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Identification of Resistance Gene Analogs (RGAs) linked to Powdery Mildew Resistance in Peas

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Abstract— ***Powdery Mildew caused by Erysiphe pisi, is one of the most common fungal disease in garden peas worldwide. As studied, few pea plants are resistance for powdery mildew and few pea plants are susceptible for powdery mildew disease. All the resistance variety plants contains resistance genes (R-genes), which are the genes in plant genomes that conveys plant disease resistance against pathogens by producing R-proteins. Most of resistance genes encode highly conserved nucleotide binding site and leucine rich repeat structure (NBS-LRR) which helps to isolate specific gene linked to powdery mildew. Degenerative primers based on NBS conserved motif of NBS-LRR resistance protein can be used as molecular RGA markers in PCR amplification for cloning of resistance genes. Later on the screening of genotype with degenerative primers can be done for both resistance genotype and susceptible genotype in order to identifying resistance gene in resistance genotype. Based on resistance gene sequence RGAs were identified, these RGAs would help in identifying marker linked to Powdery mildew resistance and improvise in further Peas Marker Assisted Selection.***

Keywords— *R-genes, degenerative primers, powdery mildew disease resistance, RGAs identification.*

I. INTRODUCTION

The peas (*pisum sativum*) are small spherical seed with life cycle of one year [1]. These are usually grown in cool climate in many parts of the world. It is an nutritionally important crop which contain 81kcal of energy with high protein content with almost zero percent of fat per 100g of raw green pea [2].

Scientific Classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Fabales
Family:	Fabaceae
Subfamily:	Faboideae
Tribe:	Vicieae
Genus:	*Pisum*
Species:	*P. sativum*

Table 1: Scientific classification of peas

Peas, green, raw	
Nutritional value per 100 g (3.5 oz)	
Energy	339 kJ (81 kcal)
Carbohydrates	14.45 g
Sugars	5.67 g
Dietary fiber	5.1 g
Fat	0.4 g
Protein	5.42 g

Table 2: Nutritional value of peas

Powdery mildew caused by *erysiphe pisi* is the common disease in many types of plants including peas, beans, beets, carrot, melons and tomatoes [3]. Pea plants are infected by powdery mildew disease mostly occurs sporadically when warm humid conditions favour its growth late in the season. The symptoms of this infection are white powdery formation throughout the plant and severely infected foliage is blue-white in colour, tissue below these infected areas may turn purple. The *erysiphe pisi* is the fungi that reproduce both sexually and asexually through the production of spores [4]. The spores may spread long distance by air or water, or they may be soil borne. Many soil inhabiting fungi are capable of living saprotrophically, carrying out the part of their life cycle in the soil. There are known as facultative saprotrops. Powdery mildew reduces plant biomass, number of pods, number of seeds, plant height and number of nodes. Severe infection may cause 25–50% yield reduction [5]. The disease is of economic importance due to yield reduction and reduced seed quality under severe conditions where 33 to 69% of pea foliage was found to be infected with powdery mildew.

In India powdery mildew disease appears in epidemic form almost every year when plants are in podding stage [6]. During transitional period between the end of winter and the onset of spring season, the weather becomes favourable for the epidemic emergence of powdery mildew disease on pea crops. Different sulphur based synthetic fungicides are used to reduce the yield losses caused by powdery mildew disease [7]. But the excessive use of fungicide has developed resistance in *E.pisi* against high doses of chemical sprays used during previous years. By identifying resistance gene analogs for such disease can

- avoid harmful chemical used to reduce powdery mildew disease in plant
- make all garden pea plant resistance to powdery mildew infection
- reduce the economic loss of pea yield

II. PLANT DISEASE RESISTANCE GENE

Resistance genes (R-gene) are the genes in plant genome that functions for plant disease resistance against pathogen. This works by production of protein called R-protein. R-genes mainly consists of a nucleotide binding site (NBS) and leucine rich repeats (LRR), called as NBS-LRR Domain [8]. When a single isolate of a plant pathogen species is inoculated onto a collection of host genotypes, it is common to find that some are resistant and some are susceptible to that pathogen. Thus the pathogen isolate distinguishes host plant variation that is manifested as clear differences in disease reaction. The existence of polymorphism for resistance/susceptibility provides the opportunity to carry out simultaneous genetic analysis of inheritance of resistance and susceptibility in host species and inheritance of virulence and avirulence in the pathogen species [9].

- Single gene difference between resistance and susceptible host genotype determines the resistance to pathogen.
- Resistance varieties are most commonly dominant.
- Multiple resistance genes can occur in a single species. Each resistance gene frequently encodes resistance to some but not all isolates of a pathogen species [10]. The resistance gene's ability or inability to determine resistance to different pathogen isolates can distinguish one resistance gene from another. This difference between resistance genes is referred to as resistance gene specificity.

III. DEGENERATIVE PRIMERS

According to researchers, degenerative primers can be employed because these primers are useful in pulling out one part of a gene sequence when you know the gene sequence of the related organisms [11]. The more distant those related organisms, the more difficult it can be to design primers. One solution is to gather sequence from large range of organism, translate them to amino acid sequence and align them. Based on these alignments, you can identify regions of the sequence which are highly conserved at the amino acid level. These conserved regions become possible locations for degenerate primers. Degenerate primers are designed to match an amino acid sequence.

IV. TECHNIQUE OF SCREENING

Screening for powdery mildew resistance can be done under natural field conditions or in the greenhouse where epiphytotic conditions exist [12]. Susceptible varieties pea plants are not attacked every year under field conditions due to environmental influences on pathogen development; therefore, tests are usually carried out during the winter months where environmental conditions conducive to pathogen development can be created at optimum air temperature 20ºC. It is important that the pathogen should be identified to verify resistance level of pea genotypes. This may be accomplished using differentiating molecular markers. Severity of disease may be measured visually on individual plants using a 0 to 9 scale based on percent of foliage covered with hyphae, where 0 = no infection, 1 = <1%, 2 = 1–5%, 3 = 5–10%, 4 = 10–20%, 5 = 20–40%, 6 = 40–60%, 7 = 60–80%, 8 = 80–90% and 9 = >90% diseased area. Disease scoring is carried out when a susceptible check is heavily infected. Scores of 0–4 are considered resistant, and 5–9 susceptible [13].

The consensuses among researchers are that powdery mildew resistance is controlled qualitatively with the recessive allele conferring resistance. According to disease-scoring pattern, it is suggested as to record susceptible or resistant with some degree of an intermediate reaction resulting from the interaction of qualitatively inherited genes for powdery mildew resistance. Disease reaction characterized by varying intensity of symptoms has been observed indicating the presence of more than one gene/allele for powdery mildew [14]. Therefore, investigating the genetics of powdery mildew resistance using parental lines from diverse origins and known genetic backgrounds is suggested. The *er1* has been reported on linkage group VI, whereas the er2 gene has been localized on pea LGIII, a position different from that of *er1*, and the research has resolved the long standing controversy in the literature regarding the existence and genomic location of *er2* gene.

V. APPROACH TO IDENTIFYING RGA

Genomic DNA from the leaf samples of peas is isolated [15]. Degenerative primers were used to amplify the conserved region between p-loop and GLPL of plant R-genes. PCR amplification reaction conducted in a total volume of 10µl containing 40ng of 2µl templet DNA, 1µl of 10X taq buffer, 0.5µl of 25mM magnesium chloride, 1µl of 1mM dNTPs, 2.5µl of 10pmol both forward and reverse primers, 0.3µl of taq polymerase and 1.2µl of milli-Q water. The PCR

reaction was as follows Initial Denaturation at 94ºC for 2min, Denaturation for 91ºC 10sec, Annealing at 60ºC for 45sec, Extension at 72ºC for 1min 30sec, number of cycles is 35, final extension at 72ºC for 5min and final hold at 4ºC. The PCR products were separated by electrophoresis on 2% (wt/vol) Agarose Gel and purified using AXYGEN biosciences DNA gel extraction kit. Further the purified plasmid DNA were sequenced using the BigDye Terminator cycle sequencing kit with universal M13F and M13R primers. Sequences of the amplified products were determined on the ABI Prism 310 Genetic Analyzer. Sequences were edited using GeneDoc software, to remove the primer and vector sequences. Sequence homologies were compared with the GeneBank database by searching the National Centre for Biotechnology Information (NCBI) GenBank using BLAST. The percentage of amino acid identity between the predicted protein sequences were determined using DNAMAN 8.0 computer software. Open reading frames were identified using the ORF finder module of the NCBI server.

The presence of P-loop, RNBS-A non TIR (Toll / Interleukin-1 Receptor), Kinase-2, RNBS-B, GLPL and RNBS-C region in the protein sequence confirms that it is a resistance gene analogs. P-loop (phosphate-binding loop) is a motif in proteins. The motif has the pattern GXXXXGK(T/S). It is an ATP or GTP binding motif found in many nucleotide-binding proteins [16]. The lysine (K) residue in the P-loop is crucial for nucleotide-binding. The NBS-LRR R-genes are divided as TIR or non-TIR subfamily depending on the last residue, D (Aspartate) or W (Tryptophan) of the conserved kinase 2 motif respectively within the NBS domain. Based on this, all the RGAs examined in this work has a tryptophan (W) residue at the end of the kinase-2 motif classifying them as non-TIR NBS-LRR class RGAs [17]. Also, the presence of the specific conserve sequence AWxCVS in the RNBS-A motif further confirms their non-TIR classification. Cannon et al. noted that the absence of the TIR domain in R genes could be predicted by the presence of the motif RNBS-A non-TIR (AWxCVS) near to the P-loop and RNBS-C non-TIR (AWxLR) near the GLPL. The absence of TIR RGAs in peas supports the view that monocotyledons are characterized by the presence of only non-TIR NBS-LRR class R-genes. TIR-NBS-LRRs has not been found in major monocots such as rice, wheat, maize and other cereals. Tarr and Alexander reported that although TIR-NBS-LRR sequences were present in early land plants, they have been reduced significantly in monocots and the TIR sequences from the *Triticum ethinopyrum* line remain the only reported monocot TIR-NBS-LRR sequences.

VI. GENERAL DISCUSSION

The most effective means of controlling such disease is to grow resistance variety. Selection of plants in a segregating population that contains appropriate combination of genes is the critical component of many crop improvement programs [18]. All the plant breeders works with large populations, MAS may help in increasing the efficiency and effectiveness of selection in plant breeding as compared to the conventional methods. Once markers that are closely linked to genes of interest are identified, breeders will use specific DNA markers as a diagnostic tool to identify plants carrying resistance genes.

Marker assisted selection saves time and helps eliminating undesirable phenotypic evaluation that could be strongly influenced by environmental effects. Such selections can be made at the seeding stage even for traits with low inheritability. The cost of using MAS compared to conventional methods of plant breeding varies considerably between studies depending on the crop and nature of trait/s of interest.

Powdery mildew cause serious loss in quality and quantity of both fresh pods and dry seed. Although powdery mildew can be controlled with fungicides, genetic resistance is preferred for economic reasons and current ease of selection. Inheritance of resistance was initially reported as a single recessive gene, *er1*. Resistance has been reported to be controlled by one too many genes. However, scientists are reaching to the consensus the either one or more of three genes (*er1*, *er2*, *Er3*) may present in resistance line where *er1* has been characterised and widely used in developing powdery mildew resistance pea cultivars. Whereas, the genes er2 has somewhat understood and Er3 have not yet fully characterized and *Er3* in pea breeding for better resistance to powdery mildew, there is a need to characterize newly identified gene as this gene that has been reported form wild pea [19].

VII. CONCLUSION

The *Erysiphe pisi* is the most important biotrophic fungus infecting peas and responsible for losses in crops. Two recessive genes (*er1* and *er2*) have been reported as resistance to *E.pisi* in cultivated peas, whereas, a dominant gene (*Er3*) was identified recently in a wild relative for pea. All the three genes noticed that they are independent to each other. The most effective and economical strategy to control such pathogens is the use of resistant cultivars considering the nutritional and economic importance of *pisum sativum*, identification of new sources of disease resistance has been one of the top priority in peas improvement. According to researchers, degenerative primers were used to amplify and characterize the conserved domains of NBS-LRR class R-genes from other plant species [20].

RGA may successfully isolated. Multiple sequence alignment of the deduced amino acids with that of known RGAs and R-genes other plants revealed AsRGA sequences with conserved subdomains of P-loop, kinase 2, Kinase 3a and GLPL. This suggests that their possible function as disease resistance protein genes [21].

In conclusion, this study might result in identifying RGA sequences from powdery mildew resistant pea genotypes resistance variety that can form the basis towards powdery mildew resistance. The identified RGAs can act as a valuable

resource towards differentiating resistant variety of peas over susceptible variety and development of RGA based molecular markers for genetic mapping in peas [22].

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REFERENCES

[1] Rogers and Speed, Man and the Biological World Read Books, ISBN; 169–170, 2007.
[2] Jegtvig and Shereen, "Peas", Nutrition, About.com, 2007.
[3] Lumbsch TH and Huhndorf SM, "Outline of Ascomycota – 2007". Myconet (The Field Museum, Department of Botany, Chicago, USA); vol 13:1–58, 2007.
[4] Nicole Davis, "Genome of Irish potato famine pathogen decoded". Haas et al., Broad Institute of MIT and Harvard, 2009, Retrieved 2012.
[5] Munjal RL, Chenulu VV and Hora TS, Assessment of losses due to powdery mildew (Erysiphe polygoni) on pea, Indian Phytopathol, vol 19; 260–267, 1963.
[6] Maurya, O. P., G. N. Singh and R. K. S. Kushwaha, An economic analysis of production and marketing of potato in district Varanasi (UP), In: Encyclopaedia of Agricultural Marketing, Vol 8: 229-38, 2001.
[7] Khunt, K. A. and D. B. Desai, Economic feasibility and marketing of perennial vegetables in South Gujarat. Financing Agriculture, vol 28: 9-14, 1996.
[8] Knepper, Caleb and Day Brad, "From Perception to Activation: The Molecular-Genetic and Biochemical Landscape of Disease Resistance Signaling in Plants", The Arabidopsis Book; 1–17, 2010.
[9] Ellis J and Jones D, Structure and function of proteins controlling strain-specific pathogen resistance in plants, Curr Opin Plant Biol, vol 1: 288–293, 1988.
[10] Harlands SC, Inheritance of immunity to powdery mildew in Peruvian forms of *Pisum sativum*, Heredity, vol 2: 263–269, 1948.
[11] S. Patricia, Stock; John, Vanderberg; Itamar, Glazer; Noel and Boemare, "1.6.2. Primers development and virus identification strategies". Insect Pathogens: Molecular Approaches and Techniques, CAB International, 2009.
[12] Nisar M, Ghafoor A, Khan MR and Qureshi AS, Screening of *Pisum sativum* (L.) Germplasm against *Erysiphe pisi* Syd. in relation with vegetative traits and SDS-PAGE profile, Acta Biologica Cracoviensia Series Botanica, vol 48: 33–37, 2011.
[13] Moon J, "The Ubiquitin-Proteasome Pathway and Plant Development", The Plant Cell Online, vol 16 (12): 3181–3195, 2004.
[14] Timmerman GM, Frew TJ, Weeden NF, Miller AL and Goulden DS, Linkage analysis of er-1, a recessive *Pisum sativum* gene for resistance to powdery mildew fungus (*Erysiphe pisi* D. C.), Theor Appl Genet, vol 88: 1050-1055, 1994.
[15] Doyle JJ, Doyle JL, Isolation of plant DNA from fresh tissue. Focus 12: 13-15, 1990.
[16] Bent A.F, Plant disease resistance genes, Function meets structure, Plant Cell 8: 1757–1771, 1996.
[17] Juansheng Ren, Yuchao Yu, Fangyuan Gao, Lihua Zeng, Xianjun Lu, Xianting Wu, Wengui Yan, Guangjun Ren, Application of resistance gene analog markers to analyses of genetic structure and diversity in rice, 56(7): 377-387, 2013.
[18] Abdul Ghafoor and Kevin McPhee, Marker assisted selection (MAS) for developing powdery mildew resistance pea cultivars, springer; 2011.
[19] Mohler V.A., Klahr G., Wenzel G. Schwarz, A resistance gene analog useful for targeting disease resistance genes against different pathogens on group 1S chromosomes of barley, wheat and rye. Theor Appl Genet. 105:364–368, 2012.
[20] Alexopoulus, C.J., C.W. Mims, and M. Blackwell, Introductory Mycology, NY: John Wiley and Sons. C.J., C.W. Mims, and M. Blackwell, Introductory Mycology, 1996.
[21] Cannon SB, Zhu H, Baumgarten AM, Spangler R, May G, Cook DR, et al. Diversity, distribution and ancient taxonomic relationships within the TIR and non-TIR NBSeLRR resistance gene subfamilies. J Mol Evol; 54: 548-62, 2002.
[22] Tarr DEK, Alexander HM. TIR-NBS genes are rare in monocots: evidence from diverse monocot orders. BMC Res Notes; 2:197, 2009

