Reproductive frost tolerance in field pea (*Pisum sativum* L.)

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By

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Abbreviations

AFLP: Amplified Fragment Length Polymorphism

ANOVA: Analysis of variance

ATFCC: Australian Temperate Field Crops Collection

BC: Backcross

CO_2: Carbon dioxide

CRD: Completely Randomised Design

CTAB: hexa-decyl, tri-methyl, ammonium bromide

DNA: Deoxyribonucleic Acid

dNTP: Deoxyribonucleoside triphosphate

EST: Expression Sequence Tag

FAO: Food and Agriculture Organisation of the United Nations

FS: Flowering Stage

HPLC: High Performance Liquid Chromatography

ICP-AES: Inductively Coupled Plasma Atomic Emission Spectrometry

NPQ: Non-photochemical quenching

OsO_4: Osmium tetraoxide

PBS: Phosphate Buffered Saline

PCR: Polymerase Chain Reaction

PDS: Pod Development Stage

PGER: Pulse Germplasm Enhancement Research

PPFD: Photosynthetic Photon Flux Density

PSII: Photosystem II

qP: Photochemical quenching

QTL: Quantitative Trait Locus

RAPD: Randomly Amplified Polymorphic DNA

RFT: Reproductive frost tolerance

RWC: Relative Water Content

SARDI: South Australian Research and Development Institute

SNP: Single Nucleotide Polymorphism

SSR: Simple Sequence Repeat

STMS: Sequence Tagged Microsatellite Site

STS: Sequence Tagged Site

TEM: Transmission Electron Microscopy

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Abstract

Radiant frost during spring is a significant problem for field pea (*Pisum sativum* L.) grown in Mediterranean environments as plants are at the vulnerable reproductive stage when frost occurs. In such environments, radiant frost events after the commencement of flowering of field pea may lead to severe frost injuries on plants, and can adversely affect the grain yield. Despite the importance of the impact of frost on grain yield, no dedicated study has been conducted on reproductive frost tolerance (RFT) in field pea.

One aim of this research was to develop a simple and reliable screening method to evaluate frost tolerance of eight reproductive organs (from immature buds to mature pods) which are often present at the same time on a single plant. A controlled environment screening method that exposed plants to a defined temperature regime, including a minimum temperature of -4.8 ^{o}C for 4 hr, was developed. A scoring key was devised to record frost symptoms on each reproductive organ, and five categories were defined to evaluate frost damage on seeds. Using this screening method, a diverse collection of germplasm was screened, including 83 accessions sourced from high altitude and frost prone areas in 39 countries. A locally adapted variety, Kaspa, the most widely cultivated field pea variety in southern Australia, was included in the screening. The flowering stage was found to be more susceptible to frost than the pod development stage. Buds and set pods were found to be the most frost-susceptible reproductive organs, and mature pods were the most frost-tolerant reproductive organs. Genetic variation was found among field pea genotypes for frost tolerance at the flowering stage. Eight accessions, ATC 104, ATC 377, ATC 947, ATC 968, ATC 1564, ATC 3489, ATC 3992 and ATC 4204, each from a different country, were identified with more than 20 % frost survival of flowering stage organs. Kaspa was highly susceptible to frost at reproductive stages, with no buds, flowers or pods surviving the frost treatment.

A BC_1F_1 population was derived from frost- tolerant ATC 1564 and frost-sensitive Kaspa, and segregation of the frost survival trait and SSR markers was studied. Little marker polymorphism was observed between the two genotypes, with only 41 (12.3 %) of the 332 primer pairs assayed on DNA samples of the parental lines, exhibiting

polymorphic products in polyacrylamide gel electrophoresis. Unfortunately, most of these markers were not linked with any other loci, and only two linkage groups were developed: one with three markers, and the other with only two. No strong marker-trait associations were observed for frost tolerance.

Responses of reproductive-stage plants to low positive temperature (10/5 ^{o}C day/night, and 150 – 250 µmole m^{-2} s^{-1} PPFD) for 7, 14 and 21 days were studiedas were the effects of these cold treatments on survival of vegetative and reproductive tissues after frost, for frost-tolerant (ATC 968 and ATC 1564) and frost-sensitive (ATC 1040 and Kaspa) genotypes. Under long exposures (21 days), all genotypes exhibited an ability to maintain the photosynthetic rate. All genotypes were found to be adversely affected by chilling at the reproductive stage, however frost-sensitive genotypes were more responsive to low positive temperatures (cold) than frost tolerant genotypes. Evidence of symptoms of chilling injuries was found in the frost-sensitive genotype: distortion in the ultrastructure of chloroplasts was observed in parenchyma cells of stipules in Kaspa. A decrease and/or non-accumulation of soluble sugar in vegetative and reproductive tissues found in all genotypes under cold conditions reflected the inability in reproductive stage plants to acclimate. In contrast to what has previously been observed for pea seedlings, cold treatment of reproductive-stage pea plants did not result in acclimation, did not improve reproductive frost tolerance, and in fact reduced frost tolerance.

In conclusion, a drop in temperature under radiant frost conditions is lethal for reproductive stage pea plants. Reproductive organs are inherently sensitive to frost, and severe frost damage may lead to abortion of buds, flowers and set pods, and significantly reduce the seed weight.

Declaration

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.

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Shaista Shafiq
Dec, 2012

Statement of authorship

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Shaista Shafiq (Candidate)

Designed experiment, performed experimental work, analysed and interpreted data, and wrote the manuscript.

Jeffery Paull (Co-author, and Principal Supervisor)

Edited the manuscript, and gave advice and suggestions on presenting results and the manuscript. Acted as a corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the article in the thesis.

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Diane Mather (Co-author, and Co-Supervisor)

Edited the manuscript, and gave useful criticism on the manuscript and particularly advice on statistical analyses of data.

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Supervised the candidate during the design and execution of the experiments, and provided financial support through South Australian Grain Industry Trust project.

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