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INSILICO IDENTIFICATION OF ALTERNATE RESISTANCE GENE AGAINST YELLOW RUST IN WHEAT

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Abstract

Yellow rust, also known as stripe rust, is one of the 3 wheat rust diseases principally found in wheat grown in cooler environments. Yellow rust, grounds by *Puccinia striiformis Westend. f.sp. tritici*, which is an important foliar disease of wheat and development resistant cultivars are the most economical method of control. Of the three rust diseases of wheat, stripe (yellow) rust (YR) is the most damaging to grain yields in cool, moist environments. Chemical control of rusts is expensive and hazardous to the environment. Yr5 and Yr10 are resistance genes among many other resistance genes while PsMAPK1 is fungus protein causing yellow rust in wheat. The protein sequences of Yr5 and Yr10 was retrieved from NCBI and PsMAPK1 protein sequence was retrieved from UNIPROT. By I-TASSER 3D models of Yr5, Yr10 and PsMAPK1 were generated. The models were evaluated by energy minimization technique. By finding the physiochemical properties of the templates and by docking technique alternate resistance gene had been found from the Yr5 and Yr10 templates. The physiochemical properties of Yr5 and Yr10 genes were compared with physiochemical properties of templates to find the suitable alternate against this disease in wheat. Docking results shows the interactions among receptor and ligand. These results show that template 2OF7 has closer results of physiochemical properties to Yr5 and template 3IZA has closer results of physiochemical properties to Yr10.

Introduction

Stripe rust is an important fungal disease of wheat in many countries (Roelfs *et al*., 1992). Genetic resistance is the most effective, economical and environment-friendly method of managing stripe rust. More than 30 genes that confer resistance to stripe rust in wheat have been cataloged (McIntosh *et al*., 1998, Rasheed *et al*., 2012). Yellow (stripe) rust is caused by *Puccinia striiformis Westend* it also infects barley, rye and other grasses. Yellow rust dispersed in all directions from this center, reaching Australia only in 1979. Till now it has come to know that the fungus *Puccinia striiformis Westend* does not attack oats, rice or maize. The one infecting barley is *Puccinia striiformis f.sp. tritici hordei* and the form infecting wheat is *P. striiformis f.sp. tritici* (Brien, 1980). Stripe (yellow) rust (YR) is the most damaging to grain yields in the cool, moist environments. The control of rusts through chemicals is expensive and hazardous to the environment (Johnson, 1988). Wheat genotypes is a type of interaction between the host and pathogen in spring and winter in adult-plant resistance (APR), in which regardless of seedling compatibility, the adult plant is partially resistant (Johnson, 1980; Milus, 1986; Singh, 1994; Bariana, 1995; Chen, 1995; Wagoire, 1998; Bariana, 2001; Zhang, 2001).

Approximately all of the genes for resistance have become fruitless to the 2 races from Yr1 to Yr10, and Yr15 (Wang, 1996). Different approaches like cultivar mixing, cultivar diversification and gene pyramiding may escort to increased resistance to yellow rust. The availability of a range of resistance genes has great impact on the effective use of such approaches (Chao, 1989; Kam-Morgan, 1989).

Using resistant varieties is considered as the favored way of controlling the disease. A number of genes are present that show resistance to yellow rust. In 1966 Macer described first time about Yr5 in *Triticum spelta album* (Macer, 1966). Yr10 confers race specific resistance to yellow rust and it is a dominant gene. It consists of two exons interrupted by an intron and it is 3630 bp long. mRNA transcript of Yr10 is 2475 bps long (Temel, 2008). The purpose of this study is to build the 3D models of the resistance genes Yr5, Yr10 and fungus protein to see their interactions. The aim of study is to find an alternate resistance gene by finding template having close resemblance with Yr5 and Yr10 genes and to find protein-protein interactions.

Material and Methods

Sequence retrieval: The protein sequences of Yr5 (UniProt Entry No.G3M5Q4), Yr10 (UniProt Entry No.Q9FR63) and PsMAPK1 proteins (UniProt Entry No. E1A882) was retrieved from the UniProt database (http://www.uniprot.org/). The first stride to determine structures of these genes is the retrieval of the sequences.

Tertiary structure prediction: 3D structures of Yr5, Yr10 and PsMAPK1 were not predicted. We used threading technique and for this purpose the I-TASSER web based server was used for predicting 3D models (http://zhanglab.ccmb.med.umich.edu/I-TASSER/).

Physiochemical properties: ProtParam was used to find the physiochemical properties. ProtParam is a tool which allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for a user entered sequence. The computed parameters include the molecular weight, theoretical pI (isoelectric point), amino acid composition, instability index, and grand average of hydropathicity (GRAVY). **(**http://web.expasy.org/protparam/). Physiochemical properties of Yr5 and Yr10 genes were compared with their templates to find the gene having close resemblance to its model gene.

Energy minimization: Energy minimization was performed by Chiron Rapid Energy Minimization Server (http://troll.med.unc.edu/chiron/documentation.php). This server reduces clash ratio of the model. Chiron minimizes the number of nonphysical atomic interactions (clashes) in the given protein structure.

Template finding: Protein-protein BLAST was performed to obtain the templates of Yr5 and Yr10 genes (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins). Structures of all the templates of Yr5 and Yr10 genes were retrieved from RSCB PDB database (http://www.pdb.org/pdb/home/home.do).

Docking: Protein-protein Docking was performed between templates and with the original structures predicted of both Yr5 and Yr10 with PsMAPK1 protein to find the interactions. Hex 4.5 protein docking tool was used to perform protein-protein docking.

Results and Discussion

The protein sequences of Yr5 (UniProt Entry No.G3M5Q4), Yr10 (UniProt Entry No.Q9FR63) and PsMAPK1 proteins (UniProt Entry No. E1A882) were retrieved from the UniProt database (http://www.uniprot.org/). These proteins Yr5 and Yr10 are involved in conferring resistance against yellow rust in wheat. The three dimensional structures of Yr5, Yr10 and PsMAPK1 were generated by I-TASSER web server. I-TASSER predicts 5 models for every input structure, out of which best structure on the basis of C-score, were selected. Yr5 models have C-score -0.08, -2.03, -2.28, -1.68 and -1.97. Model 1 with C-score -0.08 was selected. Similarly the model selected for Yr10 and PsMAPK1 have C-score -1.49 and -1.06. This tool shows the accuracy of the model, by providing TM score and RMSD. The value of TM for Yr5, Yr10 and PsMAPK1 are 0.70±0.12, 0.53±0.15 and 0.58±0.14. The RMSD value for Yr5, Yr10 and PsMAPK1 are 4.9±3.2Å, 12.1±4.4Å and 9.3±4.6Å (Figs. 1-3).

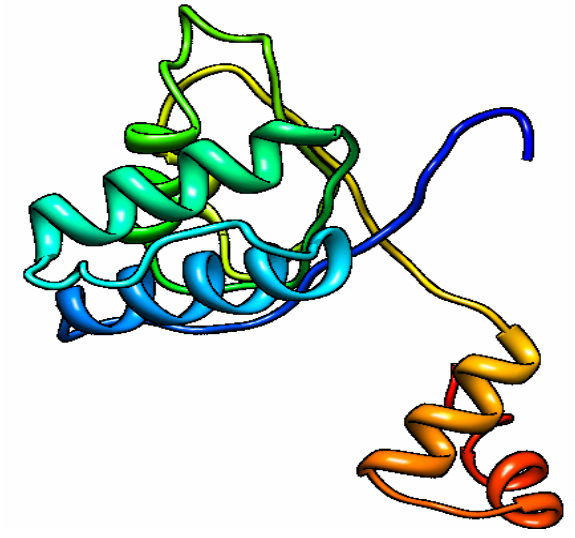

Fig. 2. 3D Model of Yr10 gene generated by I-TASSER web server.

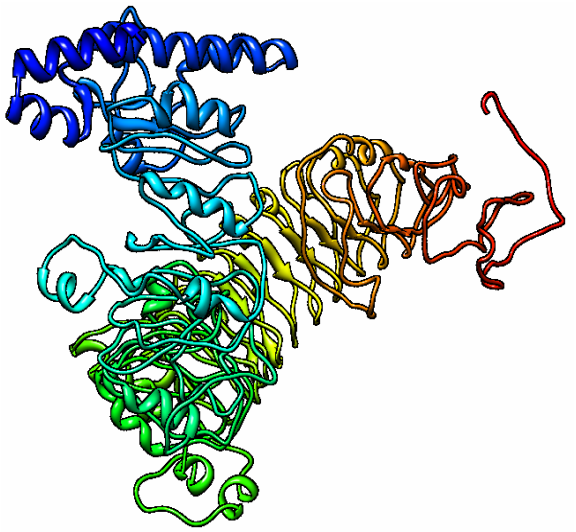

Fig. 1. 3D Model of Yr5 Gene generated by I-TASSER web server.

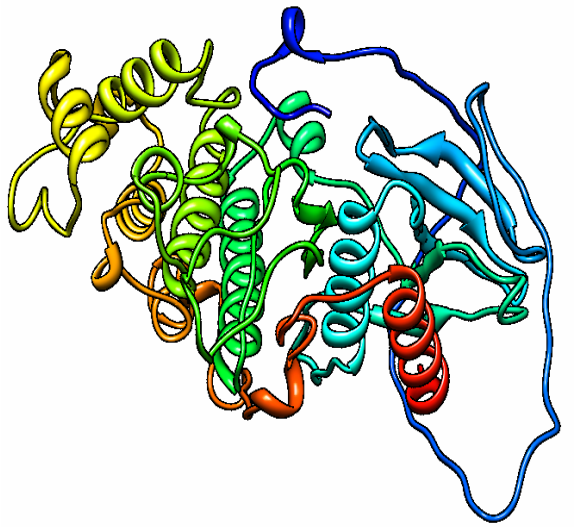

Fig. 3. 3D Model of PsMAPK1 Protein generated by I-TASSER web server.

C-score is a confidence score for estimating the quality of predicted models by I-TASSER. It is calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. C-score is typically in the range of [-5, 2], where a C-score of higher value signifies a model with a high confidence and vice-versa. TM-score and RMSD are known standards for measuring structural similarity between two structures which are usually used to measure the accuracy of structure modeling when the native structure is known. In case where the native structure is not known, it becomes necessary to predict the quality of the modeling prediction, i.e. what is the distance between the predicted model and the native structures? To answer this question, we tried predicted the TM-score and RMSD of the predicted models relative the native structures based on the C-score (http://zhanglab.ccmb.med.umich.edu/I-TASSER/output/S89282/cscore.txt).

ProtParam was used to find the physiochemical properties (http://web.expasy.org/protparam/). The Isoelectric point, pI, is the pH of an aqueous solution of an amino acid (or peptide) at which the molecules on average have no net charge. A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable. Molecular weight of the Yr5 gene is 17138.5 g/mol and theoretical pI (Isoelectric point) is 6.35. The instability index (II) is computed to be 27.47 and grand average of hydropathicity (GRAVY) value is -0.581. Molecular weight of the Yr10 gene is 93219.0 g/mol and theoretical pI (Isoelectric point) is 7.24. The instability index (II) is computed to be 39.97 and grand average of hydropathicity (GRAVY) value is -0.197. Physiochemical properties of template 2OF7 have close resemblance with Yr5 and 3IZA has close resemblance with Yr10 (Tables 1 & 2).

Table 1. Physiochemical properties of Yr5 and its templates.

Templates of Yr5	Isoelectric point (pI)	Molecular weight	Instability index (II)	Grand average of hydropathicity (GRAVY)
1Z6T	6.20	67762.0	49.47	-0.324
3IZA	6.14	143897.1	39.94	-0.303
3SFZ	5.98	141002.9	40.97	-0.221
3SHF	5.89	141681.6	40.86	-0.219
2ANN	9.23	19169.1	34.08	-0.187
2ANR	9.23	19169.1	34.08	-0.187
2QFC	6.47	35009.2	50.91	-0.479
2OF7	6.03	28762.6	35.37	-0.768
Yr5	6.35	17138.5	27.47	-0.581

Table 2. Physiochemical properties of Yr10 and its templates.

Templates of Yr10	Isoelectric point (pI)	Molecular weight	Instability index (II)	Grand average of hydropathicity (GRAVY)
3QFL	6.37	12998.3	29.06	-0.510
1Z6T	6.20	67762.0	49.47	-0.324
3IZA	6.14	143897.1	39.94	-0.303
3SFZ	5.98	141002.9	40.97	-0.221
3SHF	5.89	141681.6	40.86	-0.219
1XEZ	5.10	79576.1	36.28	-0.417
1HQC	5.99	35999.7	43.84	-0.126
1IXS	6.13	35367.0	43.54	-0.119
1IXR	6.30	34749.3	44.59	-0.124
1DGR	4.61	10260.5	41.22	-0.325
3KJE	6.03	27958.6	29.43	-0.068
2WFH	8.39	21431.6	24.18	-0.211
1AB5	4.87	13620.7	32.44	-0.032
1F48	5.83	64012.2	41.79	-0.105
3OLV	4.89	14125.4	30.14	0.046
Yr10	7.24	93219.0	39.97	-0.197

Energy minimization was performed by Chiron Rapid Energy Minimization Server (http://troll.med.unc.edu/chiron/documentation.php). This server reduces clash ratio of the model. Chiron minimizes the number of nonphysical atomic interactions (clashes) in the given protein structure. For Yr5 gene initially total numbers of clashes were 261, Total VDW repulsion energy was 286.861kcal/mol and clash ratio was 0.102231. The final numbers of clashes after energy minimizations were 41, total VDW repulsion energy was 26.38kcal/mol and clash ratio was 0.0177643 (Table 3). To compare the clash-score of the input and minimized structures to the benchmark set of 4300 high-resolution structures present in Chiron energy server, we plot the distribution of the normalized clash-score of the benchmark set and indicate the initial and final clash-score in the plot with respect to the distribution (Fig. 4).

For Yr10 gene initially total numbers of clashes were 2970, Total VDW repulsion energy was 3447.25kcal/mol and clash ratio was 0.154585. The final numbers of clashes after energy minimization was 333, total VDW repulsion energy was 204.973kcal/mol and clash ratio was 0.017378 (Table 4). To compare the clash-score of the input and minimized structures to the benchmark set of 4300 high-resolution structures present in Chiron energy server, we plot the distribution of the normalized clash-score of the benchmark set and indicate the initial and final clash-score in the plot with respect to the distribution (Fig. 5).

Table 3. Energy minimization score of Yr5 model by Chiron server.

	Number of residues	Number of clashes	Clash ratio	Total VDW repulsion energy (kcal/mol)
Initial Results (without energy minimization)	146	261	0.102231	286.861
Final results (with energy minimization)	146	41	0.0177643	26.38

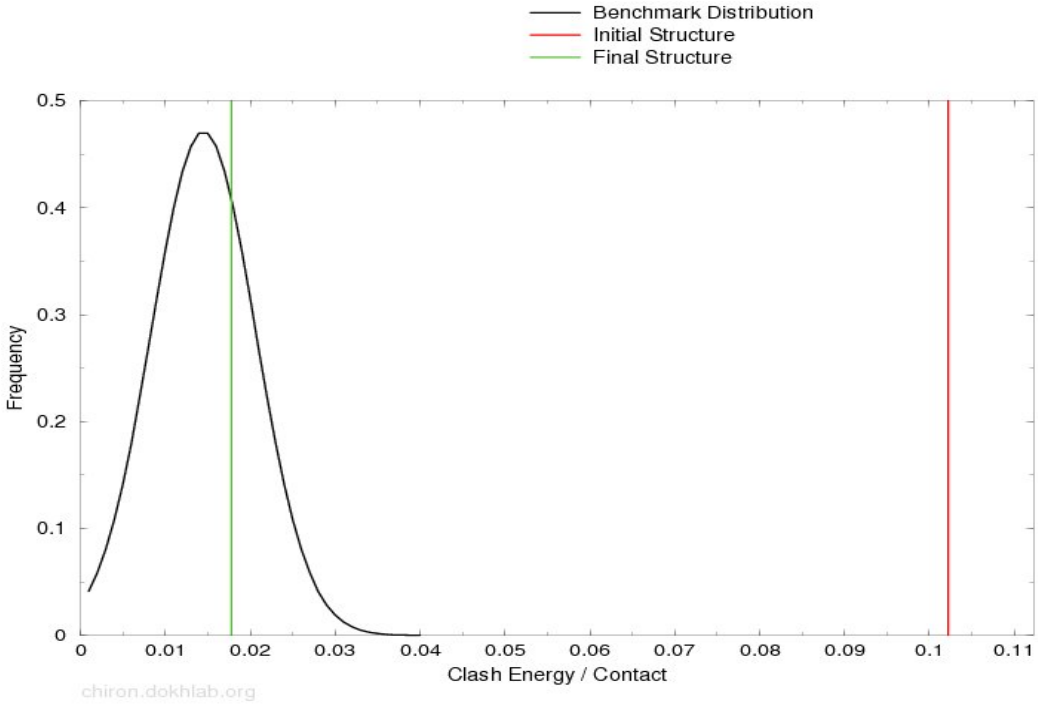

Fig. 4. Energy minimization graph of Yr5 gene.

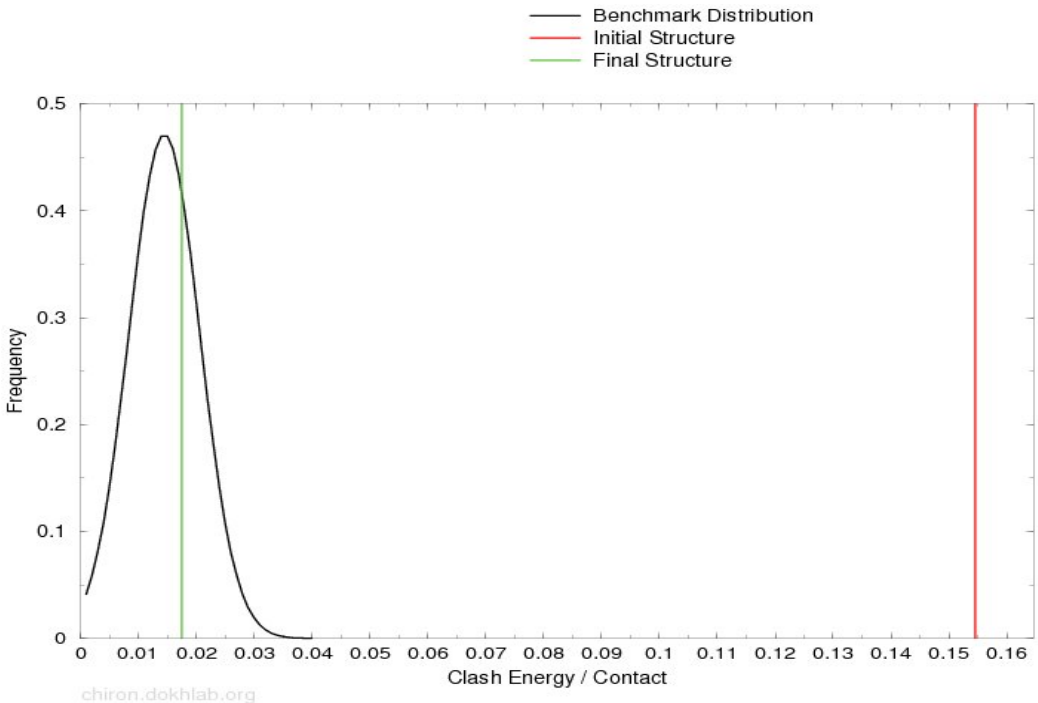

Fig. 5. Energy minimization graph of Yr10 gene.

Table 4. Energy minimization of Yr10 model by Chiron server.

	Number of residues	Number of clashes	Clash ratio	Total VDW repulsion energy (kcal/mol)
Initial Results (without energy minimization)	824	2970	0.154585	3447.25
Final Results (with energy minimization)	824	333	0.017378	204.973

Protein-protein BLAST was performed to obtain the templates of Yr5 and Yr10 genes (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins). Structures of all the templates of Yr5 and Yr10 genes were retrieved from RSCB PDB database (http://www.pdb.org/pdb/home/home.do). BLAST tables show the templates of Yr5 and Yr10 genes (Tables 5 & 6).

Table 5. Results of Yr5 BLAST table generated by protein BLAST.

Templates of Yr5	Query coverage	E-value
1Z6T	46%	0.010
3IZA	46%	0.015
3SFZ	46%	0.031
3SHF	46%	0.21
2ANN	24%	5.2
2ANR	24%	5.4
2QFC	52%	6.2
2OF7	18%	8.3

Table 6. Results of Yr10 BLAST table generated by protein BLAST.

Templates of Yr10	Query coverage	E-value
3QFL	14%	2e-20
1Z6T	37%	3e-06
3IZA	37%	3e-05
3SFZ	37%	1e-04
3SHF	37%	5e-04
1XEZ	12%	0.26
1HQC	13%	0.28
1IXS	13%	0.34
1IXR	13%	0.50
1DGR	7%	1.2
3KJE	9%	1.4
2WFH	10%	2.0
1AB5	11%	2.7
1F48	7%	4.0
3OLV	11%	8.6

Hex is an Interactive Molecular Graphics Program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex can also calculate Protein-Ligand Docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes (David, 2002). It uses Spherical Polar Fourier (SPF) correlations to accelerate the calculations and its one of the few docking programs which has built in graphics to view the result (Ritchie & Kemp, 2000).

Protein-protein Docking was performed between templates and also the original structures predicted of both Yr5 and Yr10 with PsMAPK1 protein to find the interactions. Hex 4.5 protein docking tool was used to perform protein-protein docking. Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. The results were generated on the basis of Etotal which is actually the energy total. Etotal is the total calculated interaction energy of the system. Hex doesn't calculate internal energies, and so doesn't report separate individual molecular energies.

When the receptor Yr5 was docked with the fungus protein (PsMAPK1) the energy value obtained was -644.75. Energy values on the basis of interactions between fungus protein PsMAPK1 and templates of Yr5 (Table 7). The receptor Yr10 has energy value obtained was (0.00) when docked with the fungus protein (PsMAPK1) but template of Yr10 with closer physiochemical properties has interaction value. Energy values on the basis of interactions between fungus protein PsMAPK1 and templates of Yr10 (Table 8).

Table 7. Results of Yr5 docking generated by Hex 4.5 protein docking tool.

Templates of Yr5	Etotal
1Z6T	-5.86
3IZA	-702.74
3SFZ	-588.84
3SHF	-706.41
2ANN	-519.96
2ANR	-459.05
2QFC	-637.01
2OF7	-722.48
Yr5	-644.75

Table 8. Results of Yr10 docking generated by Hex 4.5 protein docking tool.

Templates of Yr10	Etotal
3QFL	-0.63
1Z6T	-5.86
3IZA	-702.74
3SFZ	-588.84
3SHF	-706.41
1XEZ	-2.11
1HQC	-0.00
1IXS	-499.96
1IXR	-388.37
1DGR	-499.20
3KJE	-639.11
2WFH	-629.99
1AB5	-760.03
1F48	-160.32
3OLV	-640.79
Yr10	0.00

Conclusion

The physiochemical properties of templates 2OF7 and 3IZA has close resemblance with physiochemical properties of Yr5 and Yr10. These results show that both the templates can be used as alternate resistance gene against yellow rust in wheat. 2OF7 can be used in place of Yr5 and 3IZA in place of Yr10. The interaction value of 2OF7 is -722.48 while the interaction value of 3IZA is -702.74.

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