Physiological and Genetic Investigations of Iron Deficiency in Field Peas (*Pisum sativum* L.)

by

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ABSTRACT

Iron (Fe) deficiency chlorosis affects both yield and quality of many species, including cool-season food legumes and the chlorosis symptom is especially prevalent in crops grown on calcareous soils which are widely distributed in the southern region of Australia. Although Fe fertilizers have been used to correct the chlorosis and are effective for short term control, cultivation of tolerant cultivars could reduce the damage in the long term for all sensitive crops including field peas. The present study was conducted to investigate various aspects of the genetic tolerance of field pea cultivars Santi and Parafield, in particular, with the objective of providing the information to implement an efficient breeding strategy for the long-term control of Fe deficiency chlorosis.

Methods to screen field peas for tolerance to Fe deficiency were developed by utilizing both solution and pot soil cultures. Nutrient solution with a high concentration (10 mM) of bicarbonate ($HCO3^-$) in either the sodium (Na) or potassium (K) forms induced symptoms of Fe deficiency and it was possible to discriminate between tolerant and sensitive field pea genotypes. Plants grown in $NaHCO_3$ developed symptoms indicative of Na toxicity and therefore $KHCO_3$ was selected for solution culture studies. On the basis of this result, 37 accessions were screened in solution culture containing 10 mM $KHCO_3$ and eight accessions that were representative of the range of response to Fe deficiency chlorosis and variation in plant morphologies were selected for physiological and genetic studies. These included Santi, Px-95-183-7-1, Px-89-82-1 and Px-97-58-1 (tolerant genotypes) and Parafield, Glenroy, Px-97-9-4, and Px-96-83-1-1 (moderately sensitive to sensitive genotypes).

Three cultivars, namely Santi, Glenroy and Parafield were grown in pots to identify the effect of three types of calcareous soils obtained from Wangary, Glenroy and Millicent and UC soil as a control, on the Fe chlorosis symptoms. Severe symptoms indicative of Fe deficiency were induced in plants grown in Wangary and Millicent soils and were most severe for Parafield. Imposing a high soil moisture treatment of 120% of field capacity induced more severe chlorosis symptoms than 100% or 80% of field capacity, and in all three treatments Parafield was the most sensitive, Glenroy intermediate and Santi remained green. Fe chelates in the forms of Fe-EDDHA and Fe-EDTA were applied as both foliar and soil treatments to Parafield plants, grown in Millicent soil, that were exhibiting severe chlorosis. All combinations of fertilizer type x method of application were effective in

reducing shoot chlorosis of the top leaves at the time of application and also subsequent growth, indicating that the leaf chlorosis was due to Fe deficiency.

The physiological mechanism controlling genetic variation in tolerance to Fe deficiency chlorosis, between field pea cultivars Santi and Parafield, and derived backcross lines was investigated. The major mechanism was not related to acquisition as Fe(III) reductase activity of roots, and the concentration of total Fe in leaves, were not significantly different between tolerant and sensitive genotypes. There was also little or no association with distribution within the plant as the pattern of distribution of total Fe from shoot tips to lower leaves was the same for both cultivars. However, the main variation between Santi and Parafield was in maintaining active Fe in young leaves and stipules and active Fe in young tissues of Santi was significantly greater than in Parafield. There was a highly significant correlation between chlorosis and active Fe and the concentration of active Fe increased from shoot tips which were chlorotic to lower leaves which maintained a high concentration of chlorophyll. The association between active Fe and chlorosis was also observed in backcross and F_2 populations confirming that this is a direct relationship, and not just a chance association between the two traits in two unrelated cultivars.

The genetic control of tolerance to Fe deficiency chlorosis in the cross between tolerant Santi and sensitive Parafield was investigated. Reciprocal F_1 hybrids, the F_2, F_3 generations, and BC_1F_1 plants were tested for responses to Fe deficiency using the Millicent soil at 120% field capacity. There was no difference in response between the reciprocal F_1 hybrids and their response indicated that tolerance was a partially dominant trait. Segregation of the F_2, F_3 and backcross generations revealed ratios, and population variances, that were consistent with tolerance being conferred by two partially dominant genes.

As tolerance to Fe deficiency chlorosis is under major gene control with high heritability, and the trait is already present in adapted Australian cultivars, it could be introduced to other breeding material either through bi-parental crosses or via backcrossing, depending on other target traits in the populations. Selection could be undertaken effectively in early generations, for example individual F2 plants with progeny testing in the F3, to identify homozogyous tolerant selections. Although this project was not successful in identifying molecular markers linked to tolerance to Fe deficiency chlorosis, as molecular maps for field pea are further developed it is highly probable that linked markers could be idenfied.

Tolerance to Fe deficiency chlorosis was inherited independently of major genes for seed colour, plant height and leaf type, and could therefore be readily transferred to a range of plant types.

The specific tolerance of Px-95-183-7-1 and Px-89-82-1 (tolerant), Px-96-83-1-1 (moderately tolerant) and Px-97-9-4 (sensitive), all of which are breeding lines of the South Australia field pea breeding program, was compared with Santi and Parafield. These lines were crossed to Parafield and Santi and F_1 hybrids and the F_2 of each cross was grown in Millicent soil at 120% of field capacity and tested for reaction to the Fe deficiency. Results indicated that the number of genes controlling tolerance to Fe deficiency chlorosis varied, depending on the parental combinations. A cross between sensitive and tolerant parents segregated at two genes, but crosses between sensitive and intermediate-tolerant, or between intermediate-tolerant and tolerant parents segregated at a single gene. Investigations of the pedigrees of all lines tested in the project also revealed evidence of major gene control of tolerance. All tolerant lines included the breeding line M150-1 in their pedigrees and one of the parents of M150-1 is likely to be the source of Fe efficiency. Further investigations are required to identify the specific line.

The outcome of this project should assist in the breeding of Fe deficiency chlorosis tolerant cultivars of not only field peas but also the other pulse crops grown in southern Australia. The screening methods should be applicable to all crops, while it is likely that the genetic control of tolerance would also be similar among the closely related cool season pulse species.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Uyek Malik Yakop

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ABBREVIATIONS

ABARE	Australian Bureau of Agricultural and Resource Economics
ABS	Australian Bureau of Statistics
ANOVA	analysis of variance
BC	Backcross
CSBP	CSBP Plant and Soil Laboratory
DAS	days after sowing
DAT	days after treatment
DNA	deoxyribonucleic acid
EC	electrical conductivity
FAO	Food and Agricultural Organisation
FC	field capacity
F_n	Filial generation, eg F_2 is the second filial generation
Fe	iron
Fe (II)	Fe^{2+}
Fe (III)	Fe^{3+}
Fe-EDDHA	Fe-ethylendiamine di(*o*-hydroxyphenylacetic) acid
Fe-EDDHMA	Fe- ethylendiamine di(2-hydroxy-4-methylphenylacetic) acid
Fe-EDTA	Fe-ethylenediaminetetraacetic acid
ICP-AES	inductively coupled plasma – atomic emission spectrometry
HCO_3^-	bicarbonate
LSD	least significant difference
M	molar
MES	2-[N-Morpholino]ethanesulfonic acid
mM	millimolar
NA	nicotianamine
RO	Reverse Osmosis water
SARDI	South Australian Research and Development Institute
SDW	shoot dry weight
SPAD	Soil Plant Analysis Development
UC	University of California
YOL	youngest open leaf (3^{rd} YOL: third youngest open leaf)
YOS	youngest open stipule (3^{rd} YOS : third youngest open stipule)