

Inheritance and Response to Selection for Seed Weight Using the Large Seeded Landrace Oman 2 of Lucerne

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Abstract: Seed weight in lucerne (*Medicago sativa*) may affect subsequent seedling vigour and stand establishment. A landrace of lucerne (Oman 2) from Oman has a 100-seed weight over 60% larger than the largest seeded parent used in previous studies. Crosses were made between Oman 2 and the smaller-seeded cultivar Titan 9, and segregating families were produced for genetic analysis and measurement of response to selection for seed size. There were significant differences in 100-seed weights between the parents (Oman 2 and Titan 9) and subsequent families. Regression of 100-seed weights of F2 families versus F1 parents was highly significant ($p < 0.001$), as well as 100-seed weights of the F3 families versus F1 parents. Analysis of diallel crossing among large and small-seeded F1 plants revealed highly significant general (GCAs) and specific (SCAs) combining abilities, as well as highly significant reciprocals. The GCA effect was much greater than the SCA effect with a GCA/SCA ratio of 15.9. This large ratio agrees with the significant regression coefficients and indicates that 100-seed weight in lucerne has high heritability. The significance of reciprocals was due to a large maternal effect in which large-seeded maternal parents produced progenies with significantly larger seeds relative to small-seeded parents. These results indicate that large-seeded plants should be used as the maternal parents in crosses and for recurrent selection to increase the seed size of progenies.

Keywords: alfalfa; diallel; parent-offspring regression; seed size



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1. Introduction

Agronomists have long been interested in the relationship between seed weight and seedling vigour in plants, and the impact of seed weight on establishment and subsequent performance. In lucerne (*Medicago sativa* L. (Fabaceae) and its sub-species), researchers have demonstrated a positive correlation between seed weight and seedling vigour [1] and seed weight and forage yield [2] whereas others reported only a weak relationship [3], which diminished with plant age [4]. Because lucerne is often grown on heavy clay soils and its seed size is relatively small, it may be that heavier seeds will produce more vigorous seedlings and better stand establishment. Carnahan [5] reported positive correlations in lucerne between seed weight, seedling height at 4 and 8 weeks, and forage yield at 9 weeks. These correlations were attributed to heavier seeds producing seedlings with greater unifoliate leaf areas.

Pedersen and Barnes [6] hypothesised that since seed size in lucerne is determined by embryo size rather than embryo plus endosperm as in the grasses, it is a good system for research on the relationship between heterosis and seed size. Hereafter, in this paper, the terms seed size and seed weight will be used interchangeably. Pedersen and Barnes [6] demonstrated a close relationship between seed weight and seed size as measured by sieves ($r = 0.992$, $p < 0.01$), a finding substantiated by Gjuric et al. [7] who used digital imaging to measure seed size. Pedersen and Barnes [6] measured F1 seed size in lucerne and observed 100-seed weights from 0.198 to 0.260 g. They concluded that both the pollen and seed

parents had a measurable effect on seed size, and that interaction between the seed and pollen parents also influenced seed size. In contrast, Gjuric and Smith [8] used a North Carolina Design II mating scheme and demonstrated that the genotype of the seed parent had a major role in determining seed size in lucerne, and that the genotype of the seed had no influence. They concluded that for genetic studies, the effects of the pollen and seed parents on seed size should be measured on seed harvested from progeny (F1) plants. In these studies, 100-seed weights of parental populations ranged from 0.237 to 0.291 g for small and large seeded lines, respectively. In contrast to the studies described above in which large-seeded parents had a 100-seed weight of <0.3 g, this paper describes research conducted on the inheritance of 100-seed weight and response to selection in an Omani landrace (Oman 2) [9] with a 100-seed weight of up to 0.5 g, over 60% larger than the largest seeded parent used in the previously mentioned works. F1 plants were generated between Oman 2 and Titan 9 (an Australian cultivar with a 100-seed weight of 0.25 g), and F1 plants used for subsequent genetic analysis and measurement of response to selection were chosen based on half-sib family performance after polycrossing. Because of the extreme size of the Oman 2 seed relative to other lucernes, studies on its heritability and response to selection will assist in the transfer of the trait in lucerne breeding. Large and small-seeded families generated will also be useful tools in agronomic research designed to test the influence of seed size on seedling establishment.

2. Materials and Methods

The crossing scheme used to study the inheritance of seed weight and response to selection is presented in Figure 1. The large-seeded parent, Oman 2, is a landrace from Salalah, southern coastal Oman, sourced by John Irwin from Dr. Abdullah Al-Sadi, Sultan Quaboos University, Oman [9]. A total of 12 Oman 2 plants were intercrossed within the group, as were 22 plants of Titan 9 (an Australian commercial cultivar), the latter being resistant to each of Races 1, 2, and 4 of the fungal pathogen *Colletotrichum trifolii* Bain (Ascomycota, Glomerellaceae) and the former being susceptible (Figure 2). The 12 Oman 2 plants were also used as females and were pollinated with bulk pollen collected from all of the Titan 9 plants. F1 seed was bulked and seedlings grown from it were inoculated with mixed inocula of *C. trifolii* Races 1, 2 and 4. This procedure ensured that by only selecting resistant F1 seedlings, plants from self-pollination were excluded from the study.

Generation of half-sib (HS) F2 families was achieved by polycrossing among 49 F1 plants (Figure 1). On the basis of F2 HS family seed weights, 5 and 3 F1 plants which produced the highest and the lowest 100-seed weights, respectively, were selected. These F1 plants were polycrossed within their respective groups (large and small-seeded), and 100-seed weights were determined for each HS family. A total of 7 to 10 F2 plants were grown from each HS family and they were polycrossed within the large and small-seeded groups, resulting in 41 and 29 F3 HS families from the large and small-seeded groups, respectively (Figure 1). One hundred seed weights were determined for each HS F3 family. A complete diallel without self-pollinations was performed between the two F1 plants (WA3312 and WA3327) with the largest seeds and the two F1 plants (WA3210 and WA3340) with the smallest seeds, based on HS family performance.

All crosses were made in an evaporatively cooled glasshouse at St. Lucia, Brisbane, Australia, over the periods of September–October and March–April from 2009 to 2011. A measure of environmental influences on 100-seed weight was obtained by performing the polycrosses within the Oman 2 and Titan 9 populations at these 2 time periods. Gjuric et al. [7] indicated that the numbers of seeds in a pod, pods on a raceme, and racemes on a plant all contributed to seed size variability in lucerne. To reduce these effects, 2 samples each of 100 seeds were weighed for each cross made. Replication in the diallel was provided by making all crosses at two time periods (September–October and March–April). All plants were grown in 25 cm diameter pots in a potting mix fertilised with the necessary nutrients. Diseases and pests were managed by regular spraying with fungicides and insecticides, respectively.

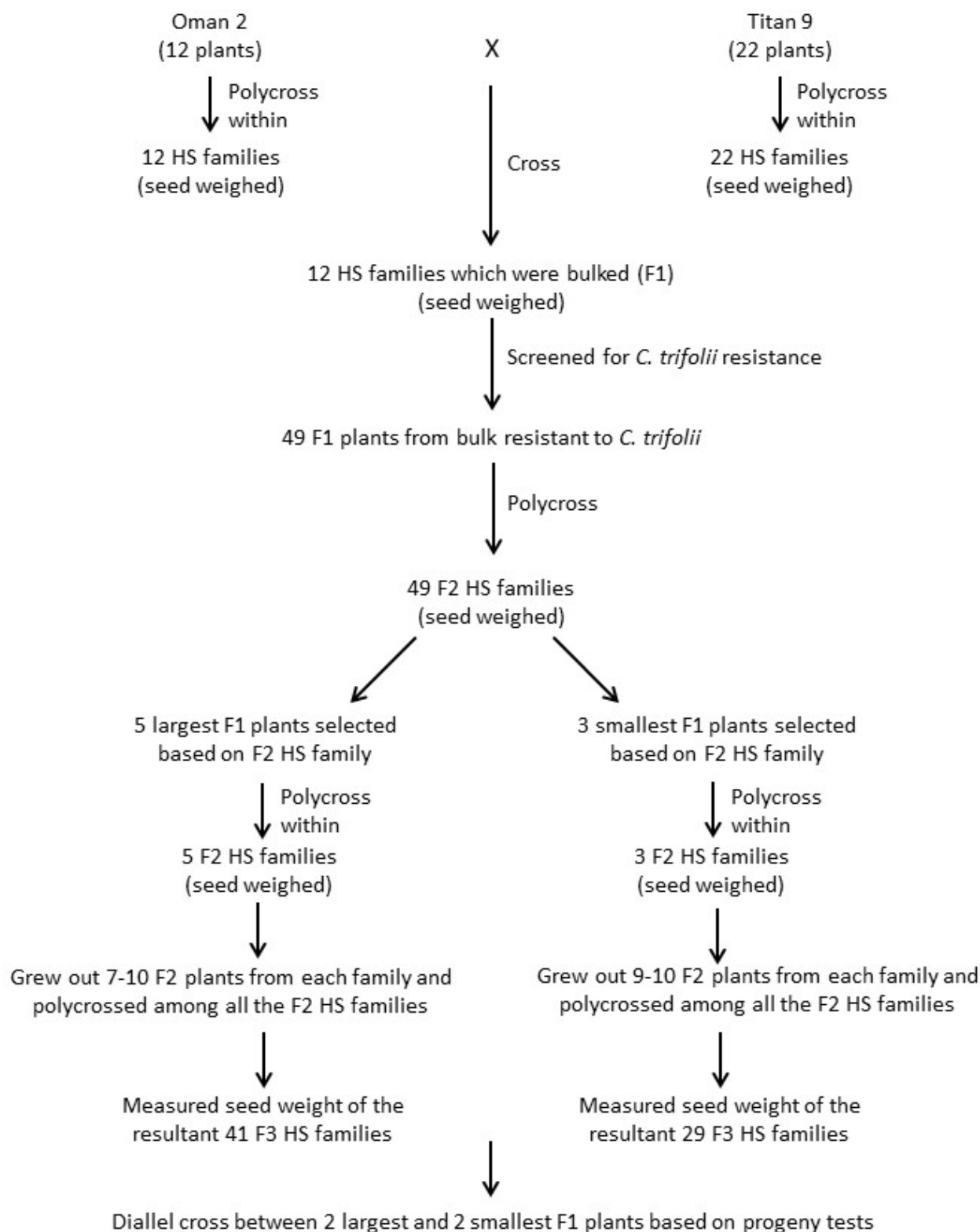


Figure 1. Mating scheme for genetic analyses of seed weights in lucerne.

Statistical analyses were completed using R studio 1 December 2023 Build 402 “Ocean Storm” Release (4da58325, 28 January 2024) for Windows (R foundation for Statistical Computing, Vienna, Austria). Analysis of variance (ANOVA), regressions, and diallel (using Griffing [10] model 3 (complete diallel with reciprocal crosses and no self-pollinations) with fixed effects) analysis were completed using 100-seed weights from parents and families.

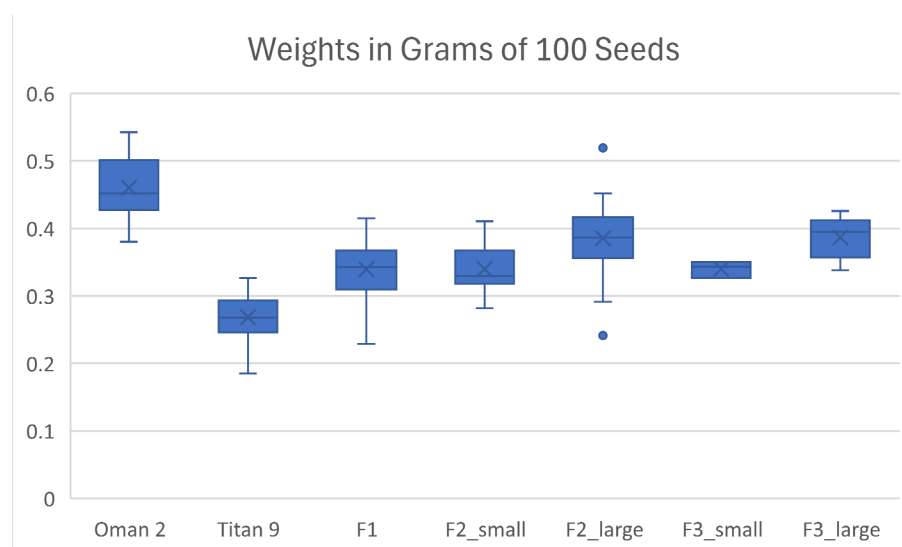


Figure 2. Boxplots of 100-seed weights of parents (Oman 2 and Titan 9) and families generated after crossing Oman 2 with Titan 9.

3. Results

There were significant ($p < 0.001$) differences between the 100-seed weights of the parents (Oman 2 and Titan 9) and subsequent families (F1, F2-Small, F2-Large, F3-Small, and F3-Large) (Table 1). As expected, Oman 2 had a significantly greater 100-seed weight compared to Titan 9, which had the smallest weight. All of the families had 100-seed weights intermediate to the parents and were not significantly different from each other (Figure 2, Table 1). The F2-Large and F3-Large families had significantly greater 100-seed weights compared to the small-seeded Titan 9; however, the F1, F2-Small, and F3-Small families were not significantly different from the Titan 9 parent (Table 1).

Table 1. Mean 100-seed weights in grams and standard deviations (sd) of parents (Oman 2 and Titan 9) and subsequent generations with least significant differences (LSD).

Parent or Generation	Mean	sd	LSD ¹
Oman 2	0.4603	0.0445	a
Titan 9	0.2683	0.0318	c
F1	0.3392	0.0417	bc
F2-Small seeds	0.3398	0.0326	bc
F2-Large seeds	0.3852	0.0504	b
F3-Small seeds	0.3401	0.0122	bc
F3-Large seeds	0.3866	0.0325	b

¹ Means followed by the same letter are not significantly different at Prob = 0.05.

Regression of 100-seed weights of the F2 families versus F1 parents was highly significant ($p < 0.001$) with a regression coefficient of 1.0058 and coefficient of determination (R^2) of 0.9763. Regression of 100-seed weights of the F3 families versus F1 parents was also highly significant ($p < 0.001$) with a regression coefficient of 1.0308 and coefficient of determination (R^2) of 0.9786. Although these significant regression coefficients are inflated due to the selection of large and small-seeded F1 plants to produce the F2 and F3 families, their significance and the large amounts of variation explained by the regressions indicate that 100-seed weight may be highly heritable.

Diallel crossing among large-seeded F1 plants produce progenies with larger seed, and smaller-seeded parents tended to produce progenies with smaller seeds (Table 2). Analysis

of the diallel cross revealed highly ($p < 0.001$) significant general (GCA) and specific (SCA) combining abilities, as well as highly significant ($p < 0.001$) reciprocals (Table 3). The GCA effect (0.00186) was much greater than the SCA effect (0.00012) yielding a GCA/SCA ratio of 15.9. This large ratio is in agreement with the significant regression coefficients and indicates that 100-seed weight in lucerne has high heritability. The GCA effects were -0.04889 and -0.02299 for the small-seeded parents (WA3210 and WA3340, respectively), indicating that small-seed parents decreased the seed weights of the progenies. The large seed parents (WA3312 and WA3327) produced progenies with larger seeds with positive GCA effects (0.04274 and 0.02914, respectively). These GCA effects are consistent with the significance of reciprocals (0.00180) in the diallel due to a large maternal effect in which large-seeded maternal parents produced progenies with significantly larger seeds relative to small-seeded parents (Table 2), which is in agreement with Pedersen and Barnes [6]. The SCA effects were predominately negative when the small-seeded parents were used as the seed parent, and positive when the large-seeded parents were used as the female in crosses (Table 4).

Table 2. Mean 100-seed weights of progenies from diallel crossing among small (WA3210 S and WA3340 S) and large (WA3312 L and WA3327 L)-seeded F1 plants of lucerne.

	WA3210 S	WA3340 S	WA3312 L	WA3327 L
WA3210 S	-	0.21335	0.2218	0.22935
WA3340 S	0.25060	-	0.2514	0.29420
WA3312 L	0.34260	0.37870	-	0.40120
WA3327 L	0.32175	0.29480	0.3503	-

Table 3. Sources of variance (SV), degrees of freedom (DF), mean squares (MS), F-Values (F), and significance (Pr) for 100-seed weights of progenies from diallel crossing among large and small-seeded F1 plants of lucerne.

SV ¹	DF	MS	F	Pr (>F) ²
GCA	3	0.0074609	1071.451	***
SCA	2	0.0002415	34.682	***
Reciprocals	6	0.0036095	518.359	***
Error	11	0.0000070		

¹ GCA = General Combining Ability and SCA = Specific Combining Ability. ² *** indicates probability < 0.001 .

Table 4. Specific combining ability effects¹ for diallel crossing among small-seeded (WA3210 and WA3340) and large-seeded (WA3312 and WA3327) F1 plants of lucerne.

		Female			
		WA3210 S	WA3340 S	WA3312 L	WA3327 L
Male	WA3210 S		0.00803	-0.00749	-0.00054
	WA3340 S	-0.01863		0.06365	0.00030
	WA3312 L	-0.06040	-0.00054		0.00803
	WA3327 L	-0.04620	-0.00749	0.02545	

¹ Negative and positive numbers indicate a reduction and increase in seed weights, respectively.

4. Discussion

There have been few previous studies on seed size in lucerne [8]. This is unexpected since larger seeds would potentially facilitate establishment, particularly on heavy soil types where lucerne is commonly grown in Australia [11]. The Oman 2 landrace has seeds approximately twice the size of most commercial cultivars of lucerne (220 versus 400 to 500 seeds per gram, respectively). For example, Gunn [12] reported an average seed count

of 464 seeds per gram for 418 seed lots from 29 cultivars of lucerne. To assist in the breeding of larger seeded lucerne, inheritance studies were conducted using Oman 2. Overall, large-seeded plants produced significantly larger seeded progenies relative to small-seeded plants. The highly significant parent–offspring regressions and GCA (Table 3) indicate that seed size is primarily controlled by additive gene action, which is in agreement with Petersen and Barnes [13] who completed crosses among nine germplasm sources. There was a significant maternal effect on seed weights (Table 3), which is in agreement with Pedersen and Barnes [6] and Katepa-Mupondwa et al. [14]. Therefore, large-seeded plants should be used as the maternal parents in crosses and for recurrent selection towards larger seed progenies. In our study, SCA for seed weight was also highly significant (Table 3), but much smaller than GCA ($GCA/SCA = 15.9$). The significant SCA indicates that there are important nuclear effects and some F1 progenies generated using Oman 2 plants as the maternal parent had smaller seed weights. Although this study and others [13] have shown significant additive gene action for seed size, Petersen and Barnes [13] reported that seedling vigour was controlled largely by non-additive gene action. These results would suggest that selecting for seed size alone may not lead to increased seedling vigour. Nevertheless, studies across different genetic backgrounds to those used in this study and with smaller seeded parents are in agreement with our findings for Oman 2. Although this study was completed in the greenhouse, the large additive effects indicate that selection under field conditions may successfully increase seed size in lucerne. The segregating families would also be useful for molecular mapping and identification of quantitative trait loci affecting seed size in lucerne.

Medicago arborea L. (Fabaceae) has larger seeds with a 100-seed weight of approximately 1 g. Crosses have been made between *M. arborea* and lucerne, resulting in partial hybrids called Alborea [11,15]. It has been found that some Alborea plants exhibit seed sizes approaching that of *M. arborea*. It remains to be determined whether the inheritance of seed size in Alborea is similar to that of Oman 2. The work reported in this paper, along with the large-seeded Alborea, provides an opportunity to select for increased seed size in lucerne, which may enhance the establishment of the crop over a wide range of soil types.

5. Conclusions

The 100-seed weight of the lucerne landrace Oman 2, which has seeds twice the average size of current cultivars, was found to be highly heritable and to show significant maternal effects. This indicates that in breeding for increased seed size, large-seeded plants such as Oman 2 should be used as maternal parents in crosses, and recurrent selection should be effective in increasing seed weights in lucerne.

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Conflicts of Interest: The authors declare no conflict of interest.

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