Progress Towards Reducing Seed Toxin Levels in Common Vetch (*Vicia sativa* L.)

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ABSTRACT

Common vetch (*Vicia sativa* L.) is well adapted to the low rainfall areas of southern Australia however, the seed contains high levels of a toxin, γ-glutamyl-β-cyanoalanine, which limits its use. Reducing the concentration of this toxin may allow greater marketing options and help develop vetch as a viable alternative grain crop for low rainfall areas. The genetic control of seed toxin level, and cotyledon colour were studied with the aim of developing low toxin lines of vetch with distinctive seeds. More than 3000 accessions from major international collections have been screened for seed toxin level. Using conventional breeding methods, it appears that seed toxin concentration can be reduced to about 0.3-0.4%. The work has shown that it is feasible to use conventional breeding methods to develop low toxin lines of vetch, which can then be used as a viable alternative source of protein in poultry diets.

KEY WORDS

Vicia sativa, vetch, neurotoxin, genetics, plant breeding, poultry feeding.

INTRODUCTION

Common vetch (*Vicia sativa* L.) is a grain legume crop that is well adapted to the low rainfall areas of southern Australia. The crop can be used for grazing, hay production, green manure or harvested for its grain. However, high concentrations of a neurotoxin (γ-glutamyl-β-cyanoalanine) in the seed of current cultivars limit its use as a feed grain and preclude its use as a pulse. Reducing toxin levels in the seed to 0.4% or less would allow the grain to be used as a source of protein in pig and poultry feed rations (3).

It is necessary to understand the genetic basis for important traits before appropriate breeding strategies can be developed. To develop a low toxin vetch with a distinctive appearance, the genetics of cotyledon colour, as well as seed toxin levels need to be described.

There is known to be genetic variation in seed toxin levels within vetch, which can be exploited to develop low toxin lines (3). In the first instance reducing levels to about 0.4% would make it suitable for pigs and poultry, but the long-term aim is to lower the toxin level further. In this paper, progress in developing low toxin lines of vetch with a distinctive appearance is described. The benefit of reducing toxin levels was examined in a short-term feeding trial using 3-week old chicks.

MATERIALS AND METHODS

Measuring seed toxin

The concentration of toxin in the powdered seed was measured using a diffuse mid infra-red method (1). Preliminary analysis showed that the seed toxin level for a plant could be reliably measured from a single, randomly selected pod containing more than 3 seeds. This method is rapid and inexpensive allowing large numbers of lines to be analysed. Skewness in the distribution of % seed toxin concentration can occur in populations due to under-developed seeds having unusually high toxin concentrations. This is overcome by weighing each seed and calculating the toxin mass per seed.

Development of low toxin populations

More than 3000 accessions from the ICARDA, Iranian and Russian collections were screened for seed toxin levels. Two low toxin lines (IR-28 and IR-36) were identified and crossed with the well-adapted South Australian cultivars Blanchefleur, Cummins and Jericho White (an early, white-flowered farmer selection).

Previously, Rathjen made twenty crosses using the low toxin lines IFVI 3091 and IFVI 3103 (3). She advanced five crosses to the F_2 generation and this material was selected further for low seed toxin and beige cotyledon colour and advanced to the F_6 generation.

Genetic analysis

A 5x5 full diallel cross was made to study the genetics of seed toxin content and cotyledon colour. Orange and beige are the two predominant cotyledon colours in *V. sativa*. In this study two beige parents (Jericho White and Cummins) and three orange parents (Blanchfleur, IR-28 and IR-36) were used. All possible cross combinations (including reciprocal crosses) were made, giving a total of 20 different cross combinations. Back crosses were made between F_1 and both parents. In all cases the F_1 plant was used as the female parent. The data on cotyledon colour were collected from the F_1 and F_2 populations in 20 cross combinations, as well as 36 back cross populations. Data from the reciprocal crosses were pooled and analyzed statistically.

Genetic analysis of the traits percent toxin and mass of toxin per seed were based on the individual F_1 plant means using Griffing's Model-1 Method-3 of diallel analysis (2). These analyses partitioned the variation due to genotypic difference into general combining ability (GCA), specific combining ability (SCA) and reciprocal effects. Model 1 was selected because the choice of parental materials was considered fixed.

Feeding studies

Three-week old chicks were fed diets containing different levels of vetch, faba bean and *Lathyrus cicera* for 18 days (Table 1). Body weight, feed intake and excreta were measured and general health monitored. At the end of the experiment the chicks were killed by cervical dislocation.

Table 1. Description of experimental diets used in the feeding trial.

Diet	Composition	Toxin level in diet (% w/w)
1	Control: Wheat, casein and pea	0
2	12.5% Jericho White (fresh seed) whole seed	0.225
3	25% Jericho White (fresh seed) whole seed	0.450
4	25% Jericho White (fresh seed) hulled	0.450
5	25% Jericho White (old seed) whole seed	0.125
6	25% Jericho White (old seed) hulled	0.125

7	25% Jericho White whole seed autoclaved	0
8	2.5% Jericho White hulls	0
9	25% Blanchefleur whole seed	0.257
10	25% Blanchefleur hulled	0.257
11	12.5% Jericho White (fresh) hulled + 12.5% faba bean hulled	0.225
12	12.5% Jericho White (fresh) hulled + 12.5% *Lathyrus cicera* hulled	0.225
13	25% Faba bean hulled	0
14	25% *Lathyrus sativus* hulled	0

RESULTS AND DISCUSSION

Development of low toxin populations

The crossing and selection programme has produced an F_6 line with a seed toxin level of 0.4?0.1%, which is significantly less than the levels in the commercially cultivated varieties (Blanchefleur and Jericho White) and the recently released cultivar Morava (Fig. 1).

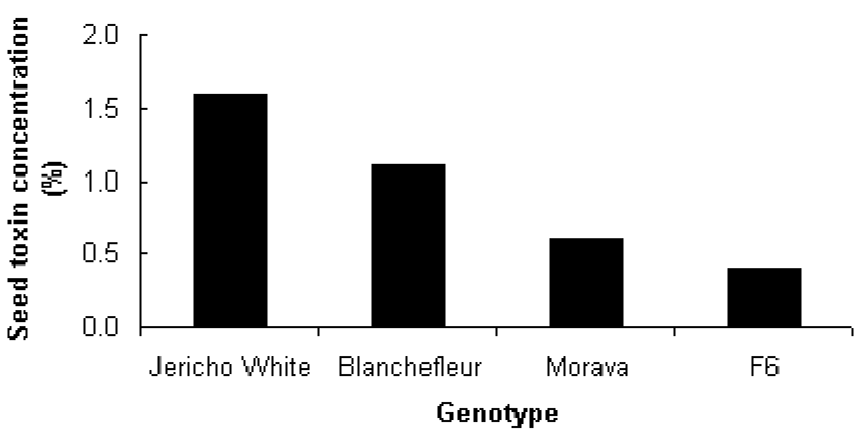

Figure 1. Variation in seed neurotoxin concentration in cultivars of common vetch and in an F_6 population selected for low seed toxin.

These results clearly show that it is possible to reduce the seed toxin levels in vetch using conventional breeding methods in a relatively short time (*c*. 4 years). The key to achieving this was the availability of an inexpensive and rapid method of analysis, which allowed large numbers of seeds to be analysed. However, reducing the toxin levels significantly below 0.4% by conventional breeding is proving to be a greater challenge.

Genetic analysis

The distribution of toxin levels in the seed (either as % toxin or mass of toxin per seed) indicates that these are quantitative traits (Fig. 2). The genotypic variance due to GCA was found to be greater than the SCA and the reciprocal estimate, indicating that seed toxin levels were controlled by loci with additive gene effects.

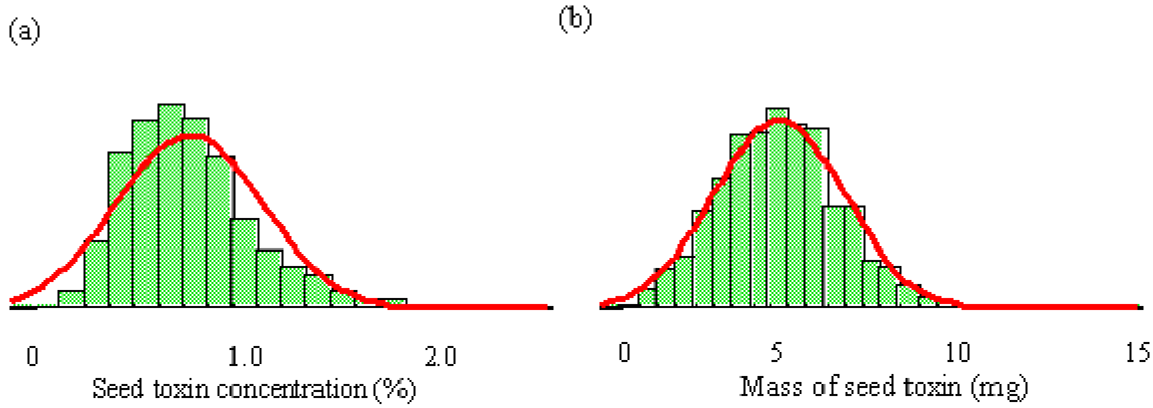

Figure 2. Frequency distribution for (a) seed toxin concentration and (b) mass of toxin per seed in F_2 populations (N=1570) of common vetch derived from crosses between low and high toxin lines.

IR-36 had the lowest GCA for percent and mass toxin followed by IR-28, indicating that these 2 lines were the best general combiners, within the genotypes examined, for reducing seed toxin levels. Therefore both IR-36 and IR-28 are lines that may be used as parents in a hybridization program to reduce toxin level in vetch. However, there were very few lines with seed toxin concentrations less than 0.4%, which suggests that further selection for lower toxin levels within these populations would not be successful.

The cross between parents with orange and beige cotyledons always produced an intermediate cotyledon colour in the F_1 population. Segregation for cotyledon colour in the F_2 progeny was 1 orange: 2 intermediate: 1 beige, indicating that colour is governed by a single gene difference with incomplete dominance. The F_1 with intermediate colour crossed with a homozygous orange cotyledon parent always produced 1 orange: 1 intermediate, while when crossed with a homozygous beige cotyledon parent, the segregation ratio was 1 beige: 1 intermediate. These observations confirmed that cotyledon colour in common vetch is controlled by a single gene difference with incomplete dominance between alleles. It is proposed that Ct_1 (orange) and Ct_2 (beige) are two allelic genes controlling cotyledon colour and showing incomplete dominance. On this basis, the genotype of Blanchfleur, IR-28 and IR-36 is Ct_1Ct_1 and the genotype of Jericho White and Cummins is Ct_2Ct_2.

Feeding trials

Body weight was significantly reduced when chicks were fed diets which contained high concentrations of toxin (Diets 3, 4 and 10) and the reduction was proportional to the amount of toxin present (Fig. 3). When the toxin level in the diet was less than 0.25% (Diets 2, 5,6) growth was similar to or exceeded that of chicks fed the control diet. Feeding a high quantity of *Lathyrus* (Diet 14) did not affect growth, unless *Lathyrus* was combined with vetch (Diet 12). This implies a negative synergism between the antinutritional factors in *L. cicera* and *V. sativa*.

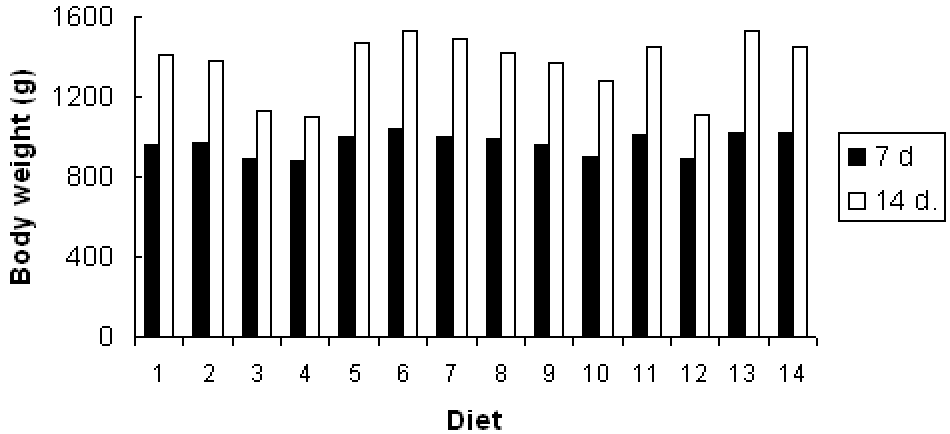

Fig. 3. Body weights of chicks after 7 and 14 days feeding on diets containing different quantities of vetch. Details of the diets are given in Table 1. Standard errors of the difference at 7 and 14 days are 31g and 52g, respectively.

Growth of young chickens is sensitive to the level of neurotoxin in the feed ration. This study has shown that once the toxin level in the diet exceeded 0.25% (w/w), growth of 3-week old chickens was impaired. Rathjen (3) found that 50% mortality of week old chicks and turkey poults occurred when there was 0.2% of toxin in the diet and no mortality at about 0.1%. If the desirable toxin level in the diet is assumed to be 0.1%, reducing the seed toxin level to 0.4% would allow up to 25% vetch to be included in the diet safely. This would allow vetch to be a useful alternative protein source in feed rations for pigs and poultry.

ACKNOWLEDGEMENT

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REFERENCES

1. Eichinger, P.C.H., Rothnie, N.E., Delaere, I., and Tate, M.E. (1999). In: Linking Research and Marketing Opportunities for Pulses in the 21st Century (Ed: R. Knight). pp. 685-692.

2. Griffing, B (1956). Heredity **10**: 31-50.

3. Rathjen, J. (1997) The potential of *Vicia sativa* L. as a grain legume for South Australia. PhD thesis Department of Plant Science, University of Adelaide.