SCREENING OF PEA (*Pisum sativum* L.) RESISTANT TO POWDERY MILDEW DISEASE FOR KUNDASANG HIGHLAND IN SABAH

EVENNI POILI

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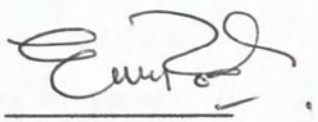

24 November 2009

Evenni Poili

PS04-001-04

CERTIFICATION

NAME : **EVENNI POILI**

MATRIC NO : **PS04-001-04**

TITLE : **IDENTIFICATION OF PEA (*Pisum sativum*) RESISTANT TO POWDERY MILDEW DISEASE IN SABAH**

DEGREE : **MASTER OF SCIENCE (PLANT PATHOLOGY)**

VIVA DATE : **25 AUGUST 2009**

DECLARED BY

SIGNATURE

SUPERVISOR

ASS. PROF. DR. MARKUS ATONG

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ABSTRACT

SCREENING OF PEA (*Pisum sativum* L.) RESISTANT TO POWDERY MILDEW DISEASE FOR KUNDASANG HIGHLAND IN SABAH

Pea (*Pisum sativum* L.) cultivation in Sabah is affected by powdery mildew caused by *Erysiphe pisi* DC. Fungicide application is the most preferred method of disease control by the growers. However, the non-judicious use of fungicides has led to excessive pesticide residues in the vegetable. Therefore planting of resistant variety is the best option because it is safe, cost effective and practical. Screening of the powdery mildew resistant pea variety was carried out in this study. A total of seven pea varieties were assessed for their response to powdery mildew infection at Agriculture Research Station Mesilou, Kundasang, Ranau. Their plant characters and field performances were also evaluated. Variety S7 was found to be resistant with 0 DSI score in all of the three trials conducted, the rest of the varieties were susceptible with DSI scores of 4.17 – 5.90. Marker linked to the resistance to powdery mildew was identified at about 600bp using PCR-RAPD primer 5'-ATT AGT AGT TGT TGT TG-3'. This band was found in resistant variety (S7) but absent in the susceptible varieties. The powdery mildew resistant pea identified in this study not only has the potential use in the production of resistant cultivars, but the resistant gene once identified and further characterised, can be utilised in the improvement of leguminous plants.

ABSTRAK

Penanaman kacang pis (Pisum sativum L.) di Sabah terjejas oleh jangkitan penyakit kulapok berdebu yang disebabkan oleh Erysiphe pisi DC. Penyakit ini biasanya dikawal dengan menggunakan racun. Penggunaan racun yang tidak berhemah menyebabkan sayuran ini mengandungi sisa racun yang tinggi. Oleh itu penanaman menggunakan variety yang rintang kepada penyakit ini adalah opsyen yang paling baik kerana ianya selamat, efektif dari segi kos dan praktikal. Kajian ini dijalankan untuk mengenalpasti varieti kacang pis yang rintang kepada penyakit kulapok berdebu. Sejumlah tujuh varieti kacang pis telah ditakbir untuk menilai jangkitan penyakit kulapok berdebu di Stesen Penyelidikan Pertanian Mesilou, Kundasang, Ranau. Ciri-ciri dan pengeluaran hasil tanaman ini di ladang telah juga dinilai. Varieti S7 didapati rintang terhadap kulapok berdebu dengan skor 0 DSI sementara varieti yang lain adalah rentan dengan skor 4.17 – 5.90 DSI. PCR-RAPD menggunakan primer 5'-ATT AGT AGT TGT TGT TG-3' menghasilkan jalur DNA yang unik pada kedudukan lebihkurang 600 bp pada varieti rintang (S7) yang mana tidak ada pada varieti-varieti lain yang rentan. Varieti kacang pis hasil daripada kajian ini selain daripada berpotensi untuk menjana kultivar yang rintang penyakit, juga sekiranya gen ini dapat dikenalpasti ianya dapat digunakan untuk pembaikbiak tanaman berkenaan.

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LIST OF ABBREVIATIONS

BC	-	Before Christ
bp	-	base pair
DAS	-	Days After Sowing
DM	-	Dry matter
DNA	-	Deoxyribonucliec Acid
DSI	-	Disease Severity Index
E	-	East
FAO	-	Food and Agriculture Organisation
HCL	-	Hydrocloride Acid
HR	-	Hypersensitive Response
KCL	-	Potassium Chloride
K_2O	-	Potash
M	-	Molar
Mg^{2+}	-	Magnesium ion
$MgCl_2$	-	Magnesium Chloride
N	-	North, Nitrogen
NaCL	-	Sodium Chloride
PCR	-	Polymerase Chain Reaction
P_2O_5	-	Phosphorus
QTL	-	Quantitative Traits Loci
RAPD	-	Randomly Amplified Polymorphic DNA
RNA	-	Ribonucleic Acid
Syn	-	Synonym

LIST OF SYMBOLS

^{o}C	-	Degree Celcius
cm	-	Centimeter
g	-	Gram
h	-	hour
kg	-	Kilogram
$kgha^{-1}$	-	Kilogram/hectare
m	-	Meter
mg	-	Milligram
ml	-	Milliliter
mM	-	Millimolar
min	-	Minute
mm	-	Millimeter
ng	-	Nanogram
%RH	-	% Relative Humidity
rpm	-	Rotation per minute
tha^{-1}	-	Tonne/hectare
µl	-	Microliter
µM	-	Micromolar
µg	-	Microgram

CHAPTER 1

INTRODUCTION

Pea (*Pisum sativum* L.) is a leguminous plant that belong to the family Papilionaceae and one of the most important food legume with high nutritive value especially in protein and carbohydrate (Gebhardt and Thomas, 2002). It is suitable for use as vegetable such as green peas (fresh, frozen, preserved) and mature peas (cooked for soup, broth), also as forage crop, for hay, pasturage or silage (Kay, 1979). As a legume crop rotation with peas could improve soil fertility subsequently yield of the succeeding crop. Pea is believed to have originated in southwestern Asia, possibly northwestern India, Pakistan or adjacent areas of former United of Soviet Socialist Republics and Afghanistan and thereafter spread to the temperate zones of Europe (Makasheva, 1983; Kay, 1979). The spread to central of Europe was reportedly happened around 4000 BC and by 2000 BC it had spread throughout Europe and into India (Sauer, 1993). Peas are predominantly grown in Europe, China, India and North America. The first field grown pea arose from a wild type *P. elatius* and *P. fulvum* which is found from the Mediterranean basin and Near East (Abbo *et al*, 2007; Zohary and Hopf, 1993). However, based on genetic diversity four centre of origins namely Central Asia, the Near East, Abyssinia and the Mediterranean have been recognized (Gritton, 1980). Peas originally were grown to full size and stored dry. However, in modern times they are picked green before maturity and eaten fresh. Peas were initially composed of two species *Pisum sativum* L. and *Pisum fulvum* Sibth. & Sm., which are represented by two self-pollinating diploid species (2n = 2x = 14) (Davies, 1995). *P. sativum* has been further divided to include several subspecies, *P.s.*ssp.*sativum, P.s.*ssp *elatius, P.s.ssp humile, P.s.*ssp *arvense* and *P.s.*ssp *hortense*. *P.s.*ssp *elatius* and *P.s.*ssp *humile* are the progenitors of pea *P.s.*ssp *sativum*. *P.s.*ssp *arvense*, includes the field pea and the 'Austrian' winter pea, both possessing coloured flowers and variously pigmented seeds (McPhee, 2003).

Like any other crops peas are subject to a number of diseases, which because of their serious effect on yield and market value are a major factor of crop production. One of the common diseases is the *Ascochyta* disease complex causing leaf, pod, stem and foot rots (Deshpande and Adsule, 1988). *Mycosphaerella pinodes* (conidial stage, *Ascochyta pinodes*) causes *Mycosphaerella* blight that produces numerous, tiny, irregular shaped purplish-black spots on all parts of the plant; *Ascochyta pisi* causes Ascochyta leaf and pod spot that produces large, sunken, brownish lesions on the leaves and pods, sometimes penetrating the pod-wall and causing brown stains on the peas whereas *Phoma medicaginis* var. *pinodella* (=*Ascochyta pinodella*) caused foot rots. *Ascochyta pinodella* causes purplish-black lesions on the stem. All the organisms of the *Ascochyta* complex are seed-borne and can survive in the soil from season to season (Tivoli and Banniza, 2007). Another disease of peas is the root rots caused by *Fusarium solani*, *Phythium ultimum* and *Rhizoctonia solani* (Grunwald *et al.*, 2004). All are more severe during abnormally wet seasons and on poorly drained soil. Peas grown on impoverished soils are also more subjected to infection. A common but serious disease of peas is the powdery mildew caused by *Erysiphe pisi* (also referred to as *E. polygoni*) which is easily differentiated from the other diseases by the visual formation of a white dust or powder on the leaves, stems and pods (Yarwood, 1978). Other than the leaves, the stems and pods may be infected causing death of the vine, withering of foliage and occasional plant death (Dixon, 1978). In pod infection, the fungus penetrated to the seeds causing it to become grey-brown while the severely infected plants have reduced yields or shortened production times (Rathi and Tripathi, 1994). The powdery mildews not only common in many host plants but caused serious diseases regardless of the climatic areas be it cool or warm, humid, or warm and dry. It only takes a long warm and dry daytime followed by cool night to develop enough dew formation which provide the moisture needed by the disease to multiply. Thus it is difficult to control the disease as their spores can be released, germinate and cause infection even when there is no film of water on the plant surfaces as long as the relative humidity in the air is fairly high (Dixon, 1978). Once infection has begun, the mycelium continues to spread on the plant surface regardless of the moisture conditions in the atmosphere. Chemical usage was the most preferred method of

controlling the powdery mildews as it is practical and easy to use. In pea crops fungicides have been used extensively to minimise damage by the powdery mildews. Sulphur was the most highly used pesticide to control powdery mildews. Other fungicides include dinitrophenols, quinomethionate, drazoxolon, difalimfos, benzimidazoles, triflorine (McGrath, 2007; Janousek *et al.*, 2006).

In Malaysia, particularly Sabah planting of peas has been carried out since the early eighties in the highland of Kundasang with an annual cultivated area of about 40 ha. (Department of Agriculture Sabah, 2004). Local production was inadequate because of diseases particularly powdery mildew. Most of the varieties planted were susceptible to the disease. Powdery mildew is usually controlled by applying fungicides. However, the non-judicious use of fungicides has led to excessive pesticide residues in vegetables. Fungicides are normally applied in the early stages of growth but frequency of spraying increases during the fruiting stage. Samplings for fungicide residues in Kundasang between 1993 and 1997 revealed that sweet pea was tainted with ethylene *bis*-dithio-carbamate (EBDC) above the maximum residual limit (MRL) (Department of Agriculture Sabah, 1996 and 1997). Brunei and Sarawak subsequently banned the import of sweet peas from Sabah (Jipanin *et al.*, 2000 and 2001). The export of sweet peas fell from 42.26 t in 1995 (Department of Agriculture Sabah, 1998) to 13.95 t in 2002 (Department of Agriculture Sabah, 2004). Powdery mildew is a menace in pea cultivation in Kundasang. Attempts to control the disease with fungicides have not been successful. The best option now is to plant tolerant varieties, which is safe, cost effective and practical.

Thus this research was carried out to select pea variety resistant to powdery mildew under Sabah conditions. Resistance is expressed as the ability to survive when expose to high level of inocula in an environment favourable for the disease. The research was carried with the following objectives;

i. To screen for pea varieties resistant to powdery mildew infection under field conditions.

ii. To carry out field assessment of yield and plant characters the pea varieties, and

iii. To detect potential gene(s) of resistance

Hypotheses of the research were:

i. Pea varieties resistant to powdery mildew can be identified based on field screening and RAPD-PCR, and

ii. Plant character and yield of the pea varieties can be determined based on field assessment.

CHAPTER 2

LITERATURE REVIEW

2.1 Pea (*Pisum sativum* L.)

2.1.1 Historical Background

Pea, a leguminous plant is among the first crop (which includes wheat, barley, lentils, chickpea and flax) that has been domesticated and consume by people since 12000 years ago (Schmidl *et al.*, 2005). Makasheva (1983) and Kay (1979) suggested that pea could have originated in southwestern Asia, possibly northwestern India, Pakistan or adjacent areas of former USSR and Afghanistan and thereafter spread to the temperate zones of Europe. The spread to central of Europe was reportedly to happen around 4000 BC and by 2000 BC it had spread throughout Europe and into India (Sauer, 1993). Non-pigmented peas were soon grown as vegetable in United Kingdom in the middle Ages (Davies *et al.*, 1985). Peas were introduced into the America soon after Columbus, while to China in the first century (Makasheva, 1983). However, based on genetic diversity four centres of origins namely Central Asia, the Near East, Abyssinia and the Mediterranean have been recognized (Gritton, 1980). It was believed that peas originated from a wild type *P. elatius* and *P. fulvum* which was found from the Mediterranean basin and Near East (Abbo *et al*, 2007; Zohary and Hopf, 1993). These wild peas have tough pods when immature which split open upon maturity, with small black seeds, only 3-5 mm in diameter. The initial stage of domestication was apparently to reduce the amount of pod dropping and shattering (Ladizinsky, 1979) out of which arose the first field pea then the white-flowered garden pea. Garden peas are divided into green peas (round smooth seeds) and marrowfats (seeds with wrinkled skins). Marrowfats with high amylose content are grown for starch production. Peas were initially composed of two species *Pisum sativum* L. and *Pisum fulvum* Sibth. & Sm., which are represented by two self-pollinating diploid species (2n = 2x = 14) (Davies, 1995). *P. sativum* has been further divided to

include several subspecies, *P.s.*ssp.*sativum, P.s.*ssp *elatius, P.s.ssp humile, P.s.*ssp *arvense* and *P.s.*ssp *hortense*. *P.s.*ssp *elatius* and *P.s.*ssp *humile* are the progenitors of pea *P.s.*ssp *sativum*. *P.s.*ssp *arvense*, includes the field pea and the 'Austrian' winter pea, both possessing coloured flowers and variously pigmented seeds (McPhee, 2003).

2.1.2 World Pea Production

Pea is among the four important cultivated legumes next to soybean, groundnut and beans. Canada, France and the Russian Federation produced most of the world pea production which totaled around 21 million tonnes (FAO, 2004). Other important production areas include China, India, United States of America (USA), Ukraine, Germany, Australia, United Kingdom, Ethiopia, Spain, Austria, Belarus, Sweden, Czech Republic, Denmark, Pakistan, Peru and Romania (Smith and Jimmerson, 2005). The peas are produced in the form of dry pea (58%) and green pea (42%). The breakdown of countries that produced these peas is as shown in Table 2.1.

2.1.3 Varieties

All edible peas are classified under the *Pisum sativum*. Kay (1979) described the field pea, as *P. sativum* var *arvense* and the garden pea as *P. sativum* var. *sativum*. The field pea which has reddish-purple flowers and small pods and seeds is frequently grown as a sprawling plant on a field-scale for its dried seeds. The garden pea, white in flowers has larger pods and seeds, and is further subdivided into cv. *macrocarpon*, the edible-podded or sugar peas, which lack the parchment-like endocarp, and cv. *humile*, the ordinary garden pea. Deshpande and Adsule(1988) however categorized pea under the var. humile and macrocarpon. They further described the commonly known peas worldwide as (i) Baby garden pea; *P. sativum* var. humile, an early dwarf pea (ii) Dry pea; refers to pea which pods and seeds are left to full maturity prior to harvesting (iii) Edible podded pea; *P. sativum* var. macrocarpon, which pods are consumed as food and picked just as the seeds commence to form; also popularly known as oriental or Chinese pea, French pea, snow pea, sugar pea, sugar snap pea and Turkey pea (iv) English pea; common green peas, shelling peas, or Austrian winter pea in honour of the

Austrian monk, Gregor Mendel, who carried out and established the principles of genetic heredity based on his work with garden pea cultivars (v) Field pea; *P. arvense*, used mainly as cattle fodder in the developed countries, ripe mature seeds are now commonly used as grain legume in human nutrition while the unripe young seeds can also be used as vegetable (vi) Green pea; pods are picked before the seeds are fully mature, as contrasted with dry pea, which is harvested after the seeds have fully matured. (viii) Petit pois pea; it is the French equivalent for pea and is also used to describe a variety of pea known as the French Canner pea or the Turkey pea (ix) Smooth-and-wrinkled-skinned pea; the seeds of several varieties of peas have perfectly smooth skins, as contrasted with the highly wrinkled skins of other varieties. The wrinkled-skinned varieties generally are regarded favourably for their flavor although the marrowfat variety with smooth skin is also quite popular. Recently, the "garden" and "field" peas are regarded as subspecies within *P. sativum*; garden peas as *P. sativum* ssp. hortense and field peas as *P.sativum* ssp. arvense (McPhee, 2003).

2.1.4 Growth Habit

Pea is a self-pollinated annual herbaceous plant 30 cm – 150 cm in height. The plant is bushy or climbing with weak, round and slender stem. The leaves are alternate, pinnate, ovate or elliptic in shape, 1.5 cm – 6.0 cm long with 1-3 pairs of leaflets that has terminal branched tendril (Oelke *et al*, 1990; Davies *et al.*, 1985; Duke, 1981; Gritton, 1980). The leaf could be conventional, semi-leafless and leafless (Davies *et al.*, 1985). Leaf size in most cases increases up to the first node bearing the first flower. Stipules are large, leaflike and up to 10 cm long. Pea inflorescence is a raceme arising from the axil of the leaf. The corolla is white, pink or purple; pods swollen or compressed, short-stalked, straight or curved, 4-15 cm long, 1.5-2.5 cm wide, 2-10 seeded, 2-valved, dehiscent on both sutures (Duke, 1981; Gritton, 1980). The node at which the first flower emerges is characteristic of a given variety; in temperate regions the number of nodes at which the first flower emerges is reported to vary from 4 in the earliest to about 25 in late maturing types under field conditions (Gritton, 1980). Flowers borne on the same peduncle produce pods that mature at different times, the youngest being at the tip. On a whole plant basis, flowering is sequential and upwards from node to

node. Seeds are globose or angled, smooth or wrinkled, exalbuminous, whitish, gray, green or brownish; 100 seeds can weigh from 10-36 grams; germination cryptocotylar (Duke, 1981)

Table 2.1 Pea Production (Tonnes) in 2004

Country	Dry Pea	Green Pea	Total
Canada	3338200	67114	3405314
France	1671477	441400	2112877
Russia Federation	1242500	45800	1288300
China	1160000	2108663	3268663
India	900000	3200000	4100000
Ukraine	636300	15000	651300
USA	558740	901675	1460415
Germany	464000	66000	530000
Australia	321000	29471	350471
United Kingdom	242000	313300	555300
Spain	195200	75200	270400
Ethiopia	170365	1000	171365
Austria	122100	3864	125964
Belarus	110000	0	110000
Sweden	88000	44500	132500
Denmark	83300	36000	119300
Czech Republic	71962	5600	77562
Romania	58036	17411	75447
Pakistan	57500	76444	133944
Peru	38044	65465	103509

Data sourced from FAOSTAT, 2004

2.1.5 Ecology

Peas are produced mainly in the temperate zones as cool-season crop in the subtropics and at higher altitudes in the tropics between the Tropics of Cancer and 50°N (Davies, 1995). This is because peas require a cool and relatively humid climate with temperatures ranging from 7 to 30°C (Davies, 1995; Duke, 1981). As a winter annual, pea tolerates frost to -2°C in the seedling stage, although top growth may be affected at -6°C meanwhile winter hardy peas can withstand -10°C, and with snow cover protection, tolerance can be increased to -40°C (Slinkard *et al.*, 1994). The optimum temperature levels for the vegetative and reproductive periods of peas were reported to be 21° and 16°C and 16° and 10°C (day and night), respectively (Slinkard *et al.*, 1994). However temperatures above 27°C may shorten the growing period and adversely affect pollination. A hot spell is more damaging to peas than light frost. Peas can be grown successfully during midsummer and early fall in those areas having relatively low temperatures and a good rainfall, or where irrigation is practiced. For very early crops, a sandy loam is preferred; for large yields where earliness is not a factor, a well-drained clay loam or silt is preferred (Duke, 1981).

2.1.6 Nutritive Value

Peas as well as other legumes are good source of protein, carbohydrate, vitamins and minerals to human diets as well as for animal feeding. One cup (187 g) of raw peas, split or mature seeds contain 672.0 calories, 48.4 g Protein, 2.3 g Total Fats, 119.0 g Carbohydrate, 50.2 g Total Fiber, 15.8 g sugars, 22.2 g water, 5.2 g ash, 108.0 mg Calcium, 8.7 mg Iron, 227.0 mg Magnesium, 721.0 mg Phosphorus, 1933.0 mg Potassium, 29.5 mg Sodium, 5.9 mg Zinc, 2.7 mg Manganese, 1.7 mg Copper, 3.2 mg Selenium, 3.5 mg Vitamin C, 294 IU Vitamin A, 0.2 mg Vitamin E, 28.6 mcg Vitamin K, 1.4 mg Thiamin, 0.4 mg Riboflavin, 5.7 mg Niacin, 0.3 mg Vitamin B6, 540 mcg Dietary Folate Equivalent (DFE), 3.5 g Panthothenic acid, 188.0 mg Choline, 0.3 g saturated fats, 0.5 g monosaturated fats, and 1.0 polysaturated fats (Gebhardt and Thomas, 2002). However this composition changed when the peas are processed; soaking and cooking increased protein content, Calcium, Manganese and Phosphorus whereas ash, Iron, Potassium, Magnesium, Zink, sucrose and oligosaccharides are reduced meanwhile dehulling

increased crude protein, starch, Potassium and Phosphorus but reduced Calcium, Iron, Magnesium and Manganese (Wang *et al.*, 2008). In green peas the fraction of amino acid in the protein by percentage composition is Arginine 7.2%, Histidine 2.4%, Isolecine 4.5%, Leucine 7.8%, Lysine 7.0%, Methionine 0.8%, Phenylalanine 5.2%, Threonine 3.5%, Tryptophan 0.7%, Valine 5.0%, Alanine 5.2%, Aspartic acid 11.0%, Cystine 1.8%, Glutamic acid 17.5%, Glycine 4.5%, Proline 5.1% , Serine 5.1% and Tryrosine 3.7% (Iqbal *et al.*, 2006). All of this essential amino acid except S-containing and Tryptophan are reportedly exceeded the amino acid standard for human set by FAO/WHO in 1985 thus making pea as a cheap, but nutritious plant protein sources. Meanwhile pea hay for livestock feeding (at 88.6% DM) contains (zero moisture basis): 10.7-21.6% crude protein, 1.5-3.7% fat, 16.8-36.1% crude fiber, 6.0-9.3% ash and 41.9-50.6% N-free extract (Muehlbauer and Tullu, 1997).

2.1.7 Diseases

Peas are subject to a number of diseases (CABI, 2002) which caused serious effect on the yield and market value of the crop. The pathogenic organisms causing the diseases are bacteria, virus, nematode and fungi. The attack caused the plant to produce symptoms such as seedling decay, root rots, wilts, leaf spots, stunted growth and death of plants. Plant pathogenic nematodes are microscopic unsegmented round-worms, where more than 20 different genera are known to attack pea roots. Economically important nematodes affecting pea include the pea cyst nematode *Heterodera goettingiana*, the root-knot nematode *Meloidogyne* species, particularly *M. incognita* (Kofoid & White) Chitwood and the the most common root-lesion nematodes *Pratylenchus penetrans* (Cobb) Filip. & Schuur-Stek (Riggs and Niblack 1993; Johnson and Fassuliotis 1984). Virus diseases such as *Pea Early Browning Virus* (PEBV), *Pea Enation Mosaic Virus* (PEMV), *Pea Mosaic Virus* (PMV), *Pea Top Yellows* (PTY), *Pea seed-borne Mosaic Virus* (PSbMV) and *Pea Streak Virus* (PSV) could cause serious crop losses particularly where there are large aphid populations (McPhee, 2003; Davies *et al.*, 1985; Kay, 1979). There are two economically important bacterial diseases of pea, the bacterial blight and brown spot which caused serious losses in pea crop (Mansfield *et al.*, 1997; Roberts *et al.*, 1995). Bacterial blight, caused by *Pseudomonas pisi*, is a seed-borne

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