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Inheritance of Agronomic Traits and Their Interrelationship in Mungbean (*Vigna radiata* (L.) Wilczek)

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Abstract

A study was conducted to observe the variation and inheritance of agronomic traits and their interrelationship in mungbean. The objective of the study was to compare agronomic traits and hardseed percentage of 268 recombinant inbred lines (RILs) developed from the cross between wild *Vigna* subspecies *sublobata* "ACC 41" with the mungbean cultivar "Berken". The RIL population and their parents were evaluated under controlled conditions in a glass house at the University of Queensland, Brisbane, Australia. The results showed significant differences among the RILs and among the parents in all traits under study. Berken had a longer flowering date and a higher seed weight per plant, but less total leaf number and pod number per plant than ACC 41. A germination test between papers revealed that ACC 41 was 100% hard-seeded and did not germinate at all, while Berken germinated up to 100%. Their RILs distributed well between 0 to 100% hardseed. Upon scarification, all hardseed germinated within seven days. Narrow-sense heritability estimates of total leave number, hardseedness, pod length, and pod width were highly heritable at 89.9, 98.9, 93.7, and 93.2%, respectively. The heritability of seed weight per plant and number of seeds per plant were lower at 63.1 and 58.4%, respectively. Seed weight per plant showed positive transgressive segregation when compared with ACC 41 and a positive correlation with 100 seed weight. While the number of seeds per pod showed a negative transgressive segregation when compared with Berken and a negative correlation with pod length and pod width. The RILs gave a 1:1 segregation ratio in leaflet shape, growth habit, and growth pattern, indicating that these traits were controlled by a single dominant gene.

Key words: mungbean, *Vigna radiata*, agronomic traits, hard seed, heritability

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) is an important pulse crop in Asia and widely cultivated in Thailand. Mungbean can be grown up to three crops per year and fit well into cropping systems with an ability to restore soil fertility through symbiotic nitrogen fixation (Malik 1994). Both domestic and international demands for mungbean are still high. Mungbean seed has a high nutritive value. Seed that does not meet human food standards can be used as livestock feed. The average yield of mungbean is low due to its indeterminate growth habit, photoperiod sensitivity, late and non-synchronous maturity, susceptibility to lodging, pod shattering, and losses due to pests and diseases (Fernandez and Shanmugasundaram 1988). In order to raise yield per unit area, new cultivars must be developed along with the improvement of cultural practices. The knowledge of genetic variation in agronomic traits and their interrelationship should lead to more understanding of yield components and yield potential in mungbean.

V. radiata is sub-divided into two subspecies. The subspecies *sublobata* is a wild mungbean potentially useful for mungbean improvement, by extending the range of variation available to breeders for traits of interest, such as bruchids resistance (Tomooka et al. 1992), disease resistance (Lambrides and Imrie 2000), heat and cold tolerance (Bates 1985), and adaptations to environment (Lambrides et al. 2000). The subspecies radiata is an indigenous species scattered throughout the moderate to higher

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rainfall zone of tropical and sub-tropical areas.

In a genetic study of agronomic traits, Rohman et al. (2003) reported that 100 seed weight, seed yield per plant, plant height, and days to flowering were governed to a greater extent by additive gene effect. Similarly, Mak and Yap (1980) reported that additive genetic variation was higher than dominant variation in governing seed weight and pod length. However, for the number of pods per plant and the number of seeds per pod, the genetic effect of dominance variation was more pronounced. The additive gene action was also found to be important in conditioning seed yield and yield components (Khattak et al. 2002). When the relationships between agronomic characters were studied, pod length showed a positive correlation with plant height (Miah and Bhadra 1989). Upadhaya et al. (1980) found a positive correlation between seed yield with number of pods per plant and plant height. Khan et al. (2001) observed that grain yield production was due to many yield-contributing traits that were positively correlated with yield. For example, grain yield was positively correlated with number of branches and thus Reddy et al. (1991) stated that the increase in number of branches enhanced the grain yield. Malik (1994) reported that seed yield was positively correlated with number of pods and number of branches per plant. As the branches bear pods, the number of pods per plant was positively correlated with the branches per plant. Khan et al. (2001) recorded that the cultivar that produced the highest number of branches per plant also produced the highest number of pods per plant. Rubio et al. (2004) reported that, due to a positive correlation between flowering date and seed yield, the latest flowering genotype would develop more branches, leading to higher seed yield. Yield production is positively correlated with the other yield-contributing traits such as days to flowering, plant height, number of branches per plant, number of pods per plant, and pod length.

Another important character in mungbean is hardseedness. The subspecies sublobata possesses several adaptive traits of importance, particulary hardseedness which is a form of seed dormancy (Verdcourt 1970). Transient hardseedness is common in mungbean cultivars, with levels usually in the range of 0-70% (Lawn and Rebetzke 2006). Williams (1989) found more than 90% hardseeds in some mungbean lines, while Lawn et al. (1988) identified 100% hardseeds in related wild species. Moreover, many small-seeded mungbean genotypes are hard-seeded but no large seed varieties have persistent hardseedness (Imrie et al. 1991). The extent of hardseedness in mungbean depends on the species itself as well as on climatic and other management factors. Temperature and relative humidity are common phenomena relating to the development of hardseed (Hazarika and Barua 2002). Hardseedness was probably under monogenic control (Williams 1989). Singh et al. (1983) reported that the F_2 generation of a cross between ssp. *radiata* with ssp. *sublobata* segregated at the ratio of 3 hard to 1 normal seed, indicating that this trait is conditioned by a single dominant gene. Likewise, Plhak et al. (1989) identified one major QTL associating with hardseedness in mungbean. Hamphry et al. (2005) analyzed an RIL population derived from a cross between a completely soft seed and a hard seed and found four

Table 1. Range and Mean ± SD of germination and some agronomic traits of ACC 41, Berken, and their RILs. The narrow-sense heritabilities are presented in the right-hand column.

Traits[1]	RILs		Parents				h^2 (%)
			Berken		ACC 41		
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	
Agronomic traits							
TL	5.55-47.00	19.18±8.68	3.00-9.00	5.92±2.06	31.00-85.00	62.25±16.41	89.9
DTF	34.00-62.00	47.00±7.20	60.00-69.00	61.40±2.55	34.00-40.00	37.08±1.88	88.0
PL	4.00-8.50	6.44±0.93	6.67-9.60	8.80±0.84	4.50-5.40	4.83±0.33	93.7
PW	0.28-0.51	0.40±0.03	0.49-0.56	0.52±0.02	0.29-0.36	0.33±0.03	93.2
PP	12.00-56.00	24.06+9.80	3.00-14.00	11.09±3.11	18.00-69.00	40.90±16.70	58.4
SP	7.50*-13.67	10.86±1.15	6.70-12.70	11.15±1.63	11.00-12.30	11.70±0.42	80.8
SW	0.75-5.00	2.30±0.57	4.81-6.09	5.44±0.42	0.61-4.69	1.12±1.26	87.0
Y	1.55-11.60*	6.13±2.03	1.14-9.17	6.89±2.22	1.77-12.59	4.48±3.11	63.1
Germination (%)							
G3D	0-100	12.1±1.84	96-100	99.2±0.96	0	0	98.9
G7D	0-100	13.2±1.02	98-100	99.6±0.63	0	0	94.8

* Transgressive segregation

[1] TL = Total number of leaves; DTF = Days to Flowering; PL = Pod length (cm); PG = Pod girth (cm); PP = Number of pods per plant; SP = Number of seeds per pod; SW = 100 seed weight (g); Y = Seed yield per plant (g); G3D = Germination at 3 days (%); G7D = Germination at 7 days (%)

QTLs associating with the genes controlling hardseedness.

The objectives of this study are to observe the variation and heritability in some agronomic traits and their interrelationship in a recombinant inbred line (RIL) population of mungbean.

Materials and Methods

Plant materials

A population of 268 recombinant inbred lines (RILs) were derived from the inter-subspecific cross between "Berken" and "Accession 41" (ACC 41), and randomly harvested in the successive generations. The parental materials are quite different in agronomic traits. Berken (*V. radiata ssp. radiata*) is an Australian commercial mungbean cultivar originally developed and released in 1962 by James Kirby, Oklahoma State University, USA. It was later released by New South Well Department of Agriculture and the Queensland Department Primary Industries in 1975 as a large-seeded dryland cultivar (Kingston 1975). Accession 41 (*Vigna radiata* ssp. *sublobata*) is a wild subspecies collected by Lawn and Cottrell (1988) near the township of Somerset Dam (lat. 27°7², long. 152°33²), Queensland, in 1984. It was described as having extremely prostrate and perennial habit. Its seed must be scarified before planting to break the dormancy. ACC 41 seed is much smaller than Berken (Rebetzke 1994).

Traits recorded

The plant materials were sown in 15 Cm pots under controlled conditions in a glass house at the University of Queensland, St Lucia Campus, Brisbane. The experimental design was a randomized complete block design with two replications. The agronomic traits were observed weekly on plant height, number of leaves on main stem, number of leaves per plant, and branches per plant. After harvest, yield and yield components were recorded on pod length, pod width, number of seeds per pod, number of pods per plant, 100 seed weight, and seed yield per plant. Morphological traits were observed on days to flowering, growth habit and pattern, and terminal leaflet shape.

Hardseedness test

Hard seed percentage was determined by germinating 50 seeds between two pieces of Whatman filter paper soaked in deionized water in a petri dish and kept at 23-25 ^{O}C in a temperature-controlled room. Seed that either germinated or imbibed water within seven days were considered as soft seeds. A hardseedness score was calculated as percentage of those seeds that did not germinate or imbibe water after three and seven days. The viability of hard seed was checked by scarifying and germinating the seed.

Data analysis

The statistical analysis was focused on genotypic variation in the RILs for each trait of interest. Comparisons were made using analysis of variance between and within three groups viz. RIL, ACC 41, and Berken. Correlations between important traits were determined only within the RIL population using Statistical Analysis System Version 6.12 (SAS Institute, 1990). A narrow-sense heritability was calculated from the variance component estimates based on plot basis according to Fehr (1987). Since the RILs are genetically homozygous, the dominant genetic variation (σ^2_d) can be disregarded and the total genetic variance would comprise only additive genetic variation (σ^2_a). Thus the heritability estimated from the RIL population is narrow-sense as shown in the formula:

$$h^2 = \sigma^2_d / [\sigma^2_a + (\sigma^2_e/r)].$$

Where h^2 is heritability in narrow-sense, σ^2_a is the additive genetic variation, σ^2_e is the experimental error variance, r is the number of replications.

Results and Discussion

Variation in agronomic traits

All traits in this study showed significant difference among the RILs and among the parents, revealing that they are polymorphic among the parents and heritable. The average plant height of the RIL at the vegetative stage of development was different from that of the parents until four weeks after planting (Fig. 1). Berken was significantly taller than ACC 41 in vegetative stage. RILs and Berken increased their height continuously until six weeks after planting, whereas ACC 41 increased its height until harvesting. The difference in number of leaves on the main stem among the treat-

Table 2. Chi-square test for goodness-of-fit against a 1:1 ratio of 268 RIL mungbean

Trait	Observed frequency		χ^2	Prob.
	"Berken"	"ACC 41"	(1:1)	
LS[1]	137	131	0.134	0.50-0.75
GH[2]	142	126	0.238	0.25-0.50
GP[3]	150	118	3.820	0.05-0.10

[1] LS = Leaflet shape; deltoid from Berken and lobed from ACC 41
[2] GB = Growth habit; erect from Berken and spreading from ACC 41
[3] GP = Growth pattern; indeterminate from Berken and determinate from ACC 41

ments was not significant at the early vegetative stage, but became significant at the flowering stage. ACC 41 produced more leaves than Berken. RILs and Berken reached the maximum leaf number and started to decrease after the flowering stage at six weeks after planting, while the number in ACC 41 still increased until nine weeks after planting (Fig. 2). The number of leaves per plant was correlated with the number of branches per plant. A similar response was observed in the number of branches per plant. ACC 41 showed indeterminate growth habit and continued increasing in branch number until ten weeks, while Berken is determinate in growth habit. ACC 41 produced more branches per plant than Berken. At six weeks after planting, Berken almost stopped increasing branch number while the average number in the RILs is halfway between both parents until harvesting (Fig. 3). The total leaves of the RILs ranged from 5.55-47.00, with the average of 19.18 falling between the parents (Table 1). ACC 41 had an earlier flowering date but a longer growing period than Berken (data not shown).

The RILs were classified into different ranges according to days to flowering, number of seeds per pod, number of pods per plant, seed weight per plant and hard seed percentage (Fig. 4 to 8). The variation within each range in very small as can be seen from their standard errors, revealing reasonable classification in each traits. The flowering date of the RILs ranged from 34-62 days with a mean of 47 days, skewing towards ACC 41. Some RILs were even earlier in flowering than ACC 41 (Fig. 4). Lawn and Rebetzke (2006) reported that the growth period of wild species is consistently longer than that of the commercial mungbean cultivars. Moreover, time to flowering is based on available nutrition, temperature, and genetic make up of the cultivars (Naidu et al. 1991).

Pod and seed characters such as pod length, pod width, and 100 seed weight were quite different among the parents and among the RILs (Table 1). ACC 41 was smaller in seed size than Berken, while both accessions showed a similar number of seeds per pod. The RIL population produced pods per plant, 100 seed weight and seed yield per plant, pod width and pod length falling between the parents as expected. They showed a negative transgressive segregation in the number of seeds per pod when compared with ACC 41 (Fig. 5), but a positive transgressive in seed yield per plant when compared with Berken. The distribution of the genes controlling this trait many not be so even among the RILs. Thus the RILs contained new genes combinations with either a positive or negative effect on the traits, allowing the RILs to fall outside the range of their parents.

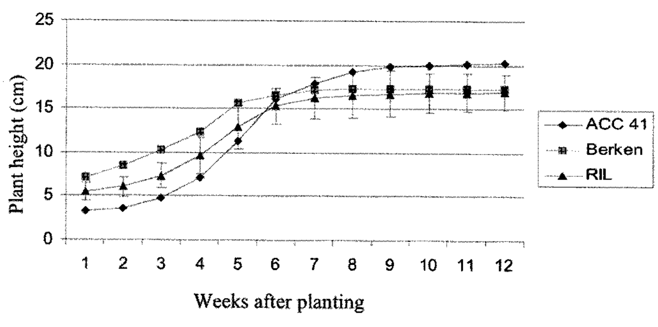

Fig. 1. Plant height of ACC 41, Berken, and RILs at different growth stages.

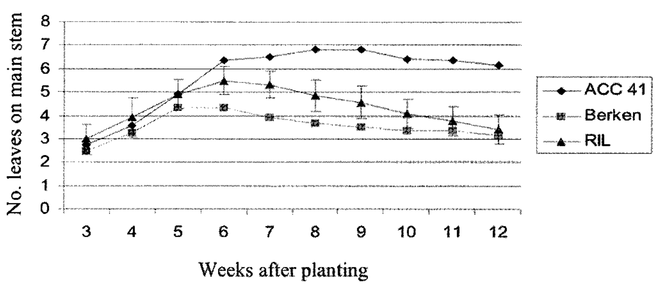

Fig. 2. Number of leaves on main stem of ACC 41, Berken, and RILs at different growth stages.

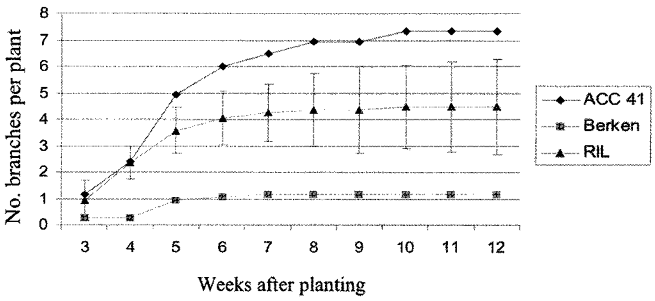

Fig. 3. Number of branches per plant of ACC 41, Berken, and RILs at different growth stages

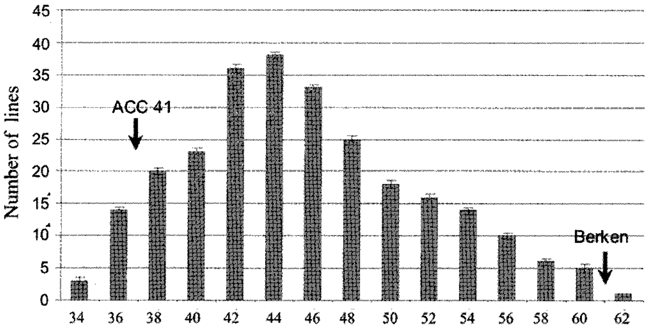

Fig. 4. Days to flowering of ACC 41, Berken and 268 RILs

The number of pod per plant distributed normally among the RILs (Fig. 6). Late flowering genotypes tended to develop more branches, and possibly give a higher seed yield. Similarly, Rubio et al. (2004) and Reddy et al. (1991) observed that various genotypes of mungbean produced different numbers of branches and pods per plant. Pod number is an important yield component and directly proportional to crop yield. Miah and Bhadra (1989) reported a difference in pod production among mungbean cultivars. An increase

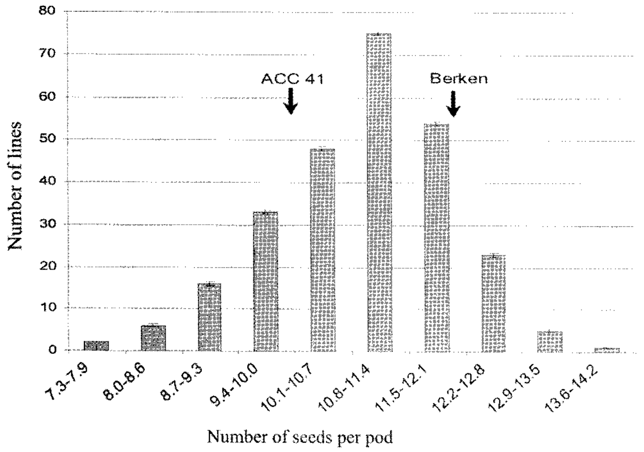

Fig. 5. Number of seeds per pod of ACC 41, Berken, and 268 RILs.

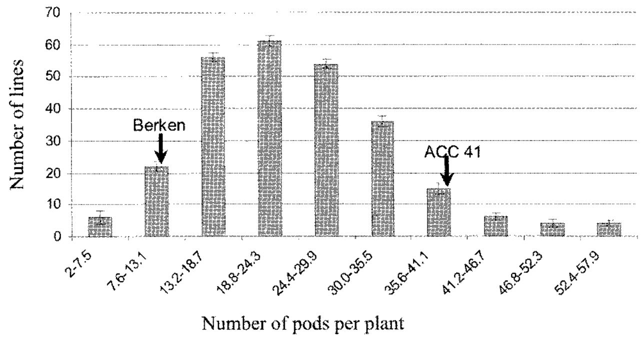

Fig. 6. Number of pods per plant of ACC 41, Berken, and 268 RILs.

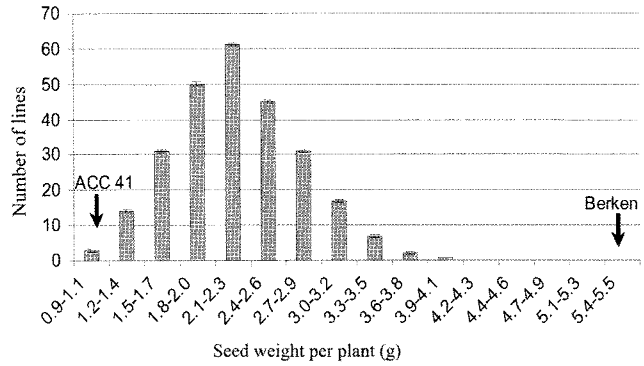

Fig. 7. Seed weight per plant (g) of ACC 41, Berken, and 268 RILs.

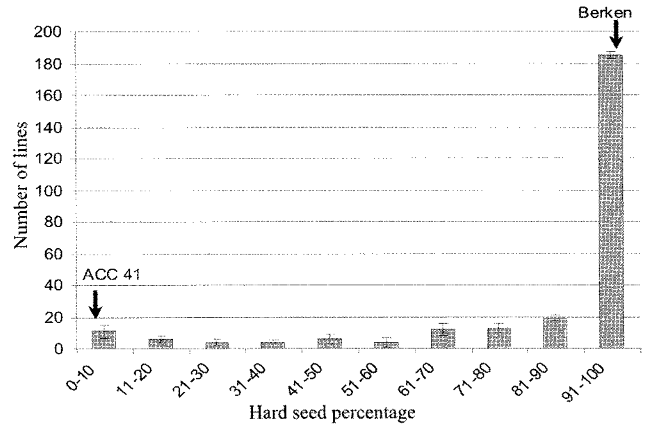

Fig. 8. Hardseed percentage of ACC 41, Berken, and 268 RILs

in number of pods per plant will also increase grain yield. In this study, the number of seeds per pod was not different among the parents and RILs. Finally, Berken produced a higher yield per plant than ACC 41, owing mainly to its larger seed size (Fig. 7). The RIL population showed a large transgressive segregation in seed yield per plant.

Variation in hard seed percentage

The germination of the RIL seed at three days after testing ranged from 0-100%, with the average of 12.1% falling between the two parents. The seed of the wild mungbean ACC 41 did not germinate due to hard seed found in it, while the commercial mungbean cultivar, Berken germinated up to 99.2%. ACC 41 eventually did not germinate at seven days after testing, during which time Berken reached 99.6% germination. The average germination of the RILs was low (13.2%), ranging from 0-100% (Table 1). All the remainder seeds germinated upon being scarified and kept in room temperature at 25 ^{o}C for seven days. This suggested that seed samples of ACC 41 were 100% hard-seeded while those of Berken were 100% soft-seeded (all germinated without the need of scarification).

Hardseedness among the RILs was not normally distributed and

Table 3. Correlation between agronomic traits of 268 RIL mungbean.

Traits	LM	TLB	LB	PL	PG	SP	PP	Y	SW
TL	0.683**	0.997**	0.851**	0.032**	0.054	0.011	0.273**	-0.105	-0.144*
LM[1]		0.625**	0.496**	0.137	0.287**	-0.147*	0.030	-0.015	0.095
TLB[2]			0.857**	-0.049	0.027	0.027	0.289**	-0.111	-0.164
LB[3]				-0.038	0.106	0.040	0.263**	-0.170	-0.159*
PL					0.658**	-0.540**	-0.191*	-0.036	0.591**
PG						-0.578**	-0.227*	-0.046	0.578**
SP							0.193*	-0.100	-0.535**
PP								-0.089	-0.272**
Y									0.571**

*,** Significant at 0.05 and 0.01 level of probability, respectively.
[1] LM = Number of leaves on main stem; [2] TLB = Total leaves on branch; [3] LB = Number of leaves per branch

yet skewed towards 100% hardseed (Fig. 8). Although the gene(s) controlling hardseedness should theoretically distribute equally among the RILs. Most RILs were small in seed size skewing toward that of ACC 41 (Table 1). Humphry et al. (2005) found a negative correlation between hardseedness and seed weight in mungbean. Similarly, Imrie et al. (1991) found that many small-seeded mungbean genotypes had a high percentage of hard seed while large-seeded varieties did not show persistent hardseedness. Consequently, large seed cultivars might possess some physical properties related to seed coat that helped easy imbibition of water into the seeds (Afzal et al. 2003a). Moreover, Imrie (1992) reported that hard seed percentage was dependent on physiological maturity, a combination of high temperature and humidity, and period of wetting and drying.

Correlation between agronomic traits

One hundred seed weight showed positive correlation with seed yield per plant, pod length, and pod width with correlation coefficients of 0.57, 0.58, and 0.59, respectively (Table 3). This implies that yield depends on seed size, and increasing pod size will increase the seed yield as well. These results were in accordance with the finding of Reddy et al. (1991) who also reported a positive association between pod length and seed weight in mungbean. Conversely, 100 seed weight was negatively correlated with seeds per pod, similar to that reported by Rohman et al. (2003). The number of seeds per pod was negatively correlated with pod width and pod length, indicating that a large-seeded genotype has a fewer number of seeds per pod than a small-seeded one. This is similar to the results reported by Sandhu et al. (1997). Berken produced higher seed yield per plant than ACC 41 due to its larger seed size, although the wild mungbean ACC 41 produces more pods than the commercial cultivar Berken. Seed size increment was essential for yield improvement in mungbean (Afzal et al. 2003b). Seed yield depends on many factors, especially seed weight, number of seeds per pod, and pods per plant. In this study, seed size is the most important yield component and directly proportional to seed yield per plant.

Heritability

Heritability was estimated using variance components from the analysis of variance of the RIL population and shown in Table 1. Heritability of days to flowering was high (88%), similar to the report of Nair et al. (2004) and was mainly controlled by additive gene effect. Heritability of pod size was the highest at 93.7% in pod length, and 93.2% in pod width. The other traits with high heritability were seed weight, number of seeds per pod and seed yield per plant, indicating high additive genetic effect governing these characters. Ilhamuddin et al. (1989) reported high heritability of 1000 seed weight at 82%. Moreover, Khattak et al. (2002) reported that additive genetic variance component was significant only in pod length and seeds per pod, and the estimates of narrow-sense and broad-sense heritability were relatively large for pods per plant, pod length, and 1000 seed weight. In this study, the number of pods per plant gave the lowest narrow-sense heritability (58.4%). Malik and Singh (1983) reported that both additive and dominant gene actions were involved in the expression of this trait. The heritability of germination at three days after testing (h^2 = 98.9%) was as high as in seven days (h^2 = 94.8%). Selection for highly heritable traits would be effective at any generation.

Testing of genetic segregation

Leaflet shape of the RILs was tested against 1 deltoid : 1 lobed ratio by Chi-square and the observed number agreed well with the expected one (Table 2). James et al. (1999) and Humphry et al. (2005) reported that this trait is conditioned by a single gene and located on linkage group B. Similarly, growth habit was not significantly deviated from 1 erect: 1 spreading ratio, while growth pattern followed the 1 indeterminate: 1 determinate ratio. Tar'an et al. (2002) studied in common bean and found that the latter trait distributed as a 1: 2: 1 ratio.

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