVA) was employed to analyze phenotypic variables for seed 100-seed weight of both RIL populations using GENSTAT ver. 4.2 (P = 0.05). Least significant difference (LSD) values were calculated to determine statistical difference among the 'size groups'. The ratios of different size groups were used to investigate the Mendelian inheritance mechanism

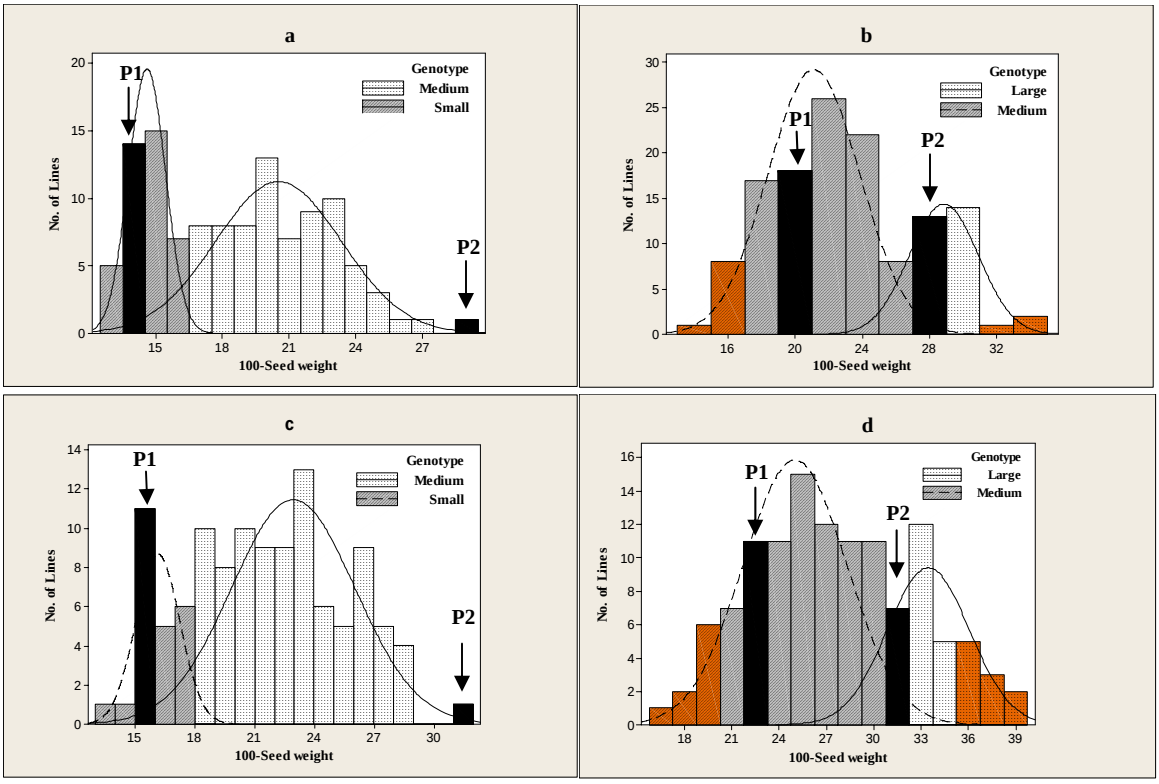

Fig 1. Distribution pattern of 100-seed weight among respective parental and RILs of the P445 and P453 populations at Horsham (a and b, respectively) and Warne (c and d, respectively). Where: ■ bars in both populations represent RILs that had seed size equal to the parental mean P_1 (small, ICC3996 or medium, Howzat) and P_2 (large, S95362), ■ bars represent RILs that had smaller or bigger seed size then the respective parental type in P453 population.

of seed size within the populations and between the two environments. The goodness-of-fit to expected segregation ratios for various inheritance models was determined by Chi-square (X^2) analysis with the threshold X^2 value 0.05.

Heritability of seed size

General linear Model (GLM) analysis was used to estimate variance components for genetic parameters of 100-seed weight, including genetic variance (σ^2_G or genotypic variance caused by genetic attributes), environmental variance (σ^2_E or the variance caused by environmental factors) and genetic *vs* environmental variance (σ^2_{G*E} or the variance caused by genotype and environmental interaction) using MINITAB® (Release 14.13). The heritability (h^2) of 100-seed weight for each population was estimated according to Nyquist (1991) using the standard formula of ratio of genotypic variance (σ^2_G) to the phenotypic variance (σ^2_P). Where, phenotypic variance was partitioned into genetic variance (σ^2_G), environmental variance (σ^2_E) and genetic *vs* environmental variance (σ^2_{G*E}). Thus, heritability was calculated as: $h^2 = \sigma^2_G / \sigma^2_P = \sigma^2_G / \sigma^2_G + \sigma^2_{G*E} + \sigma^2_E$

Table 2. Analysis of variance for 100-seed weight within the P445 and P453 RIL populations harvested from Horsham and Warne locations.

Genotype		df	Mean square	*P* value
P445 (Horsham)	Replicate	1	5.4406	0.011
	RIL	116	25.9556	< 0.001
	Residual	110	0.8114	
P453 (Horsham)	Replicate	1	1.3719	0.232
	RIL	134	33.6446	< 0.001
	Residual	122	0.9500	
P445 (Warne)	Replicate	1	14.7701	< 0.001
	RIL	119	31.1046	< 0.001
	Residual	107	0.6339	
P453 (Warne)	Replicate	1	42.3291	< 0.001
	RIL	140	42.9733	< 0.001
	Residual	116	0.6753	

Quantitative trait loci (QTL) analysis

A framework chickpea linkage map was developed using marker segregation data from the RIL P445 population (desi ICC3996 x kabuli S95362) A total of 80 SSR markers previously characterized by Winter el al. (1999) or Lichtenzveig et al. (2005) were used that were polymorphic between the parental genotypes. PCR reactions of 12.5 µl were performed in a Eppendorf

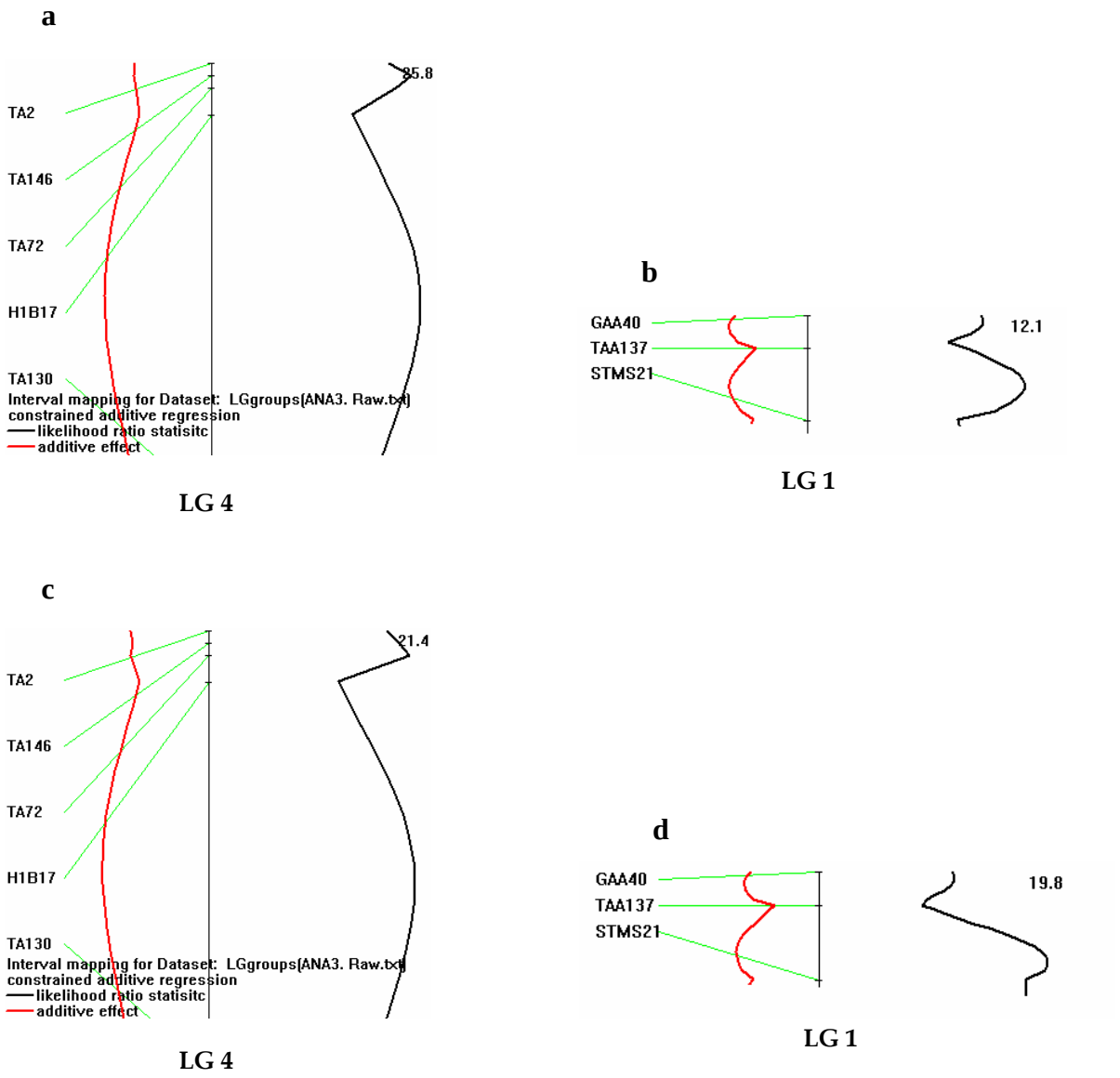

Fig 2. Two putative QTL (QTL_1 and QTL_2) detected for 100 grain weight (seed size) in two different environmental sites; Horsham (a and b) and Warne (c and d).

Mastercycler and comprised PCR buffer (Bioline, Australia), 2 mM of combined dNTPs (Bioline, Australia), 2.5 mM $MgCl_2$ (Bioline, Australia), 10 µM of each primer (Sigma-Genosys, Australia), 1 unit of *Taq* polymerase (Bioline, Australia) and 10 ng of template DNA. The PCR reaction included an initial denaturation step at 94 °C for 3 min followed by 35 cycles of 94 ºC for 30 s, 50 ºC - 60 ºC (as appropriate for the primer pair) for 1 min and 72 ºC for 90 s, with a final elongation step at 72 ºC for 5 min. Amplified SSR markers were separated by electrophoresis in TBE buffer on 1.4% agarose gel, stained and visualized. Clear and single locus-specific markers were then scored as either allele 1 (parent 1) or allele 2 (parent 2). Each segregating marker was tested for goodness of fit to the expected Mendelian segregation ratio of 1:1, representative of a co-dominantly inherited single locus in an RIL $F_{5:7}$ population using Chi-square (χ^2) analysis (P <0.05). Linkage analysis was performed on the non-distorted markers using Map Manager QTX Ver. 0.23 (Manly et al. 2001). Linkage groups (LGs) were established by assigning markers with the "make linkage groups" command at P < 0.0001 (equivalent to a LOD score of 3.0). The marker order of each linkage group was verified using the "ripple" command. Final map distances were calculated by applying the 'Kosambi' function (Kosambi 1944). LG labeling was done according to the anchoring of SSR markers from previously developed maps (Winter et al. 2000).

Both 100-seed weight and seed size index (SSI) RIL and parental data were employed to perform QTL detection for 'seed size'. QTL analysis was carried out

Table 3. $F_{5:7}$ RILs segregation for seed size based on 100-seed weight and Chi-square (χ^2) analyses for best fit to genetic models for seed size.

Site	Genotype	No. of Lines	$F_{5:7}$ generation			df	Tested ratio	χ^2	P value
	RIL P445		Small (P_1)	Medium	Large (P_2)				
Horsham	CQHO05	113	36	77		1	1 : 1	14.876	0.000
						1	1 : 3	2.835	0.092^{ns}
						1	1 : 7	38.717	0.000
Warne	CQMC05	113	29	84		1	1 : 1	26.77	0.000
						1	1 : 3	0.027	0.870^{ns}
						1	1 : 7	17.903	0.000
	RIL P453		Medium (P_1)	Large (P_2)					
Horsham	CQHO05	128	94	34		1	1 : 1	28.125	0.000
						1	3 : 1	0.167	0.683^{ns}
						1	7 : 1	23.143	0.000
Warne	CQMC05	128	88	40		1	1 : 1	18.0	0.000
						1	3 : 1	2.667	0.102^{ns}
						1	7 : 1	41.143	0.000

*ns = non significant

using MapManager QTX ver. 0.23 (Manly et al. 2001). Two approaches were applied to determine chromosomal regions associated with seed size. Firstly, single point analysis was performed on all markers to determine those significantly associated with either 100 seed-weight or SSI using the 'Links Report' command. Regions of high marker association were proposed to contain a QTL effect that was expressed as the percentage of total variance, calculated from the trait variance and residual variance. The significance of each association between marker and QTL was determined by a likelihood ratio statistics (LRS) value. Secondly, simple interval mapping (SIM) at 1 cM intervals was applied to identify putative QTL for the traits assessed among the two environments (Horsham and Warne). Multiple QTL were analyzed further by applying multiple regression to determine the cumulative QTL effect (R^2). The significance (effect) of a QTL was determined empirically using the Churchill and Doerge (1994) permutation test with 1000 replications, which gave a LRS/LOD [logarithm (base 10) of odds] value for the size of the QTL and a ~ 95% confidence indication on the location of the QTL within the marker intervals.

Results and discussion

Due to greatest discrimination between the parental genotypes at both sites, the 100-seed weight was used to investigate the inheritance of seed size within the two RIL populations (Table 1). The common kabuli parent (S95362) seed were 103%-105% and 35%-45% heavier than both of the desi parents (ICC3996; small size and Howzat; medium size) respectively. The average seed weight across all RILs for each population at both sites was within one standard error of the mid-parent value, which suggested an additive effect on seed-weight (size). Also, the mean seed-weights across all RILs at both sites (particularly for P445) were closer to the small maternal (P1) parental type for each cross (ICC3996 or Howzat) than the large paternal (P2) parental type (S95362). This indicated that the genetic effects had complimentary interactions, where the small size introduced by the maternal parent prevented full expression of the large size. This supports the findings of earlier studies by Rastogi (1979), Kumar and Singh (1995) and Malhotra et al. (1997). They reported dominance of small seed size over large seed size with additive effect. However, a contrasting result was found by Niknezad et al. (1971), who reported that large seeds were partially dominant over small seeds. Analysis of variance (ANOVA) indicated that significant differences in seed size existed among RILs within the populations based on 100 seed-weight ($P \leq 0.001$; Table 2). Although distribution of 100 seed- weight for the RIL of both populations in both environments appeared to be somewhat continuous, major weight peaks/groups were observed, which suggested some element of quantitative inheritance for seed size, based on weight, in chickpea (Figure. 1). The quantitatively inherited genes may also be overlaid by several smaller contributing qualitative genes.

Discrimination of seed size into different groups

In order to investigate possible simple Mendelian inheritance mechanisms for seed size, the RILs were classified into size groups by Fisher's least significant difference (LSD) based on the standard error of mean differences of the RILs within each population. For the P445 population, RILs with a 100-seed weight less than the ICC3996 (small) parent mean plus the LSD were considered as small seed size. Whilst RILs with a 100-seed weight more than the ICC3996 mean plus the LSD were considered as medium size. There was no RIL genotype recovered with a 100-seed weight measure as heavy as the S95362 (large) parental type at either environmental site (Figures. 1 a and c). The seed-weight range for small seed size at Horsham and Warne was 12-16 g and 14-18 g, respectively, and the medium size was 17-25 g and 19-29 g, respectively. For the P453 population, RILs with a 100-seed weight higher than the S95362 (large) parent mean minus the LSD were considered as large seed size, whereas RILs with 100-

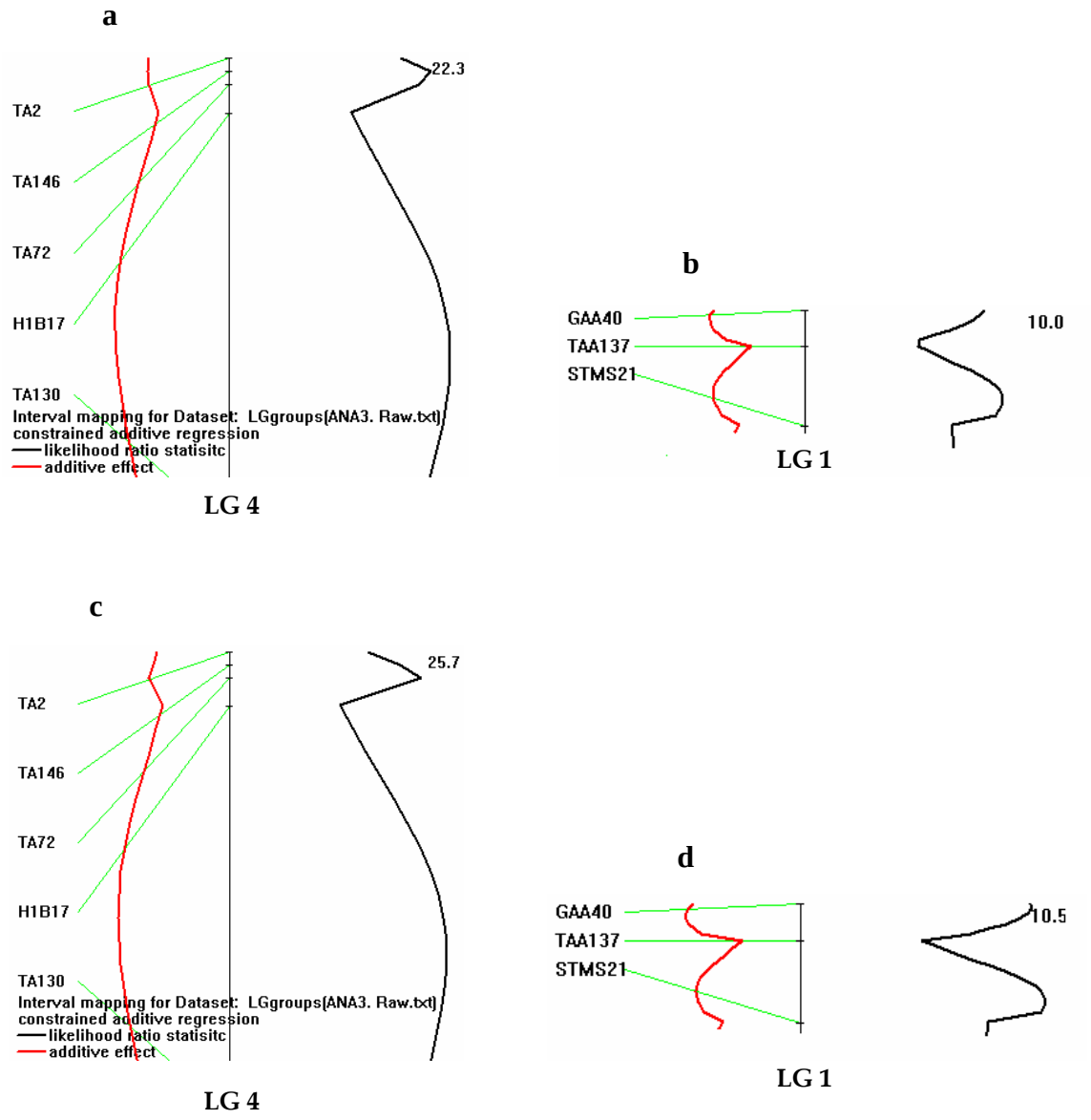

Fig 3. Two putative QTL (QTL_1 and QTL_2) detected for seed size index round (seed size) in two different environmental sites; Horsham (a and b) and Warne (c and d).

seed weight less than S95362 (large) parent mean minus the LSD were considered as medium seed size. The seed weight range for medium seed size at Horsham and Warne was 17-25 g and 21-29 g, respectively, and the large size was 26-31 g and 30-34 g, respectively. Several RILs from the P453 population at both sites had a higher 100-seed weight than their respective large S95362 (mean seed weight for each site ± LSD) parental type or a lower 100-seed weight than their respective medium Howzat (mean seed weight for each site ± LSD) parental type (Figures. 1, b and d). This suggested that the kabuli type S95362 parent may possess an allele which has a negative influence on size (represented by 100-seed weight), whilst the desi type Howzat parent may possess an allele which has a positive effect on this trait.

Mendelian inheritance of seed size in chickpea

The observed segregation ratios from 100-seed weight data for the P445 population among sites were 36 (small): 77 (medium) at Horsham and 29 (small): 84 (medium) at Warne. These both best fit the expected ratio 1 (small): 3 (medium) for a two gene model (Table 3). Since, in this particular cross none of the RILs were as large as the large parental type, a two gene complimentary model with maternal effects was proposed (Table 4). The observed 100-seed weight segregation ratios for

Table 4. Proposed model for a two gene interaction for seed size inheritance within the P445 and P453 RIL populations.

Model	Two gene complementary (eg P453)	Two gene maternal small effect with P_1 maternal and complementary dominance (eg P445)	Two gene complementary	Two gene no interaction equal effect	Two gene no interaction unequal effect
aabb	Medium	Small	Small	Small	Small
aaBB	Medium	Medium	Small	Medium	Medium low
AAbb	Medium	Medium	Small	Medium	Medium high
AABB	Large	Medium	Large	Large	Large
Ratio	3:1	1:3	3:1	1:2:1	1:1:1:1

Where: P_1 (small/ medium) = aabb and P_2 (large) = AABB in both crosses

Table 5. Estimate of genotypic and environmental variance components and heritability in the broad sense for seed size within the P445 and P453 RIL populations.

Genotype	Source of variance	Estimated value	%
RIL P445	Site	5.3477	25.88
	Replicate(Site)	0.0806	0.39
	Genotype	13.2976	64.37
	Site* Genotype	1.1988	5.80
	Error	0.7331	3.54
	Total	20.6578	100
	Heritability	0.873	87.3
RIL P453	Site	10.9818	35.10
	Replicate(Site)	0.0476	0.15
	Genotype	17.9405	57.34
	Site* Genotype	1.6217	5.18
	Error	0.6947	2.22
	Total	31.2863	100
	Heritability	0.885	88.5

the P453 population among sites were 94 (medium): 34 (large) at Horsham and 88 (medium): 40 (large) at Warne. These also best fit the expected ratio 3 (medium): 1 (large) for a two complementary gene model but with no detected maternal effects (Tables 3 and 4). Other tested expected ratios (Table 3) did not significantly fit with the observed ratio classes for either population at either site. This is in agreement with the earlier findings of Ghatge (1993) and Upadhyaya et al. (2006). Cobos et al. (2007) also investigated genetic analysis of chickpea seed size using an intraspecific RIL population derived from a desi x kabuli cross and found two QTL for seed size. In an earlier investigation Niknezad et al. (1971) reported that, one or two genes could explain the substantial genetic variation in chickpea seed size. Studies that revealed contrasting results (Patil and D'Cruz, 1964; Malhotra et al. 1997) may be due to the use of different genotypes and different approaches taken for seed size measurements.

Heritability and QTL analysis

The genetic components variance for 100-seed weight was estimated as 64.3% of the total variation in the P445 and as 57.3% of the total variation in the P453 population (Table 5). Analysis of genotype by site interaction explained 5.8% and 5.1% of total variation for P445 and P453, respectively (Table 5). This indicated that the environment had a relatively low influence on 100-seed weight within the trials of these populations. Heritability estimates for 100-seed weight were determined using combined data from both locations, and were calculated as 0.87 (87% of total variation) for P445 and 0.88 (88% of total variation) for P453 (Table 5). The high magnitude of genetic heritability also supports the earlier findings by Niknezad et al. (1971), Kumar and Singh (1995) and Cobos et al. (2007). The high heritability of the trait increased confidence in obtaining accurate data from the field trials for identifying the chromosomal location of the major genetic components. Using single point analysis, six markers (STMS21, TA2, TA146, TA72, TA130 and H1B17) were significantly associated with 100-seed weight (100GW H and 100GW W) and five (TA2, TA146, TA72, H1B17 and TA130) were associated with seed size index (SSI H and SSI W), the alternate size measurement. These associations were determined at a LRS threshold of 13.8 (equivalent to LOD score 3) and explained over 80% of total trait variation across the environments and were located on LG 1 and LG 4 (Table 6). The negative additive regression coefficient for the marker associated to the QTL suggested that the marker may be associated with a smaller seed size. Simple interval mapping identified one major QTL (QTL_1) that was significantly associated with seed size (100-seed weight and SSI) on LG 4, at the marker interval TA146 and TA72 (Figures 1 and 2), with

Table 6. Significant associations between molecular markers and putative QTL for seed size (100-seed weight and SSI) within the P445 population at two environments.

Site	QTL name	Linkage group	Marker	[1]LRS	[2]Variance(σ) %	P	[3]Additive effect
Horsham	100GW H	LG 4	TA2	20.3	18	0.000	-1.67
		LG 4	TA146	24.1	20	0.000	-1.71
		LG 4	TA72	22.7	19	0.000	-1.66
		LG 4	H1B17	19.1	16	0.000	-1.53
	SSI H	LG 4	TA2	16.6	15	0.000	-0.20
		LG 4	TA146	20.0	17	0.000	-0.21
		LG 4	TA72	19.5	17	0.000	-0.20
		LG 4	H1B17	15.4	13	0.000	-0.18
		LG 4	TA130	19.5	18	0.000	-0.21
Warne	100GW W	LG 1	STMS21	17.6	14	0.000	-1.80
		LG 4	TA2	17.0	16	0.000	-1.75
		LG 4	TA146	19.4	16	0.000	-1.75
		LG 4	TA72	20.0	17	0.000	-1.77
		LG 4	TA130	19.1	18	0.000	-1.79
	SSI W	LG 4	TA2	15.4	14	0.000	-0.20
		LG 4	TA146	19.9	17	0.000	-0.21
		LG 4	TA72	22.0	19	0.000	-0.22
		LG 4	H1B17	15.2	13	0.000	-0.18
		LG 4	TA130	23.2	21	0.000	-0.22

[1]LRS = likelihood ratio statistic for association of the trait with this locus.
[2]Variance (σ) % = the amount of total variance, which would be explained by a QTL at this locus.
Where: 100GW = 100 grain weight, SSI = seed size index and H = Horsham (an environmental location) and W = Warne (an environmental location).

Table 7. Putative QTL for seed size traits identified using simple interval mapping in the P445 population at two environments.

Trait [1]	Marker interval	QTL name	LG	LRS/LOD	Variance(σ) %	R^2%
Seed size						
100GW H	TA146 – TA72	QTL_1 100GW H	4	24.7/5.3	21	34.1
	TAA137 - STMS21	QTL_2 100GW H	1	12.1/2.6	10	
100GW W	TA146 – TA72	QTL_1 100GW W	4	20.8/4.5	18	39.0
	TAA137 - STMS21	QTL_2 100GW W	1	19.8/4.2	16	
SSI H	TA146 – TA72	QTL_1 SSI R H	4	20.4/4.4	17	9.2
	TAA137 - STMS21	QTL_2 SSI R H	1	8.0/1.7	6	
SSI W	TA146 – TA72	QTL_1 SSI R W	4	22.7/4.9	19	29.8
	TAA137 - STMS21	QTL_2 SSI R W	1	10.5/2.2	9	

[1]key for this table as per previous table

an average LRS value of 22.75 (equivalent to and LOD score of 4.9) for 100-seed weight and 21.55 (equivalent to a LOD score of 4.6) for SSI, respectively (Table 7). The QTL (QTL_1) for seed size individually explained between 17% and 21% of the total phenotypic variance in the two environments. An additional but weaker QTL (QTL_2) for seed size was also found on LG 1 between markers TAA137 and STMS21 (Figures 1 and 2) with an average LRS value of 15.95 (equivalent to a LOD score of 3.4) for 100 seed weight and 9.25 (equivalent to a LOD score of 2.0) for SSI, respectively (Table 7) and explained up to 16% of the total phenotypic variance in the two environments. Therefore, the two major QTL for seed size, detected on LG 1 and on LG 4, together explained up to 39% (100-seed weight) and 29% (seed size index) of the phenotypic variation for seed size (Table 7). The consistency of these QTL for seed size and the stability across environments, suggested that they may correspond with the two major genes found in Mendelian inheritance model. This is also consistent with the findings of Upadhyaya et al. (2006) who reported that chickpea seed weight was controlled by at least two major genes. Radhika et al. (2007) previously identified two major QTL for chickpea seed weight at a LOD > 3.0 on LG 1 and LG 4 in a different genetic background. Alternatively, Cobos et al. (2007) reported, two QTL for

chickpea seed size located in LG 4 and LG 8 that explained up to 30.4% of the phenotypic variation and Cho et al. (2002) found two putative QTL for 100-seed weight located on LG 4 and LG 9. Most recently, Cobos et al. (2009) reported two QTL for chickpea seed size located in LG 4 and LG 2 that explained up to 32% of the phenotypic variation. Again, these discrepancies may be due to different genetic backgrounds and/or different ways in which seed size or weight has been measured and highlights the need to adopt a universally agreed method to measure these traits if studies of underlying genetic components are to be compared. Further it should also be validated that the same LG nomenclature has been used for direct QTL comparison among maps. Nevertheless, it is possible that QTL_1 for seed size (100 seed-weight and SSI) identified in the present study be at the same location as the QTL for seed-weight reported by Cho et al. (2002), Cobos et al. (2007) Radhika et al. (2007) and Cobos et al. (2009) on LG 4. Also, the QTL_2 in the present study may be equivalent to that reported by Radhika et al. (2007) on LG 1. The inability to align LGs among maps due to there being no shared mapped markers in these regions makes further comparison of QTL impossible. Further map saturation, particularly in the regions where the QTL overlap with common markers will be required to better compare among present and previously mapped seed size trait loci.

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