FINAL REPORT

GRDC
GRAINS RESEARCH & DEVELOPMENT CORPORATION

UWA314

Incorporation of pea weevil resistance into a cultivar field pea

PROJECT DETAILS

PROJECT CODE:	UWA314
PROJECT TITLE:	INCORPORATION OF PEA WEEVIL RESISTANCE INTO A CULTIVAR FIELD PEA
START DATE:	01.01.2000
END DATE:	31.12.2003
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ORGANISATION:	CLIMA
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Summary

The project research has led to the development of pre-breeding material containing pea weevil resistance genes, providing a naturally derived resistance source for use by pea breeders. The aim of the project was to produce one pea weevil resistant line to third backcross by transferring resistance genes from *Pisum fulvum*, a wild pea species, into cultivated field peas. This output was successfully achieved, through a succession of backcrosses, and advanced to a stage suitable for use as parental material in germplasm enhancement programs. The techniques used to produce this outcome include plant hybridisation, insect bioassays, molecular markers and plant phenotyping.

Report Disclaimer

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Conclusions

1. Pea weevil resistance is a polygenic trait, controlled by a minimum of three major recessive genes.

Breeding populations should ideally be kept large enough to include a sufficient chance of selecting resistant lines in the population. For example, only one in 64 plants will be 100% pea weevil resistant if the hypothesis that the trait is controlled by three major recessive genes is correct. For example, in a population of 6,500 F2 plants, 100 of these will theoretically carry all three resistance genes and be 100% resistant.

2. Molecular markers

Screening a population with one marker linked to resistance has the potential to positively identify plants containing at least one resistance gene. Combining this with a marker selecting for susceptibility will also select against plants containing one gene for susceptibility. While this does not provide the complete answer as far as selecting 100% resistant plants, it does provide an efficient way to reduce the screenable population to a manageable size. The multiplex marker developed in this project falls into this category.

3. Multiple marker system

A polymerase chain reaction (PCR) system consisting of more multiplexed markers (for example, three pairs of resistant/susceptible PCR markers), representing each of the three proposed gene regions, or quantitative trait loci (QTL), as visualised by the scatter of 588 amplified fragment length polymorphism (AFLP) bands in Principal Coordinate Analysis (PCO) space should make this system very efficient in selecting resistant plants. (Byrne M, Galwey N & Hardie D (2004) - submitted paper).

4. Recurrent selection and backcrossing

Improvement in seed size increase and general variety trait performance was facilitated by regular recording of a range of traits, including flowering time, seed size, flower colour, etc. Statistical association between any one of these traits and pea weevil resistance was inconclusive. For example, flower colour would offer a very practical visual marker. A wildness index based on the association of many of the traits together provided a quick visual method for selecting resistant lines, with the greatest leaning towards variety habit.

Recommendations

The recommendations following the outcomes of this project are to encourage the fast tracking of potentially useful resistant material from the pre-breeding stage to the parental line stage of pea breeding initiatives in Australia.

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1. Seed multiplication is recommended for backcrossed lines for future national pea field testing of pea weevil resistance (at multi trial locations in Australia).

2. It is also recommended to consider screening the advanced backcross progeny lines with the multiplex marker developed in this project.

3. Backcrossing of advanced resistant lines to be continued into elite varieties (e.g. into high yielding lines, blackspot tolerant lines, etc).

Other recommendations:

Germplasm

P. fulvum X *P. sativum* hybrid and backcross lines are to be stored for future advancement. A database of all lines is to be provided with seed. Database spreadsheets to contain a plant identification code, parentage, character traits, pea weevil resistance scores, etc. Select lines used in backcross breeding programs also to be highlighted.

Seed material to be sent to Horsham for storage.

Molecular

Sequence data to be retained for molecular markers identified with linkage to the pea weevil resistance character. Specific characterised primer sequences (SCAR) also to be made available to contributing parties to the project.

Outcomes

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Economic

The prospective economic benefits to the Australian grains industry relate to the utilisation of the wider genetic pool for germplasm enhancement. Incorporating genes from wild pea germplasm to enhance pea performance against the pea weevil means, potentially, that new varieties can be introduced rapidly and without the regulations currently delaying release of transgenic plant varieties. More specifically, if a naturally bred pea weevil resistant pea variety can be released, this will not be subject to the same regulation and market vagaries as genetically modified (GM) varieties. This strategy fulfils the Industry and government drive for a 'clean and green' food industry to expand exports. Economic benefits also include future reductions in yield penalties of several million dollars annually due to pea weevil damage, and future increase in the market value of field peas by producing a quality product acceptable for human consumption that is required for certain export markets. The predicted timeframe for a flow of benefits to the Australian grains industry is still several years away and is dependent on future pea breeding initiatives.

Environmental

Environmental benefits of future pea weevil resistant field pea material include a potential reduction in the number and application rate of chemical pesticide sprays applied in cropping phases. The group of chemical sprays used most commonly for pea weevil control in-field is synthetic pyrethroids (SPs), which are non-selective against most invertebrates, including many beneficial species found within crops, the European honeybee, and farm catchment invertebrates i.e. yabbies. In the storage phase, both on-farm and at receival centres, fumigation with phosphine# or methyl bromide# will also be greatly reduced or eliminated, which will further reduce the grains industry's reliance on proven or potential ozone depletants and greenhouse gases.

Social

The Australian grains industry demonstrates its social conscience by providing an alternative to aerial and ground rig application of insecticides through the development of insect resistant varieties, in this case pea weevil resistant varieties. This is a positive message for the grains industry in the debate surrounding spray drift. Future social outcomes include the potential for a greater understanding of the biochemical basis of pea weevil resistance. This may result in an expansion of the knowledge base on biologically active compounds (e.g. bioflavonoids, phytoestrogens), which might have relevance to human and animal health if such compounds are present in the enhanced germplasm material that has been produced.

Achievements/Benefits

Background importance of the issue this project was designed to address:

Pea weevil is a major pest of field peas in all pea-growing states in Australia, impacting most in Victoria (VIC) and South Australia (SA), which account for approximately 90% of the pea growing area. Western Australia (WA) alone has the potential to produce more than 200,000t of field peas annually (currently it produces 30,000t/pa) (The Crop Variety Sowing Guide, 1999). As part of an Australian pulse industry strategic plan, the Grains Council of Australia (GCA) predicts an increase in field pea production of about 900,000t within 10 years, including 250,000t of food grade pea. If the major problems associated with the growing of field peas are overcome (such as blackspot), the scale of the benefits associated with pea weevil resistant varieties will also expand.

This project had several aims:

Backcross pea weevil resistance derived from wild peas into an agronomically superior field pea variety with the aid of molecular marker technology.

Investigate the biochemical basis of pea weevil resistance by developing an insect-feeding bioassay, with the aim of identifying the active compound(s) involved.

Provide resistance in field peas to pea weevil that does not involve a transgene and is therefore free of associated patent regulations.

Major achievements of this project:

1. Production of resistant lines

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'By the completion of the project, a minimum of one resistant field pea to BC3 generation will be produced and made available to breeders'.

Resistant backcross lines to BC3 generation have been produced by first backcrossing resistant F4 lines with Helena(Ф), a Dundale-derived variety released by the Department of Agriculture and Food, Western Australia (DAFWA) in 1999, followed by two backcrosses with Dunwa. In total, 6,500 BC1F2 seeds were produced by selfing F1 hybrid backcross plants. Preliminary selections for pea weevil resistance and variety traits were made on the BC1F2 generation and the best performing lines were screened again (BC1F3s). A population of 260 backcross (BC1F3) plants were screened for pea weevil resistance using an in-situ pod bioassay and also by testing with a multiplex molecular marker. Plants were also screened for a range of phenotypic traits, used as indicators in selecting plants with a well-adapted genetic background. In a random sampling of 157 BC1F3 plants, three plants were 100% pea weevil resistant, 94 were 14-90% resistant (with a mean of 40% resistance) and 59 plants were 90-100% susceptible. The ratios support earlier findings that pea weevil resistance fits a three-gene inheritance profile for a recessively inherited trait (1:36:27). The BC1F4 seeds were collected from the 260 BC1F3 plants and the three most resistant lines were backcrossed (BC2) to Dunwa, a WA-bred dun pea.

From the second backcross with Dunwa, 55 BC2F1 plants were screened for pea weevil resistance and genomic DNA extracted for molecular marker assessment. A third backcross (BC3) of the most promising BC2 lines with Dunwa has produced BC3F1 seed. Representative plots of all the BC1F2 lines were also planted out at Medina, in WA, in 2004 and will be screened for blackspot resistance, field performance of pea weevil resistance, earliness, plant growth habit and other phenotypic traits (as part of the field pea breeding program). Resistant lines are also being backcrossed again in 2004. The backcross material is performing very well in both glasshouse and field trials, with a large proportion of lines showing resemblance to variety plants.

2. Variety advancement

The range of phenotypic traits measured included: (i) flower colour, (ii) early/late flowering, (iii) node of first flower, (iv) basal flowering, (v) basal branching, (vi) pod number, (vii) seed size, (viii) seed shape (ix) seed coat colour (x) biomass and (xi) a 'wildness' score (0-5).

Seed size is probably one of the more important characters to the breeder when selecting lines to advance. The very small seed size of the resistant wild pea is one of the negative characters that was improved while screening for pea weevil resistance. Seed size (100 seed weight) in the original resistant and susceptible parents was 60mg and 220mg, respectively. The average seed weight in the first cross was 120mg (F2). The mean seed weight for 19 selected resistant lines in the first backcross (BCF2) into the variety Helena was 150mg, representing a 25% increase in seed size. A second backcross into the elite large seeded Dunwa has further increased seed size to an average of 237mg, or 80% that of Dunwa in glasshouse assessment. A third backcross into Dunwa is at the F1 stage and predictions for further seed size increase in F2 are very promising.

Other phenotypic traits that have been improved in the breeding component of this project include: early/late flowering lines, low 'wildness' score, selections for seed type (e.g. light coloured seed coats, dun coloured seeds). Glasshouse assessments of flowering time ranged from five (early) to 14 (late) weeks, with a mean flowering time of 10 weeks. Flower colour was recorded for all plants and included pink/purple dun pea types, white/cream pea types and brown/orange flowering types (wild genes). The resistant lines selected for backcrossing are represented within each flower colour category and in both early/late flowering types.

3. Molecular marker development

Ten polymorphic AFLP bands with statistically significant linkage to pea weevil resistance were isolated, cloned and sequenced. Primer pairs were designed from DNA sequences of candidate bands. These candidate bands were selected using PCO, a multivariate approach used to evaluate 588 polymorphic AFLP loci. To validate the statistical method of selecting candidate bands, bands were also ranked by more empirical means. There was a positive correlation between the PCO method and empirical approach to selecting the best bands. The PCO method is more useful than the empirical method because it could discriminate markers unlikely to be linked to each other, ensuring markers were not duplicates of each other, but were mapping to different loci. This is especially important because the research is dealing with a trait influenced by several genes, or QTL.

The DNA sequences of the polymorphic fragments were compared to DNA and protein sequences held on international

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genome sequence databases, using the Australian National Genomic Information Service (ANGIS) to access the information. The searches were successful in identifying sequence similarity in most of the bands to known plant or animal DNA. Two bands, PE66260 and PE25414, isolated from the susceptible parent were recognised as *P. sativum* DNA sequences. Marker fragment PE66260 was shown to have a strong association with *P. sativum* gibberellin c20-oxidase gene. Fragment PE25414 showed significant association with the plant defence-related gene, STR246N. The marker fragments derived from the wild parent showed less association with known sequences in gene databases, as would be expected.

SCAR primers were designed around the sequence of eight of the AFLP bands to produce a rapid test of the genomic DNA material. These primer pairs were tested on parental genomic DNA, hybrid progeny and unrelated varieties in validation studies.

4. Molecular marker validation studies

Three PCR markers (AE47435, AE47349, PE66260) have successfully discriminated between susceptible and resistant parents, identified resistant progeny and differentiated between susceptible unrelated varieties and wild accessions. For example, (i) AE47435: in a PCR screen of 16 F2 plants, the three 100% resistant plants contained the marker, five mixed resistant and only two susceptible plants also contained this marker. Seven F2 plants did not contain the marker. The same marker tested on 27 F3 plants discriminated all six resistant plants from mixed and susceptible plants. (ii) AE47359: in a PCR test of 29 F3 plants, the marker was present in 17 F3 plants, with a mean resistance score of 58% originating from F2 lines with a mean resistance of 62%. The average resistance score for the remaining 12 F3 plants with band absence was 12% (88% susceptibility) in F3 and 13% in F2. (iii) A PCR-based marker, associated with pea weevil susceptibility (PE66260) positively identified susceptible individuals. A 'multiplex' PCR diagnostic assay was developed using AE47359 and PE66260 to fast-track screening individuals for both markers at one time.

5. Multiplex marker validation

A multiplex PCR assay, containing a marker for insect resistance (AE47359) and a marker for insect susceptibility (PE66260) was successful in discriminating susceptible and resistant parents, susceptible and resistant F4 recombinant inbred lines (RILs) and susceptible unrelated varieties. The PCR multiplex assay was used to screen a backcross population of 247 plants of known resistance ranking. Eighteen individuals (7%) contained only the AE47359 band, indicative of resistance. Two of these individuals were 100% resistant, one was 75% resistant, 12 were of mixed resistance ranking and seven were susceptible. The PCR test was successful in identifying 25% of the resistants in the population.

There are several explanations for this:

(i) the marker set is enriching for one of the three major resistance genes, which ties in well with findings that pea weevil resistance is controlled by three major recessive genes.

(ii) It explains why some susceptible plants contain the resistant band: there will always be a percentage of heterozygous individuals in the population carrying resistance genes in the recessive form.

(iii) There is also the possibility that the marker is not closely linked enough to resistant individuals. It is very promising that the most resistant line selected for further backcrossing, using conventional screening, was one of the 100% lines identified using this multiplex PCR marker screen.

5. Pea weevil resistance and inheritance studies

The pea weevil resistance mechanism fitted a three-gene model:

In a population of 262 F2 lines produced by an interspecific cross between the wild *P. fulvum* (ATC113) and pea variety *P. sativum* (Pennant), resistance was established as operating at the level of the cotyledons. From these results, there were three groups of plants: five resistant, 148 mid range resistance and 110 highly susceptible. These ratios fit a 1:36:27 model. It would be expected to have a 1:3:3:3:9:9:9:27 ratio for a trihybrid cross. The simplest model is to explain it using a PP WW RR notation for the three genes:

27 have the PWR phenotype - complete susceptibility
36 have PWr, PwR, Pwr, pWR, PWr, or pwR - moderate susceptibility/resistance

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1 has the pwr phenotype - low (no susceptibility)

This is often the case with disease and resistance genetics in plants.
Resistance in the pod wall was also observed in some crosses. There is a strong indication that the pod wall resistance is associated with neoplastic pods (controlled by the Np gene), a phenomenon that is often associated with glasshouse cultivated peas. Lines in which the resistance was seen in the cotyledon were used for marker development in this project.

6. Biochemical basis of pea weevil resistance: the active compound(s) involved:

Pod wall and cotyledon extracts were screened for active compounds involved in the resistance. Parental lines and resistant progeny plants were tested at several stages of seed maturity to include the seed growth stage when pea weevil attacks the seed. This work is in collaboration with the Chemistry Centre (WA). A formal report on the chemistry findings will follow shortly: Expected report delivery: September 2004.

7. Naturally derived resistance

The project resulted in the production of a potential source of pea weevil resistant pea that does not involve a transgene and is, therefore, free of associated patent regulations. This fulfils one of the aims of the project.

8. Other achievements

Other achievements of the project include media releases; publication of research in articles in the West Australian, Countryman, Esperance Express, communication of research findings at international level in a refereed journal, international conference paper and several posters.

How these achievements will benefit the industry

These achievements will benefit the industry in several ways: the prospective benefits of using a natural germplasm base (wild pea), in conjunction with marker accelerated backcrossing, means that new varieties can be introduced rapidly and without the regulations currently delaying release of transgenic plant varieties. Benefits also include the reduction in cost of chemical sprays, reduction in yield penalties due to pea weevil damage and an increase in the market value of field peas by producing a quality product acceptable for human consumption and export markets.

The total number of hectares to which the benefits will apply is estimated at 316,200ha (Krieg et al, 1996). The improvement in the quality of field peas will impact on market opportunities, where more grain can be sold into the premium end of the market (human consumption and export). Pea seed which is not contaminated with live pea weevil will be much more acceptable to grain merchants (such as the Grain Pool of WA) because the export market has nil tolerance for live insect contamination. It will also be easier to market a quality pea seed that is not contaminated with pea weevil to millers (processors) for splitting or flour production, as many of the holes caused by pea weevil are only discovered at this stage. Even if fumigated, contaminated grain will still need to be cleaned.

Other research

1. Several of the pea populations developed within this GRDC project are suited to mapping. A balanced F2 population with phenotypic traits measurements already collated, DNA and leaf material presently available in cold storage (University of Western Australia (UWA) and Murdoch University, in Perth, WA).

2. R&D opportunities include investigating the gene expression of pea weevil resistance mechanisms. Investigate changes in protein expression during seed fill stage (time of pea weevil attack).

3. A preliminary investigation of the cytogenetics of the interspecific cross between the wild pea and cultivated pea has been conducted. Some sample material is presently available (in cold storage at UWA). Development of fluorescence in situ hybridization (FISH) and genomic in situ hybridisation (GISH) fluorescent tags for screening for pea weevil resistance.

4. Preliminary findings indicate there was some association between flower colour and pea weevil resistance and this might be worth further investigation.

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Intellectual property summary

Any new varieties developed will be Australian owned, the IP belonging to the pea breeders, the research team and GRDC.

Commercialisation will depend on whether a new variety developed by this research will be issued with Plant Breeder's Rights (PBR) protection, or whether it can be released directly into the public domain. This will be reflected by policy decisions at the time. Commercialisation potential will also be dependent on GRDC policy and contributing stakeholder's policy (UWA, Centre for Legumes in Mediterranean Agriculture (CLIMA) and DAFWA) involving a negotiated stance.

Additional information

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