Exploitation of the genetic variability in the genotype of Egyptian Wheat by studding Molecular Markers, Molecular Docking and Nano Composites. Regulation of leaf and stem rust.

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Abstract

Puccinia graminis f. sp. *tritici* (Pgt) and *P. triticina* (Pt), the causal agents of stem rust and leaf rust, respectively form new physiological races that significantly reduce growth and yield of wheat cultivars. Therefore, seeking for resistant cultivars and exploring it to continuously produce new wheat cultivars resistant to stem and leaf rust through breeding programs is urgent. The aim of the present study was to assess 18 Egyptian wheat genotypes for resistance to stem and leaf rust. The 18 genotypes were also analyzed for polymorphism using 20 SCoT and SRAP primers. Furthermore, the activity of chitosan-cupper composite nanoparticle in controlling stem rust is examined and its mode of action is studied using molecular docking analysis. In seedling stage, the genotypes were tested against 20 stem rust races, and the host reaction types were noticed. The lowest host reaction types (It = 0; to 2++) were recorded for Sakha 94, Sakha 95, Beni Sweif 4, Beni Sweif 7, Sohag 4, Sohag 5 and Gemmeiza 12. These genotypes except Gemmeiza 12 were resistant to all races. The remaining genotypes were susceptable to most races, but Giza 160 was highly susceptible to all races. In adult stage, the 18 genotypes were evaluated for resistant to stem rust and leaf rust in two different location, *i.e.* Giza and sids. The evaluation was

expressed as percentage of final rust severity (FRS%), Area under disease progress curve (AUDPC) and rate of rust disease increase (r-value). SCoT and SRAP analysis generated 140 and 121 polymorphic band with 97 and 99% polymorphism, respectively. Among them, 71 and 73 were unique loci for SCoT and SRAP, respectively. The 18 genotypes were divided into two main groups depending on the similarity matrix. The first cluster consists of the most resistant genotypes to leaf rust (Giza 171, Sakha 94, Misr 1, Misr 2, Misr 3, Giza 168, Gemmeiza 12 and Sids 12) in addition to two cultivars susceptible to leaf and stem rust (Beni Sweif 7, Gemmeiza 11). Meanwhile, the second ones consists of the most susceptible genotypes to stem and leaf rust (Giza 164, Sakha 69, Giza 160, Beni Sweif 4, Sohag 5 and Sohag 4) in addition to Sakha 95 (resistant to leaf rust but susceptible to stem rust) and Shandaweel 1 (resistant to stem rust but susceptible to leaf rust). Moreover, the 18 genotypes were sprayed with Cu-chitosan composite nanoparticle either before or before and after inoculation with uridiospores of stem rust to determine the effect of this solution and its application method in controlling the disease. The infection was reduced when the plant sprayed 24 h before and 24 h before and after inoculation. Incubation and latent periods were increased in treated plant genotypes. Besides, the treatment gave the lowest infection type compared to the control. The foliar spray application didn't affect the efficacy of the tested treatment.

Keywords: Molecular markers, Genetic diversity, stem rust, leaf rust, nanoparticle.

Introduction

Wheat (*Triticum aestivum*) is an important domestic and international economic food grain source. The demand for wheat specially for developing countries is projected to increase to 60% by 2050 (FAO, 2016). This increase demand for wheat is very serious, especially in the context of climate changes that would lead to decrease the final productivity by 29% (Rosegrant *et al.* 1995). Moreover, wheat is attack by three rust diseases, *i.e.* stripe, leaf and stem rust. The later caused by *Puccinia graminis* Pers. *f. sp. tritici* (Eriks. & E. Henn.) is a destructive disease of wheat crops all over the world under appropriate environmental conditions when a disease becomes epidemic (Naseri and Sabeti, 2020). Wheat stem rust fungus attacks wheat plants, especially those planted lately leading to block the vascular system, plant stunting and finally causes up to 100% yield losses , due to damage grain and tillers (Boukhatem *et al* 2002 and Kokhmetova *et al* 2011). In Egypt, stem rust causes 1.96 to 8.21% losses in the yield of local wheat varieties (Ashmawy *et al* 2013). In the last two decades, the stem rust disease has a reason again

due to arise of the new race Ug99. About 90% of wheat crop worldwide are susceptible to this destructive race (Singh *et al.* 2011) leading to up to 70% yield losses (Singh *et al.* 2006). In Kenya, yield losses due to stem rust exhibited up to 100% reduction (Njau *et al* 2010). In Egypt, up to 95% of the local commercial varieties are susceptible or highly susceptible to race Ug99, while only a few older varieties showing some level of "adult plant resistance" (APR) to this race and it's variants that have been detected in Kenya (Wanyera *et al* 2006 and Njau *et al* 2010). more recently, more than 15 confirmed Ug99 races have been reported in Africa (Singh *et al.*, 2015), and rapidly spread to another countries in Africa (Kenya, Ethiopia, Sudan, Tanzania, South Africa, Zimbabwe, Mozambique, Eritrea and Egypt) and Middle East (Yemen, Iran and Pakistan) (Wanyera *et al* 2006, Nazari *et al* 2009, Pretorios *et al* 2010, Wolday *et al* 2011 and Abou-Zeid *et al* 2014). It is expected that the Ug99 could be attack cereal gown in another geographical regions around the world in the future.

Due to the enormous yield loses caused by the Ug99 lineage of stem rust races to wheat productivity, efforts have been made to discover new sources for resistance and incorporate effective genes into new commercial varieties with high yield is urgent.

Molecular markers are useful mean for determining, characterizing, and assessing genetic diversity among organisms in the level of species[11]. It is well known as DNA sequences located within specific genome regions and are associated with the trait inheritance of linked genes[12]. recently, gene-targeted markers such as Conserved DNA-Derived Polymorphism (CDDP) and Start Codon Targeted (SCoT) [15] replaced the classic genetic markers (RAPD, AFLP, RFLP, ISSR, and SSR) [13]. These gene-targeted markers are widely used for studying the genetic diversity of different organisms, including plants[14]. The SCoT polymorphisms work through the short conserved region in plant genes surrounding the ATG translation start (or initiation) codon[15].

SCoT markers are successfully used to assess the genetic diversity, structure, cultivars identification, quantitative trait loci (QTL) mapping, and DNA fingerprinting in different microorganisms species[16,17] The SCoT markers have been largely and

successfully used for evaluating the phylogenetic (Xiong et al. 2011), genetic diversity (Satya et al. 2015), and fingerprinting (Xiong et al. 2011; Mulpuri et al. 2013).

According to Li and Quiros (2001), the sequence-related amplified polymorphism (SRAP) technique is a highly reproducible DNA marker that can be used for different purposes, including genetic variation (Robarts and Wolfe 2014), quantitative trait loci mapping (Zhang et al. 2006), map construction (Li and Quiros 2001), and cDNA fingerprinting and map-based cloning (Robarts and Wolfe 2014). The advantages of SRAP markers, such as simplicity, revealing of numerous co-dominant markers, and targeting of open reading frames (ORFs) (Uzun et al. 2009; Robarts and Wolfe 2014), have prioritized it over other molecular markers. Evaluation of the genetic diversity between and within the *Carthamus* species has been done by SRAP (Peng et al. 2008; Mokhtari et al. 2013). Accordingly, the main objective of this study was to evaluate the disease response of 18 Egyptian wheat varieties against stem rust at the adult plant stage under field conditions and green house conditions. Moreover, to assess the genetic diversity relationships among eighteen species of wheat plants using combine both SRAP and SCoT molecular markers. Also, to determine the relationship between the disease parameters and molecular markers linked for these. Additionally, a preliminary screening and evaluation process of the NANO particles antifungal on 18 Egyptian wheat varieties against stem rust were performed.

Materials and Methods

Eighteen Egyptian wheat genotypes (Table 1) were assessed for stem rust resistance at seedling and adult stages. The later was evaluated in two locations at Giza and Sids Research Station in 2019/20.The wheat genotypes seeds were getting from Wheat Disease Research Department, Plant Pathology Research Institute, Agricultural Research Centre, Egypt.

Table (1): Pedigree and year of release of the tested wheat genotypes.

code	Wheat genotype	Pedigree	Year of release

1	Gemmeiza 11	B0W"S"/KVZ"S"//7C/SERI82/3/GIZA168/SAKHA61. GM7892-2GM-1GM-2GM-1GM-0GM.	2011
2	Gemmeiza 12	OTUS/3/SARA/THB//VEE .CCMSS97Y00227S-5Y-010M-010Y-010M-2Y-1M-0Y-0GM	2017
3	Sids 12	BUC//7C/ALD/5/MAYA74/ON//1160-147/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL-4SD-1SD-1SD-0SD.	2007
4	Misr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR. CMSSOYO1881T-050M-030Y-O3OM-030WGY-33M-0Y-0S.	2010
5	Misr 2	SKAUZ/BAV92. CMSS96M0361S-1M-010SY-010M-010SY-8M-0Y-0S.	2011
6	Misr 3	ATTILA*2/ABW65*2/KACHU CMSS06Y00258 2T-099TOPM-099Y-099ZTM-099Y-099M-10WGY-0B-0EGY	2018
7	Giza 168	MIL/BUC//Seri CM93046-8M-0Y-0M-2Y-0B	1999
8	Giza 171	Sakha 93 / Gemmeiza 9 S.6-1GZ-4GZ-1GZ-2GZ-0S	2013
9	Sakha 94	OPATA/RAYON//KAUZ. CMBW90Y3280-0TOPM-3Y-010M-010M-010Y-10M-015Y-0Y-0AP-0S.	2004
10	Sakha 95	PASTOR//SITE/MO/3/CHEN/AEGILOPS SQUARROSA(TAUS)//BCN /4/WBLL1CMSA01Y00158S-040P0Y-040M-030ZTM-040SY-26M-0Y-0SY-0S	2018
11	Beni Sweif 7		
12	Shandaweel 1	SITE//MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC. CMSS93B00567S-72Y-010M-010Y-010M-0HTY-0SH	2011
13	Giza 164	KVZ/Buha "s"//Kal/Bb CM33027-F-15M-500y-0M	1987
14	Sakha 69	Inia–RL4220´7C/YR"S"CM15430- 25 -65-0S-0S	1980
15	Giza 160	Chenab 70/Giza 155	1982
16	Beni Sweif 4	AUSL/5/CANDO/4/BY*2/TACE//II27655/3/TME//ZB/W*2. ICD88-1120-ABL-0TR-1BR-0TR-6AP-OSD.	2007
17	Sohag 4	Ajaia-16//Hora/Jro/3/Gan/4/Zar/5/Suok-7/6/Stot//Altar84/Ald CDSS99B00778S-OTOPY-0M-0Y-129Y-0M-0Y-1B-0SH	2016
18	Sohag 5	TRN//21563/AA/3/BD2080/4/BD2339/5/Rascon 37// Tarro 2// Rascon 3/6/Auk/Gull//GreenCDSS00B00364T-0T0PB-0B-2Y-0M-0Y-1B-0Y-0SH	2016

Greenhouse experiment (seedling stage evaluation):

This experiment has been implemented in the greenhouse of Wheat Diseases Res. Dept., Plant Pathology Res. Inst., ARC, Giza, Egypt for the years 2019-2020. Eighteen Egyptian wheat genotypes were tested against 15 stem rust races (Table 2) in three replicates totally random. Six wheat grains from each genotype were sown in plastic pots (6 cm. diam.) contain a mixture (1:1 v/v) of peat-moss and soil. One grain was sown in pots. Seven days old seedlings were inoculated by a paintbrush contaminated with the

urediniospores of each rust race. The inoculated seedlings were incubated at 18 °C in a dark dew chamber for 14 h, then moved to the benches in the greenhouse and maintained

No.	Races	A virulence (Effective genes)	Virulence (Ineffective genes)

at 20-24º C and 70-80 % relative humidity with 16 h light and 8 h dark at about 7600 lux (Ohm and Shaner, 1976). The seeding were daily observed until the development of rust pustules. Seedling response was scored 10-12 days after inoculation based on the infection types (IT) expressed on each genotype using a 0-4 scale (Jin et al. 2008). Infection types, *i.e*. ;0, 0, 1, and 2 represent low infection (IT), while 3 and 4 represent high infection (IT) (Long and Kolmer 1989).

Table (2). The avirulence/virulence pattern of 20 *Puccinia graminis f.sp. tritici* pathotypes used to study stem rust resistance of eighteen Egyptian wheat genotypes

1	BTSTC	5, 21, 9e, 7b, 17, 24, 31, 38	11, 6, 8a, 9g, 36, 9b, 30, 9a, 9d, 10, Tmp, Mcn
2	SKTTC	5, 21, 9e, 11, 36, 9b, 30, 17, 24, 31, 38	7b, 6, 8a, 9g, 9a, 9d, 10, Tmp, Mcn
3	JKGKC	5, 7b, 11, 36, 30, 17, 9a, 24, 31, 38	21,9e, 6, 8a, 9g, 9b, 9d, 10, Tmp, Mcn
4	SJDCC	5, 21, 9e, 11, 9g, 36, 9b, 17, 9a, 9d, 10, 24, 31, 38	7b, 6, 8a, 30, Tmp, Mcn
5	DKTTC	5, 21, 7b, 11, 36, 9b, 30, 17, 24, 31, 38	9e, 6, 8a, 9g, 9a, 9d, 10, Tmp, Mcn
6	PKTTC	21, 11, 36, 9b, 30, 17, 24, 31, 38	5, 9e, 7b, 6, 8a, 9g, 9a, 9d, 10, Tmp, Mcn
7	PKTTH	21, 11, 36, 9b, 30, 17, 24, 31, 38	5, 9e, 7b, 6, 8a, 9g, 9a, 9d, 10, Tmp, Mcn
8	PCTKC	21, 11, 6, 8a, 36, 9b, 30, 17, 9a, 24, 31, 38	5, 9e, 7b, 9g, 9d, 10, Tmp, Mcn
9	STKTC	5, 21, 9e, 36, 24, 31, 38	7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, Tmp, Mcn
10	TKTPC	11, 36, 9b, 30, 17, 9d, 24, 31, 38	5, 21, 9e, 7b, 6, 8a, 9g, 9a, 10, Tmp, Mcn
11	TKPTC	11, 9b, 24, 31, 38	5, 21, 9e, 7b, 6, 8a, 9g, 36, 30, 17, 9a, 9d, 10, Tmp, Mcn
12	TKSTC	11, 17, 24, 31, 38	5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 9a, 9d, 10, Tmp, Mcn
13	TKTTC	11, 36, 9b, 30, 17, 24, 31, 38	5, 21, 9e, 7b, 6, 8a, 9g, 9a, 9d, 10, Tmp, Mcn
14	TTKSC	36, Tmp, 24, 31, 38	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, Mcn
15	TTTSK	36, 9b, 30, 17, Tmp, 24,	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9a, 9d, 10, Mcn
16	PKTTH	21, 11, 36, 9b, 30, 17, 24, 38	5, 9e, 7b, 6, 8a, 9g, 9a, 9d, 10, Tmp, Mcn
17	PCTKC	21, 11, 6, 8a, 36, 9b, 30, 17, 9a, 24, 31, 38	5, 9e, 7b, 9g, 9d, 10, Tmp, Mcn
18	STKTC	5, 21, 9e, 36, 24, 31, 38	7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, Tmp, Mcn
19	TKTPC	11, 36, 9b, 30, 17, 9d, 24, 31, 38	5, 21, 9e, 7b, 6, 8a, 9g, 9a, 10, Tmp, Mcn
20	TKPTC	11, 9b, 24, 31, 38	5, 21, 9e, 7b, 6, 8a, 9g, 36, 30, 17, 9a, 9d, 10, Tmp Mcn

Field experiment (adult stage evaluation):

This experiment was performed at Giza and Sids Agricultural Research Station, Agric. Res. Center of Egypt during 2019-20 growing season in a randomized complete block design (RCB) with three replicates. Each genotype was sown in plots divided into two rows of three-meter length and 30 cm apart. All plots were surrounded by a highly susceptible wheat (variety Morocco and Thatcher) plant which planted over an area 1 m^2 to provide high and uniform disease pressure. All recommended cultural practices *i.e.* fertilization, irrigation and other management were applied. To enhance the development of stem rust epidemics under field conditions, experiment was irrigated and inoculated by

dusting a mixture of urediniospores of the common and more aggressive stem rust pathotypes mixed with talcum powder at a rate of one volume of fresh urediniospores mixture to 20 volumes of talcum powder according to the method described by Tervet and Cassell (1951).

Characterized and disease development was assessed through the use of the two epidemiological parameters i.e. final rust severity (FRS %), area under disease progress curve (AUDPC) and rate of stem and leaf rust disease increase (r-value). Stem and leaf rust severity (%) which was expressed as a percentage of stem area covered with stem rust (0 % to 100 %) was recorded according to the Modified Cob's scale (Peterson *et al*., 1948). Disease severity (%) for each genotype was assessed three times at 15 days intervals. The adult-plant reactions were also recorded using the description of Roelfs *et al*. (1992). Final rust severity (FRS %) was also recorded for each of the tested cultivars as disease severity (%) when the highly susceptible (check) variety was severely rusted and the disease rate reached it's the highest or final level of severity (Das *et al*., 1993). The area under disease progress curve (AUDPC) is calculated by using the formula suggested by Pandey *et al*. (1989).

$$\text{AUDPC} = D\left[\frac{1}{2}(Y1 + Yk) + (Y2 + Y3 + \ldots\ldots + Yk\text{-}1)\right]$$

Where:

D = days between two consecutive records (time intervals)

Y1 + Yk = Sum of the first and last disease scores.

Y2 + Y3 + …….. + Yk-1 = Sum of all in between disease scores.

Rate of stem rust disease increase (r-value), as a function of times was also estimated, according to the formula of Van der Plank (1963), as estimated using the following formula.

$$\text{r-value} = \frac{1}{t_2 - t_1}\left(\log_e \frac{X_2}{1 - X_2} - \log_e \frac{X_1}{1 - X_1}\right)$$ Vander Plank, (1963)

Where: X1 = the proportion of the susceptible infected tissue (disease severity) at date t1.

X2 = the proportion of the susceptible infected tissue (disease severity) at date t2; t2 - t1 = the interval in days between these dates.

Statistical analysis:

Combined analysis of variance over the two seasons was carried out (Table 2). The significance of difference among the studied cultivars was tested by the analysis of variance (ANOVA) test as outlined by Snedecor and Cochran (1967). Mean comparisons for variables were made among genotypes using least significant differences (LSD at 5%) tests.

2.2. Molecular analysis

2..1. DNA Extraction

High-quality genomic DNA was isolated from fresh leaves (100 mg) of the collected 18 wheat plants using CTAB method (Doyle and Doyle 1990). DNA concentrations were determined using spectrophotometry (260/280) and gel electrophoresis (1% agarose gel) was used for PCR analysis in the final concentration of 25 ng/µl.

SCoT --PCR amplification

A set of 20-mer primers established by Collard and Mackill [15] were used for genetic diversity analysis in 18 wheat plant samples. The PCR reaction was performed in a total volume of 25 µl containing 1X reaction buffer, 1.5 mM$MgCl_2$, 0.1 mM dNTP, 0.3 µM of a primer , 60 ng genomic DNA and 2U of Taq DNA polymerase. The amplification of PCR was programmed at 94 °C for 3 min. Followed by 36 cycles of 94 °C for 50 °C for 1 min. and 72 °C for 2 min; the final extension at 72 °C was held for 5 min. All the PCR amplification fragments were separated by electrophoresis on 1.5% agarose gels in 1X TBE buffer stained with ethidium bromide. A 100 bp DNA ladder was used as a DNA marker.

SRAP-PCR amplification

SRAP–PCR amplification

The molecular analysis of SRAP markers was done according to the procedure described by Li and Quiros (2001) with minor modifications. All SRAP primer combinations were initially screened using a group of samples according to Uzun et al. (2009) . Twelve primer combinations with scorable polymorphic bands were performed. PCR reactions were performed in a total volume of 25 µl as track: 10 µl of Master Mix (Amplicon), 2 µl of DNA template, 2 µl of forward primer and reverse primer (10 µmol/l primers), and 3 µl of ddH_2O. The SRAP markers were amplified on following parameters: 5 min at 94 °C, 5 cycles of 94 °C for 1 min, 35 °C for 1 min, 72 °C for 2 min, 30 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min, and final extension of 5 min at 72 °C. The amplification products were resolved by electrophoresis in a 1.5 % agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer. A 100 bp DNA ladder was used as molecular size standards.

2.2.4 Data analysis

The PCR products generated from SCoT and SRAP primers were visually scored. The data analysis and the final scores were determined for the clear bands Only. For each marker, the amplicons were scored as presented (1) or absent (0) to create the binary data set in all the 18 wheat plants. Polymorphism percentage calculated by dividing the quantity of amplified polymorphic bands through the total number of amplified bands by means of the same primer or the primer combination. The genetic similarity coefficients were assessed according to the Dice coefficient (Sneath and Sokal, 1973). A dendrogram was generated one through cluster analysis using the unweighted pair group approach of the mathematics averages (UPGMA) for all the exclusive marker systems. systat ver. 7 (SPSS Inc. 1997 SPSS Inc.3/9`7 standard version) (Yang and Quiros, 1993) statistical software program was used to compare the similarity matrices and the dendrograms produced by the SCoT and SRAP techniques to visualize the 18 wheat plant .

Database Search, Structural Modelling, and Model Validation.

The proteins sequences are available in NCBI (https://www.ncbi.nlm.nih.gov/) and uniprot (https://www.uniprot.org/) . in fasta format with with GenBank accession no. table 2 . the fungus proteins was selected for searching all the sequential homolog and orthologs using NCBI Blast server1 (Altschul et al., 1997) keeping the default values, and against the non-redundant protein sequences, with searching the organism. The sequences were retrieved in the FASTA format as an amino-acid length sequence and used to build. the 3D structure were built using the SWISSMODLE server (https://swissmodel.expasy.org/). The homology model of the fungs protein was achieved by using the SWISSMODLE server. A sequence sequence of Blast-P similarities for recognition of close-related structural homologs in fungus was queried against a PDB database14 (Berman et al., 2000). The first hit on the annotation Blast-p was obtained to identify the templates in which (PDB temple ID, identity, QMEAN). The Protein Data Bank collected the PDB file of the templates (PDB). Found by BLAST was the alignment data. The SWISSMODLE server began with the target sequence file, the alignment file, and the PDB file for the prototype, all the temples proteins. The fungus proteins with a QMEAN score have been developed for the development of models. In addition, for final confirmation, the protein model with an below -4 score has been chosen. The optimized models MAP kinase 1 [Puccinia triticina] **and PGTG** *Puccinia graminis f. sp. Tritici* was found to be suitable based on several qualitative backgrounds including the RAMPAGE, , ERRAT, PROCHECK (PDB Sum), and Verify-3D. The Ramachandran plot which evaluated that the predicted models were closer to the template (91% , 93%) residues lying in the favored regions). The ERRAT score values (91.56 % , 78.43%) and Verify-3D results were good enough signifying the consistency of the model prediction and explained that the predicted model was reliable and satisfactory, as it was reported that, for a model having good resolution ,the ideal score values for Verify-3D should be 80%, and that for the ERRAT around 95% (Colovos and Yeates, 1993).

Preparation of Protein and Ligands

The fungus protein was selected as a target receptor protein and was imported to the 3Drefine server to make energy minimization for 2 proteins (http://sysbio.rnet.missouri.edu/3Drefine/) For pre-docking, all water molecules and ligands were removed while hydrogen atoms were added to the target protein. The docking system was built using SAMSON software 2020 (https://www.samson-connect.net/).and then improted to SAMSON The structures were prepared using protein preparation wizard of the Autodock Vina extension . using SAMSON 2020 software. . A receptor grid X,Yand Z value of proteins are in table 2 was generated besd on the blind docking to explore the effect of the ligands on the proteins and if they will effect of not to explore the mechanism of insect die . The ligands were retrieved from the PubChem database id in table 2 . Dependent upon our previous study . (……..)The ligands was prepared an desined in chemdraw https://www.perkinelmer.com/category/chemdraw) in pdb format, then converted into MOL2 format by using openbable software (http://openbabel.org/wiki/Main_Page). then The energy minimization was done at neutral pH 7.0 ± 2.0.in SAMSON software.

Binding site prediction, Protein-ligand docking

SAMSON software (https://www.samson-connect.net/) is used for binding site prediction.[14] It uses the interaction energy between the protein and a simple van der Waals probe to locate energetically favorable binding sites. This software uses Autodocking Vina as element to maximizing the accuracy of these predictions while minimizing the computer time (Trott e al., 2010). The program works based on the quantum mechanics. It predicts the potential affinity, molecular structure, geometry optimization of structure, vibration frequencies of coordinates of atoms, bond length and bond angle. Following by the exhaustive search, 100 poses were analyzed and the best scoring poses were used to calculate the binding affinity of the ligands. The ligands that tightly bind to target protein with high score were selected **table2** The proteins were docked against the compounds chitosan and cu using SAMSON software (https://www.samson-connect.net/).[15] The2D interaction was carried out to find the favorable binding geometries of the ligand with the proteins by using Discovery studio software to get the 2D interaction image of the Docked protein ligand complexes with high score to the predicted active site.

Protein ligand interaction using SAMSON and Discovery studio software

The ligands were docked with the target protein, and the best docking poses were identifying. Figures 1-2 shows 2D and 3D structure of the binding poses of the compounds.

Results

Evaluation of 18 wheat genotypes against 20 stem rust races under greenhouse conditions:

Great difference in the responses of 18 wheat genotypes to stem rust races was noticed in this experiment. Resistant was noticed in 10 genotypes (Misr 3, Sakha 94, Sakha 95, Beni Sweif 4, Beni Sweif 7, Sohag 4 and Sohag 5, Gemmeiza 12, Shandaweel 1 and Giza 171). Furthermore, susceptible was noticed in 8 genotypes (Gemmeiza 11, Sids 12, Misr 1, Misr 2, Sakha 69, Giza 164, Giza 168 and Giza 160). Misr 3, Sakha 94, Sakha 95, Beni Sweif 4, Beni Sweif 7, Sohag 4 and Sohag 5 genotypes were resistant to all races, but Giza 160 was susceptible to all races (Table 3).

Evaluation of wheat genotypes against stem rust and leaf rust under field conditions:

Final rust severity (FRS %):

The 18 wheat genotypes in adult stage were evaluated against stem rust and leaf rust under field conditions in two different (Giza and Sids) locations. The evaluation was estimated and expressed as final rust severity (FRS%), area under disease progress curve (AUDPC) and rate of rust disease increase (r-value).

Data presented in table (4) revealed that, the FRS% was varied among genotypes and ranged from 0 to 80 S for stem rust and from Tr Mr to 70 S for leaf rust. The highest final stem rust severity percent was recorded for Giza 160, Giza 164, Misr 2, Sakha 69 and Misr1genotypes either in Giza or Sids, but the lowest final stem rust severity was noticed

Table (3). Seedling reaction with *pgt* races.

	BTSTC	SKTTC	IKGKC	SIDCC	DKTTC	PKTTC	PKTTH	PCTKC	STKTC	TKTPC	TKPTC	TKSTC	TKTTS	TTKSC	TTTSK	PKTTH	PCTKC	STKTC	TKTPC	TKPTC
Gemmeiza 11	0	3	0	0	1	1	4	3	0	0	0	4	0	3	3	0	4	0	3	0
Gemmeiza 12	0	2	0	0	2	0	2	2	4	2	0	0	; 0	0	2	; 0	0	; 0	0	0
Sids 12	2+	3	3	0	3	4	3	4	2	0	; 0	4	4	3	3	4	4	0	3	3
Misr 1	1	3	3	1	3	0	; 0	4	4	1	4	2	2	3	1	0	3	3	1	0
Misr 2	1	4	3	; 0	4	2+	1	3	3	0	3	1	1, 2	3	2	; 0	3	3	0	; 0
Misr 3	0	2+	2	0	0	0	1	0	1	2++	0	1	1	1	1	0	0	; 0	0	0
Giza 164	3	1	3	0	1	0	4	4	3	0	4	3	4	3	0	; 0	4	0	4	; 0
Giza 168	0	4	0	1	1	1	4	3	4	0	4	4	3	3	3	0	3	0	3	0
Giza 171	0	2	2	; 0	0	0	4	2	4	0	4	2	3	2	1	0	4	; 0	0	0
Sakha 69	0	1	3	1	0	2++	4	3	1	2	4	4	1	0	1	0	3	0	3	0
Sakha 94	2	0	1	0	0	0	1	2	2	2	0	0	; 0	0	; 0	; 0	0	0	0	0
Sakha 95	0	0	1	; 0	1	0	1	1, 2	1	1	0	0	0	0	1	0	; 0	; 0	0	0
Shandaweel 1	2	2	0	2	4	2	1	1	1	1	1, 2	0	1	1	3	3	0	0	0	4
Beni Sweif 4	0	; 0	1	; 0	1, 2	0	0	1	2	; 0	1	1	1	2	1	0	0	0	0	; 0
Beni Sweif 7	; 0	1	1	0	2	1	0	; 0	1	1	1	0	0	2++	1	; 0	0	; 0	0	0
Sohag 4	1	1	1	; 0	2	2	2	2	2++	2	2	0	2++	2++	2	2	0	0	0	0
Sohag 5	1	1	2	0	0	0	1	1	2	0	1	1	2	2	1	0	0	0	0	0
Giza 160	3	3	3	4	3	3	4	3	3	3	3	3	4	3	3	4	3	3	3	4

for Giza 171 and shandweel 1 genotypes in both locations. On the other hand, the highest final leaf rust severity was recorded for Giza 160, Giza 164, Sakha 69 and Gemmiza 11 in both locations, but the lowest final leaf rust severity was recorded for Giza 168 and Sakha 95 in both locations.

Table 4: Final stem rust severity (FRS %) of 18 wheat genotypes of some Egyptian wheat cultivars, under field conditions at Giza and Sids Research Station during 2019/20 growing season.

Genotype	SR		LR	
	Giza	Sids	Giza	Sids
Gemmeiza 11	20 MS	10 MS	40 MS	50 MS
Gemmeiza 12	Tr MS	5 MS	30 MR	20 MR
Sids 12	20 S	40 S	20 MR	20 MR
Misr 1	40 S	60 S	Tr MR	5 MR
Misr 2	60 S	60 S	5 MR	5 MR
Misr 3	Tr S	5 S	10 MR	10 MR
Giza 168	5 S	10 S	20 M	30 M
Giza 171	10 M	0	10 R	20 R
Sakha 94	5 MS	20 MS	5 R	10 R
Sakha 95	20 S	30 S	10 R	20 R
Shandaweel 1	10 MR	5 MR	20 MS	30 MS
Giza 164	70 S	60 S	60 S	70 S
Sakha 69	50 S	40 S	70 S	70 S
Beni Sweif 7	30 MS	10 MS	10 S	20 S
Beni Sweif 4	20 MS	5 MS	30 S	40 S
Sohag 4	10 MS	Tr MS	20 S	30 S
Sohag 5	5 S	Tr S	20 S	30 S
Giza 160	80 S	80 S	50 S	60 S

Area under disease progress curve (AUDPC) and rate of rust disease increase (r-value):

Area under disease progress curve (AUDPC), as useful and reliable quantitative data of plant disease severity over time, was also considered to evaluate the 18 genotypes for resistant to stem rust and leaf rust in Giza and sids stations. The AUDPC values were differed among the tested genotypes, the disease and locations (Table 5). The AUDPC more than 250 classified as highly susceptible to rusts but those with values less than 250 classified as highly resistant genotypes. Therefore, the genotypes, Giza 164, Sakha 69 and Giza 160 were highly susceptible to stem rust and leaf rust in Giza and sids stations with highly AUDPC values. Misr 1 and Misr 2 genotypes were highly susceptible to stem rust

but not leaf rust. Beni Sweif 4 and Gemmeiza 11 were highly susceptible to leaf rust but not stem rust. Sids 12 and Sakha 95 were highly susceptible to stem rust in sids station only. The remaining genotypes displayed the lowest AUDPC values (less than 250) either for stem rust or leaf rust in both locations.

On the other hand, the rate of stem and leaf rust progress

Stem rust was developed more slowly and increased at relatively lower rates (r-value) on all tested wheat cultivars this third parameter used, The first group included eleven wheat , (Gemmeiza 12, Misr 3, Giza 168, Giza 171, Sakha 94, Sakha 95, Shandaweel 1, Bany Swif 7, Bany Swif 4, Sohag 4 and Sohag 5) cultivars, which exhibited stem rust developed more slowly and increased at relatively lower rates of disease increase (r-values), ranged from 0.00 to 0.100 at the growing season, The second group included the wheat verities (Gemmeiza 11, Sids 12, Misr 1, Misr 2, Giza 164, Sakha 69and Giza 160) which showed the lowest levels of partial resistance to stem rust infection in comparison with the other cultivars under this study, where the r-values reached the maximum level more than 0.100 were classified as fast rusting or highly susceptible wheat genotypes.

Leaf rust disease severity in the wheat cultivars also could be ranked in to main groups: The first group included eleven wheat cultivars, (Gemmeiza 12, Misr 1, Misr 2, Misr 3,Giza 168, Giza 171, Sakha 94, Sakha 95 and Sohag 5) which exhibited stem rust developed more slowly and increased at relatively lower rates of disease increase (r-values). These cultivars were designated as the partially resistance, slow rusting ones. Since they displayed the highest level of adult plant resistance or field resistance under the stress of leaf rust infection through this study, they may be have durable resistance to leaf rust. On the other, hand the fast rusting or highly susceptible varieties Gemmeiza 11, Sids 12, Shandaweel 1, Bany Swif 7, Bany Swif 4, Sohag 4, Giza 164, Sakha 69 and Giza 160.

Table 5: Area under disease progress curve (AUDPC) and rate of rust disease increase (r-value) of 18 wheat genotypes grown at Giza and Sids Agric. Res. Station, during 2019/20 growing seasons.

Genotype	AUDPC				r-value			
	SR		LR		SR		LR	
	Giza	Sids	Giza	Sids	Giza	Sids	Giza	Sids
Gemmeiza 11	143.50	87.50	283.50	297.50	0.183	0.149	0.121	0.121
Gemmeiza 12	24.50	59.50	199.50	164.50	0.067	0.067	0.069	0.040

Sids 12	178.50	525.00	101.50	164.50	0.170	0.185	0.140	0.140
Misr 1	560.00	1050.00	45.50	66.50	0.192	0.189	0.067	0.067
Misr 2	735.00	980.00	42.00	45.50	0.215	0.283	0.053	0.067
Misr 3	31.50	63.00	38.50	66.50	0.079	0.079	0.088	0.103
Giza 168	63.00	122.50	94.50	171.50	0.104	0.036	0.032	0.007
Giza 171	66.50	0.00	63.00	164.50	0.103	0.00	0.096	0.140
Sakha 94	52.50	52.50	52.50	101.50	0.067	0.067	0.067	0.088
Sakha 95	178.50	318.50	42.00	171.50	0.100	0.108	0.096	0.032
Shandaweel 1	66.50	52.50	129.50	227.50	0.100	0.067	0.132	0.164
Giza 164	910.00	1050.00	735.00	910.00	0.245	0.285	0.224	0.245
Sakha 69	700.00	507.50	822.50	962.50	0.205	0.221	0.281	0.281
Beni Sweif 7	234.50	115.50	80.50	178.50	0.094	0.093	0.114	0.103
Beni Sweif 4	164.50	31.50	437.50	507.50	0.040	0.067	0.100	0.121
Sohag 4	56.00	52.50	178.50	297.50	0.079	0.067	0.153	0.100
Sohag 5	94.50	52.50	262.50	283.50	0.032	0.067	0.074	0.178
Giza 160	1260.00	1260.00	560.00	910.00	0.232	0.232	0.205	0.205

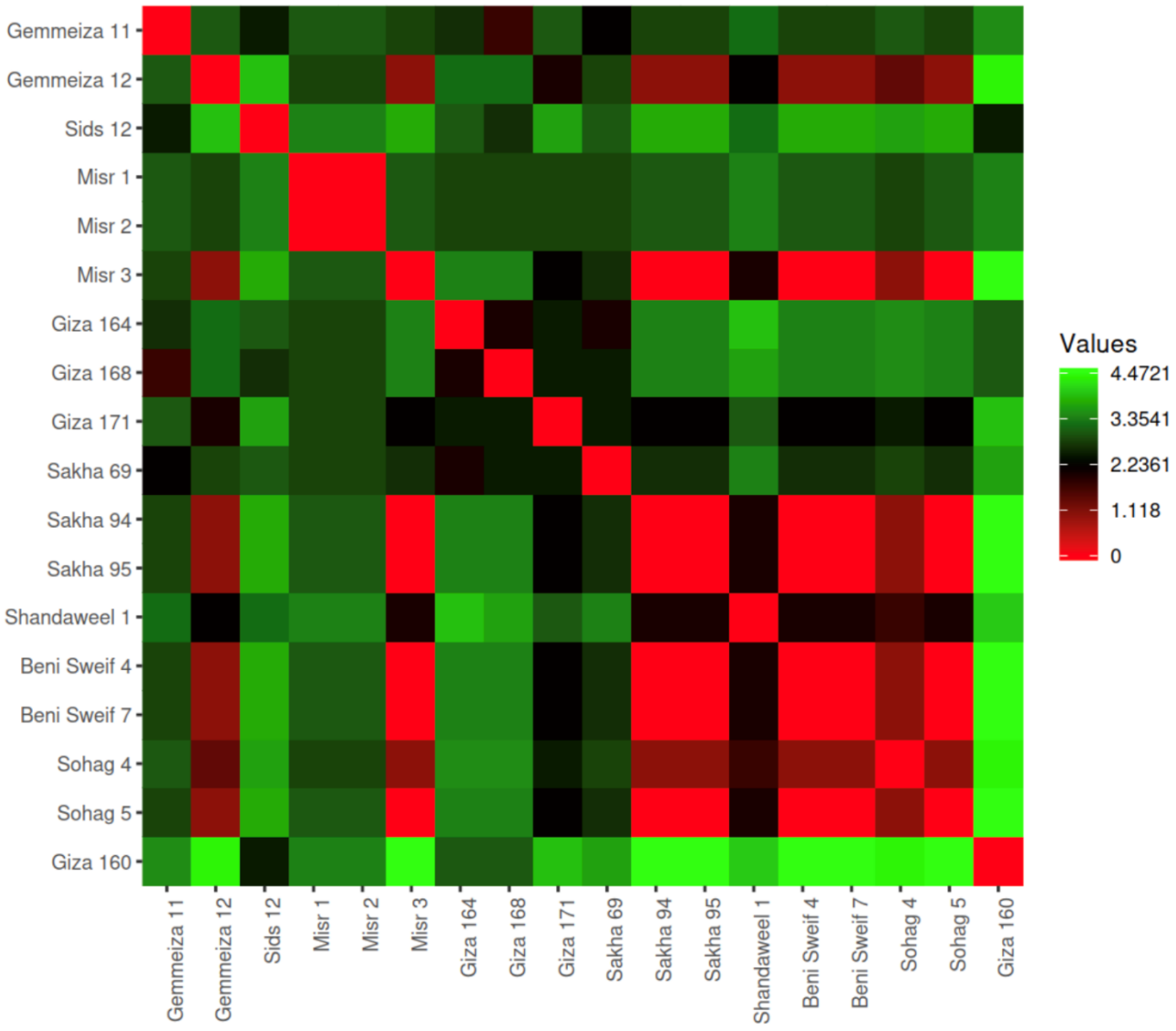

FIGURE 4 | Heat map exhibiting the correlation among the18 wheat genotypes. The red color represents a perfect relationship of each genotype with itself. The blocks of warmer colors displays clusters of non-related genotypes.

Genetic diversity of wheat genotypes by SCOT and SRAP:

1. SCoT markers diversity pattern:

In this study, out of 20 scot primers evaluated, eleven primers exhibited polymorphism in 18 wheat genotypes. The primer code, primer sequence, number of generated bands (total, polymorphic and monomorphic), unique band and percentage of polymorphism in the evaluated wheat genotypes are presented in Table (6) and Fig. (2). A maximum number of bands generated from 11 scot primer was 216 fragment with sizes ranged from 100 to 1500 base pairs (bp). These DNA fragments were characterized as 140 polymorphic and 5 monomorphic fragments (97% polymorphism). Furthermore, 71 unique fragments were exhibited in the SCoT profile. The maximum number of polymorphic bands (24 fragment) was generated by primer SCoT13 and the minimum ones (6 fragment) was generated by primer SCoT71. Moreover, the number of unique bands ranged from 1 (SCoT 70) to 23 (SCoT 13) as shown in table (6).

The similarity percentage among the 18 genotypes varied between 38 and 84%. The highest similarity value (84%) was noticed between Sakha 69 and Giza 164 which characterized as susceptible to stem and leaf rust. Meanwhile, the lowest similarity value (38%) was recorded between Shandaweel 1 and Gemmeiza 11 given that Shandweel 1 is resistant to stem rust but susceptible to leaf rust. meanwhile, Gemmeiza 11 is susceptible to stem and leaf rust (Table 7).

A dendrogram generated from scot analysis based on the UPGMA clustering grouped the 18 wheat genotypes into three main clusters (Fig. 3). The first cluster consists of Beni Sweif 7 and Sohag 4 of which susceptible to stem and leaf rust. The second cluster garbed the most susceptible genotypes to leaf and stem rust (Giza 164, Sakha 69, Sohag 5, Bani Sweif 4, Giza 160 and Shandaweel 1) in addition to three genotypes resistant to leaf rust (Giza 171, Sakha 94 and Sakha 95). The third cluster consists of the most resistant genotypes to leaf rust (Misr 1, Misr 2, Misr 3, Gemmiza 12

and Sids 12) and some susceptible to stem and leaf rust (Giza 168 and Gemmiza 11) remaining genotypes.

Table 6. Primer code, primer sequence, percentage of polymorphism, total, polymorphic and unique bands in the profile of SCoT primer.

Primer Code	Primer sequence	Number of bands			Unique band	Polymorphism %
		Total	Polymorphic	Monomorphic		
SCOT13	5'ACGACATGGCGACCATCG3'	47	24	-	23	100
SCOT14	5'ACGACATGGCGACCACGG3'	20	13	1	6	95
SCOT24	5'CACCATGGCTACCACCAT3'	19	12	1	6	94.7
SCOT26	5'ACCATGGCTACCACCGTC3'	20	15	-	5	100
SCOT31	5'CCATGGCTACCACCGCCT3'	22	15	-	7	100
SCOT33	5'CCATGGCTACCACCGCAG3'	20	11	2	7	90
SCOT34	5'ACCATGGCTACCACCGCA3'	26	18	-	8	100
SCOT52	5'ACAATGGCTACCACTGCA3'	13	10	-	3	100
SCOT61	5'CAACAATGGCTACCACCG3'	11	8	-	3	100
SCOT70	5'ACCATGGCTACCAGCGCG3'	9	8	-	1	100
SCOT71	5'CCATGGCTACCACCGCCG3'	9	6	1	2	88.8
Total		216	140		71	97
Average		19	12		6	

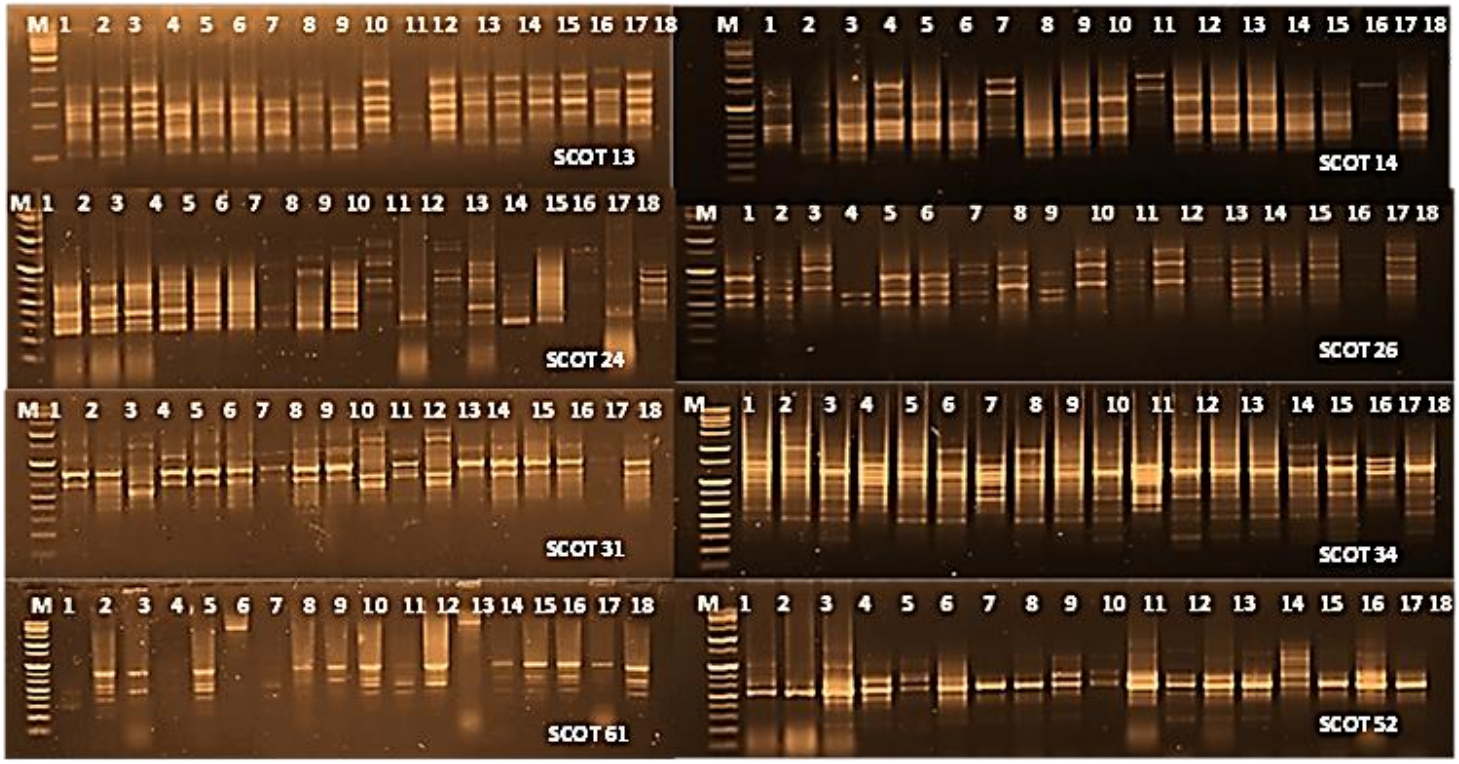

Fig. 2. Amplification profile obtained with eleven Scot primers in eighteen wheat genotypes. M: molecular marker (100 bp); lanes 1–18,

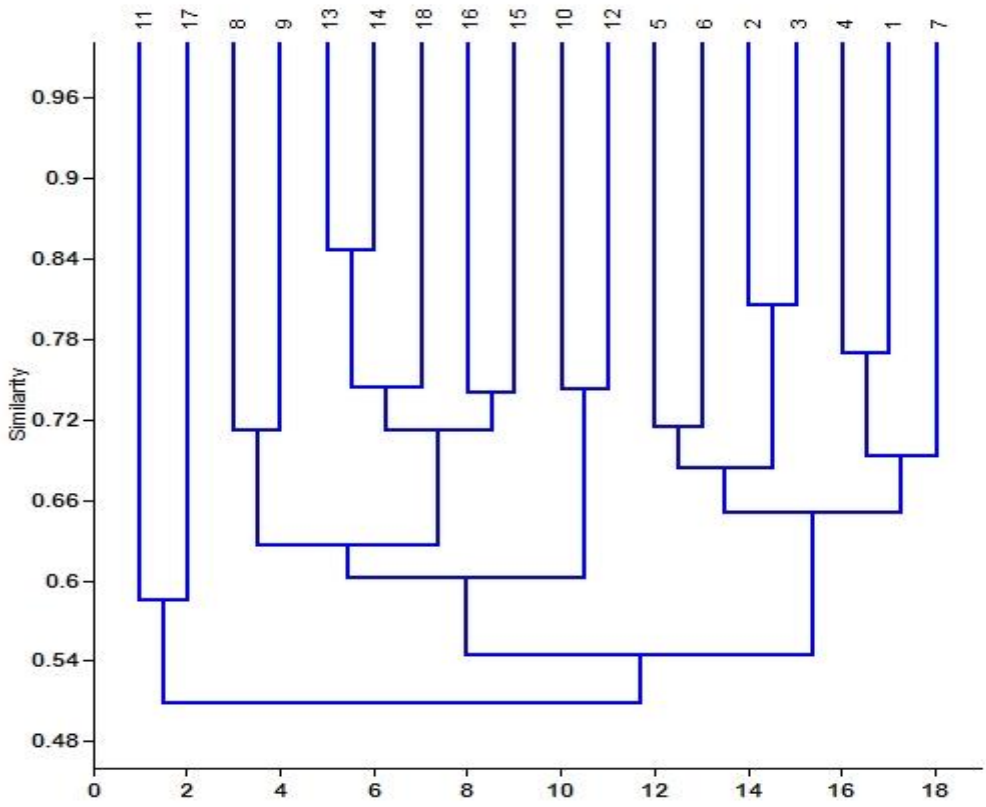

Fig. 3. A dendrogram based on UPGMA analysis using SCoT marker

Table 7. Genetic similarity matrix among the 18 wheat genotypes based on the Dice coefficient generated from scot marker.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	142	16	17	18
Gemmeiza 11	1.00																	
Gemmeiza 12	0.69	1.00																
Sids 12	0.67	0.80	1.00															
Misr 1	0.77	0.69	0.69	1.00														
Misr 2	0.65	0.73	0.67	0.77	1.00													
Misr 3	0.61	0.69	0.65	0.69	0.71	1.00												
Giza 168	0.68	0.56	0.53	0.70	0.62	0.64	1.00											
Giza 171	0.53	0.58	0.48	0.66	0.62	0.68	0.60	1.00										
Sakha 94	0.54	0.59	0.55	0.66	0.64	0.68	0.58	0.71	1.00									
Sakha 95	0.44	0.54	0.55	0.52	0.65	0.49	0.47	0.51	0.63	1.00								
Beni Sweif 7	0.53	0.49	0.55	0.57	0.55	0.50	0.50	0.50	0.57	0.53	1.00							
Shandaweel 1	0.38	0.50	0.48	0.50	0.54	0.51	0.47	0.51	0.63	0.74	0.51	1.00						
Giza 164	0.58	0.55	0.53	0.62	0.58	0.56	0.54	0.64	0.66	0.63	0.54	0.61	1.00					
Sakha 69	0.54	0.59	0.57	0.58	0.58	0.61	0.47	0.62	0.62	0.61	0.51	0.57	0.84	1.00				
Giza 160	0.50	0.52	0.51	0.63	0.57	0.59	0.53	0.65	0.71	0.57	0.53	0.62	0.74	0.74	1.00			
Beni Sweif 4	0.43	0.46	0.46	0.49	0.49	0.50	0.47	0.56	0.58	0.59	0.48	0.63	0.66	0.67	0.74	1.00		
Sohag 4	0.48	0.39	0.46	0.51	0.42	0.45	0.52	0.45	0.44	0.48	0.58	0.47	0.60	0.55	0.57	0.59	1.00	
Sohag 5	0.45	0.54	0.55	0.58	0.54	0.61	0.51	0.59	0.62	0.66	0.50	0.64	0.75	0.74	0.73	0.74	0.55	1.00

SRAP markers diversity pattern:

Out of out of 20 primer combinations examined, 14 primers exhibited polymorphism in 18 wheat genotypes. The primer code, primer sequence, number of generated bands (total, polymorphic and monomorphic), unique band and percentage of polymorphism in the evaluated wheat genotypes are presented in Table (8) and Fig. (5). A maximum number of bands generated was 230 fragment. These DNA fragments were characterized as 121 polymorphic and13 monomorphic fragments (99% polymorphism). Furthermore, 74 unique fragments were exhibited in the SRAP profile. The maximum number of polymorphic bands (11 fragments) was generated by Me1–Em2 and Me2–Em5 primers. The minimum ones (3 fragment) was generated by primer Me1- Em6. Moreover, the number of unique bands greatly varied among SRAP primers. Five (Me4-

Em5, Me4- Em6, Me5- Em2, Me5- Em5 and Me5- Em6) primers didn't generate any unique bands meanwhile Me4- Em2 and Me1- Em5 primers generated the highest number of unique bands (11 bands).

The similarity percentage among the 18 genotypes varied between 0.60 and 0.94%. The highest similarity value (0.94%) was noticed between Sakha 69 and Giza 160. Meanwhile, the lowest similarity value (0.60%) was noticed between Sohag 5 and Gemmeiza 11 (Table 9).

A dendrogram generated from SRAP analysis based on the UPGMA clustering grouped the 18 wheat genotypes into two main clusters (Fig.6). The first cluster consists of the most susceptible genotypes to stem and leaf rust (Shandaweel 1, Sohag 5, Giza 164, Sakha 69, Giza 160, Beni Sweif 4 and Sohag 4). The second cluster garbed the largest number of genotypes of which grouped the most resistant genotypes to leaf rust (Misr 1, Misr 2, Misr 3, Gemmeiza 12, Giza 171, Sakha 95 and Sakha 94) and some cultivars susceptible to stem and leaf rust (Beni Sweif 7, Giza 168, Gemmeiza 11, Sids 12).

Table 8: Primer code, primer sequence and combination total bands, polymorphic bands, unique band and percentage of polymorphism in the SRAP primer.

Primer Code SRAP	Primer combination	Primer sequence	Number of bands			Unique band	Polymorphism %
			Total	Polymorphic	Monomorphic		
Me1	**Me1- Em2**	**5′TGAGTCCAAACCGGATA3′**	20	11	-	9	100
Me2	**Me1- Em3**	**5′-TGAGTCCAAACCGGAGC-3′**	28	9	-	19	100
Me3	**Me1- Em4**	**5′-TGAGTCCAAACCGGAAT-3′**	16	10	-	6	100
Me4	**Me1- Em5**	**5′-TGAGTCCAAACCGGACC-3′**	17	6	-	11	100
Me5	**Me1- Em6**	**5′-TGAGTCCAAACCGGAAG-3′**	7	3	-	4	100
Em1	**Me2- Em1**	**5′-GACTGCGTACGAATTAAT-3′**	25	16	-	9	100
Em2	**Me2- Em3**	**5′-GACTGCGTACGAATTTGC-3′**	8	4	1	3	87.5
Em3	**Me2- Em5**	**5′-GACTGCGTACGAATTGAC-3′**	18	11	-	7	100
Em4	**Me4- Em2**	**5′-GACTGCGTACGAATTTGA-3′**	19	8	-	11	100
Em5	**Me4- Em4**	**5′-GACTGCGTACGAATTAAC-3′**	16	10	4	2	75
Em6	**Me4- Em5**	**5′-GACTGCGTACGAATTGCA-3**	7	6	1	-	85.7
	Me4- Em6		6	6	-	-	100
	Me5- Em2		11	7	4	-	63.6
	Me5- Em5		12	9	3	-	75
	Me5- Em6		20	11	-	-	100
		Total	230	121		74	99
		Average	16	8		5	

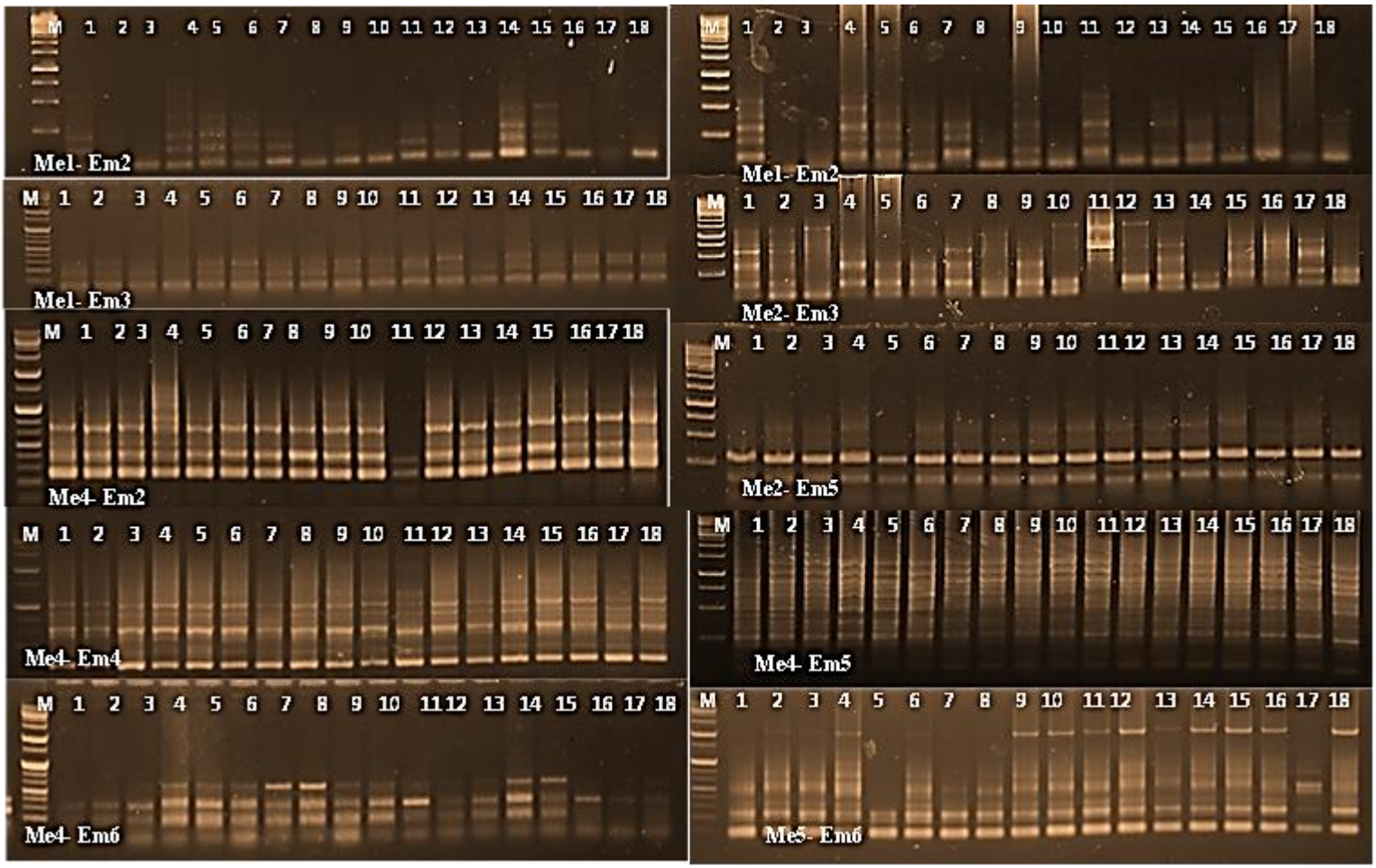

Fig. 5. Amplification profile generated by 14 SRAP primers in 18 wheat genotypes. M: molecular marker (100 bp); lanes 1–18,

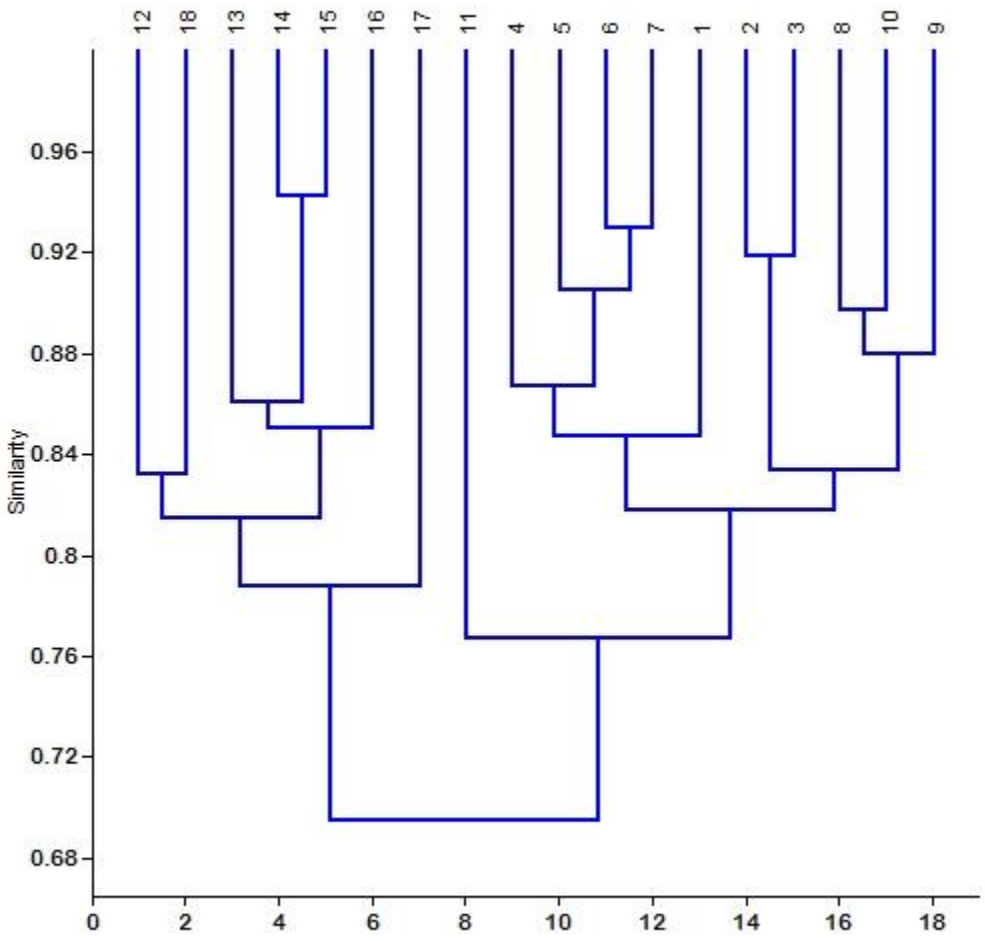

Fig .6. A dendrogram analysis based on UPGMA illustrating the genetic diversity produced by 14 SRAP primers among 18 wheat genotypes.

Table 9. Genetic similarity matrix among the 18 wheat genotypes based on the Dice coefficient generated from scot marker.

Column1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	142	16	17	18
Gemmeiza 11	1.00																	
Gemmeiza 12	0.85	1.00																
Sids 12	0.81	0.92	1.00															
Misr 1	0.83	0.77	0.81	1.00														
Misr 2	0.86	0.75	0.77	0.86	1.00													
Misr 3	0.85	0.85	0.87	0.87	0.89	1.00												
Giza 168	0.85	0.82	0.82	0.87	0.92	0.93	1.00											
Giza 171	0.79	0.85	0.91	0.81	0.81	0.89	0.86	1.00										
Sakha 94	0.80	0.78	0.81	0.85	0.83	0.85	0.86	0.88	1.00									
Sakha 95	0.76	0.81	0.84	0.81	0.78	0.82	0.81	0.90	0.88	1.00								
Beni Sweif 7	0.71	0.70	0.76	0.75	0.74	0.77	0.77	0.79	0.81	0.86	1.00							
Shandaweel 1	0.67	0.71	0.76	0.70	0.71	0.76	0.73	0.76	0.78	0.80	0.79	1.00						
Giza 164	0.67	0.70	0.70	0.68	0.70	0.70	0.70	0.74	0.75	0.77	0.74	0.81	1.00					
Sakha 69	0.66	0.67	0.70	0.71	0.72	0.72	0.70	0.70	0.73	0.75	0.77	0.85	0.86	1.00				
Giza 160	0.67	0.67	0.69	0.69	0.70	0.70	0.70	0.68	0.74	0.73	0.75	0.84	0.86	0.94	1.00			
Beni Sweif 4	0.63	0.64	0.66	0.67	0.64	0.66	0.66	0.67	0.70	0.73	0.72	0.78	0.85	0.84	0.86	1.00		
Sohag 4	0.61	0.64	0.68	0.65	0.67	0.66	0.66	0.71	0.67	0.70	0.68	0.77	0.81	0.79	0.78	0.83	1.00	
Sohag 5	0.60	0.61	0.68	0.62	0.61	0.63	0.62	0.68	0.71	0.73	0.73	0.83	0.78	0.82	0.83	0.83	0.75	1.00

Diversity analysis by combined data:

The generic dendrogram set up using the combined data generated from SCoT and SRAP analysis are presented in Fig. (10). A dendrogram divided the genotypes into two main clusters. The first cluster consists of the most resistant genotypes to leaf rust (Beni Sweif 7, Giza 171, Sakha 94, Misr 1, Misr 2, Misr 3, Giza 168, Gemmeiza 11, Gemmeiza 12 and Sids 12). Meanwhile, the second ones consists of the most susceptible genotypes to stem and leaf rust (Sakha 95, Shandaweel 1, Giza 164, Sakha 69, Giza 160, Beni Sweif 4, Sohag 5 and Sohag 4).

The similarity percentage among the 18 genotypes varied between 0.53 and 0.85%. The highest similarity value (0.85%) was noticed between Sakha 69 and Giza 160. Meanwhile, the lowest similarity value (0.35%) was noticed between three pairs of genotypes (Sohag 5 and Gemmeiza 11); (Shandaweel 1 and Gemmeiza 11) and (Sohag 4 and Gemmeiza 12) as presented in Table (10).

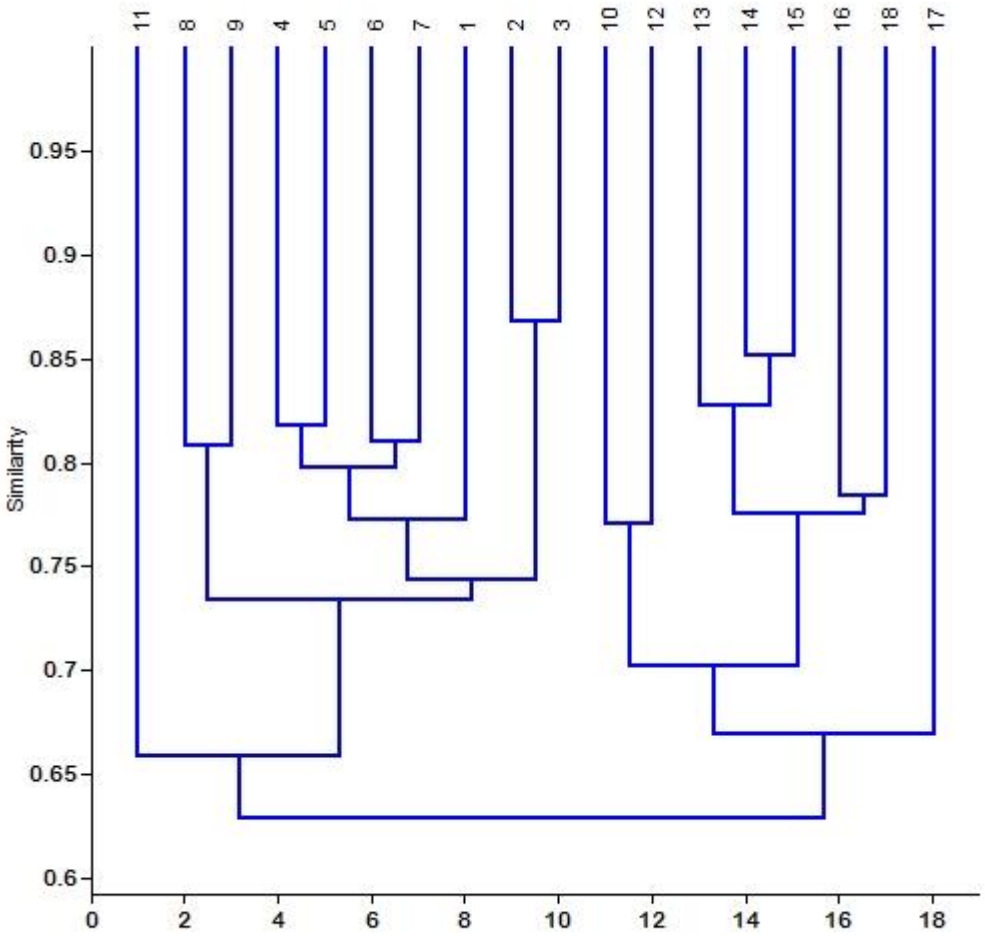

Fig .7. A dendrogram analysis based on UPGMA illustrating the genetic diversity produced by combined data among 18 wheat genotypes.

Table 10. Genetic similarity matrix among the 18 wheat genotypes based on the Dice coefficient generated from combined data.

Column1	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**142**	**16**	**17**	**18**
Gemmeiza 11	1.00																	
Gemmeiza 12	0.78	1.00																
Sids 12	0.74	0.87	1.00															
Misr 1	0.80	0.74	0.75	1.00														
Misr 2	0.76	0.74	0.72	0.82	1.00													
Misr 3	0.75	0.78	0.77	0.79	0.81	1.00												
Giza 168	0.78	0.71	0.69	0.80	0.79	0.81	1.00											
Giza 171	0.68	0.74	0.72	0.74	0.72	0.80	0.75	1.00										
Sakha 94	0.68	0.70	0.70	0.77	0.74	0.78	0.74	0.81	1.00									
Sakha 95	0.61	0.68	0.70	0.68	0.71	0.67	0.66	0.72	0.77	1.00								
Beni Sweif 7	0.63	0.61	0.66	0.67	0.65	0.66	0.66	0.67	0.71	0.71	1.00							
Shandaweel 1	0.53	0.61	0.63	0.61	0.63	0.65	0.61	0.65	0.71	0.77	0.66	1.00						
Giza 164	0.63	0.63	0.62	0.65	0.64	0.64	0.63	0.69	0.71	0.70	0.65	0.71	1.00					
Sakha 69	0.60	0.63	0.64	0.65	0.65	0.67	0.60	0.66	0.68	0.69	0.66	0.72	0.85	1.00				
Giza 160	0.60	0.60	0.61	0.66	0.64	0.65	0.63	0.67	0.73	0.66	0.65	0.74	0.80	0.85	1.00			
Beni Sweif 4	0.54	0.56	0.57	0.58	0.57	0.59	0.58	0.62	0.65	0.66	0.61	0.70	0.76	0.76	0.81	1.00		
Sohag 4	0.55	0.53	0.58	0.59	0.56	0.57	0.60	0.60	0.57	0.60	0.64	0.63	0.71	0.68	0.69	0.72	1.00	
Sohag 5	0.53	0.58	0.62	0.60	0.58	0.62	0.57	0.64	0.67	0.70	0.62	0.74	0.76	0.78	0.78	0.78	0.66	1.00

Effect of Cu-chitosan composite nanoparticle and application methods on incubation, latent periods and infection type of wheat stem rust disease.

In this experiment, the 18 wheat genotypes were sprayed with Cu-chitosan composite nanoparticle either before or after and before inoculation with uridiospores of stem rust to determine the effect of this solution and its application method in controlling the disease. the estimation was expressed as incubation, latent periods and infection type as presented in table (11). The infection was reduced when the plant sprayed 24 h before and 24 h before and after inoculation. Incubation and latent periods were increased in treated plant genotypes. Besides, the treatment gave the lowest infection type compared to the control. The foliar spray application didn’t affect the efficacy of the tested treatment.

Table (11) Effect of Cu-chitosan NPs treatments and application methods on incubation, latent periods and infection type of wheat stem rust disease.

Treatment/ Verities	Incubation period/day			Latent period/day			Infection type		
	Control	Before*	Post**	Control	Before*	Post**	Control	Pre	post
Gemmeiza 11	8.59	14.3	15.04	12.04	19.95	19.97	3	2+	2+
Gemmeiza 12	7.23	16.27	15.54	13.28	20.05	20.31	2	1+	1
Sids 12	6.98	14.21	14.56	13.46	19.92	19.35	4	2++	2+
Misr 1	5.67	13.16	13.45	11.67	18.67	18.67	4	2+	2+
Misr 2	5.76	13.33	13.57	11.06	20.21	20.31	3	2++	2+
Misr 3	7.85	16.83	16.92	13.45	21.22	21.04	2	1	1
Giza 164	6.03	13.57	13.89	13.78	19.92	20.81	4	2++	3
Giza 168	7.07	15.76	15.78	12.86	20.05	20.09	3	1	2
Giza 171	8.45	15.37	15.62	12.35	19.07	19.08	2	1	1
Sakha 69	7.63	14.56	14.87	12.35	19.09	20.18	3	2	1++
Sakha 94	8.12	16.93	16.36	11.92	19.92	19.94	2	1	1
Sakha 95	7.95	17.56	17.45	13.00	20.63	20.97	3	1	1+
Shandaweel 1	8.31	17.93	17.97	13.57	20.46	20.93	3	2	2+
Beni Sweif 4	8.34	17.84	17.92	14.01	20.23	22.03	2	1	2
Beni Sweif 7	7.93	17.36	17.35	12.04	19.07	19.04	2	1+	1
Sohag 4	6.38	15.93	15.38	13.75	19.2	19.83	2	1+	1
Sohag 5	7.01	16.26	16.17	13.84	19.35	19.47	2	1	1
Giza 160	5.01	12.35	12.48	11.87	19.08	19.09	4	3	3+

* spray before inoculation by 24 hours, **Spray before and after inoculation by 24 hours

0, 1,1+, 2, 2+, 2++ (Low infection types) Resistance

3. 4 (High infection types) Susceptible

Database Search, Structural Modelling, and Model Validation.

the homology of the query protein sequences with the target proteins from pdb . The query coverage of proteins sequences showed MAP kinase 1 [Puccinia triticina] **and PGTG** *Puccinia graminis f. sp. tritici* (84% , 12) query coverage's with (56.14 , 56.15) identity with the template proteins (2vkn.1.A , 2fa2.1.A) they used as a template proteins for homology modeling of our target proteins

Protein	GenBank/ID:	Template	Seq Identity	Coverage	QMEAN
MAP kinase 1 [Puccinia triticina]	AAY89655.1	2fa2.1.A	56.14	**0.84**	**-1.53**

PGTG *Puccinia graminis f. sp. tritici*	10532062	2vkn.1.A	56.14	**0.12**	**-0.69**

Structural Modeling, *in silico* Characterization, and Model Validation

The SWISSMODLE server generated (25 ,35) predictive models for MAP kinase 1 [Puccinia triticina] **and PGTG** *Puccinia graminis f. sp. tritici* proteins with different (QMEAN) score values. The model with low values for QMEAN score(-1.53 and -0.69) were selected as a final model for *in silico* characterization and docking studies. The selected model was verified for their stereochemical quality assessment. Furthermore, in each case of qualitative assessment, a comparative study was done with experimentally solved crystal structures, to check the quality, reliability, accuracy, stability and compatibility of the computationally predicted protein. The Ramachandran plot obtained through The PROCHECK module of the PDBSum server revealed that the predicted model

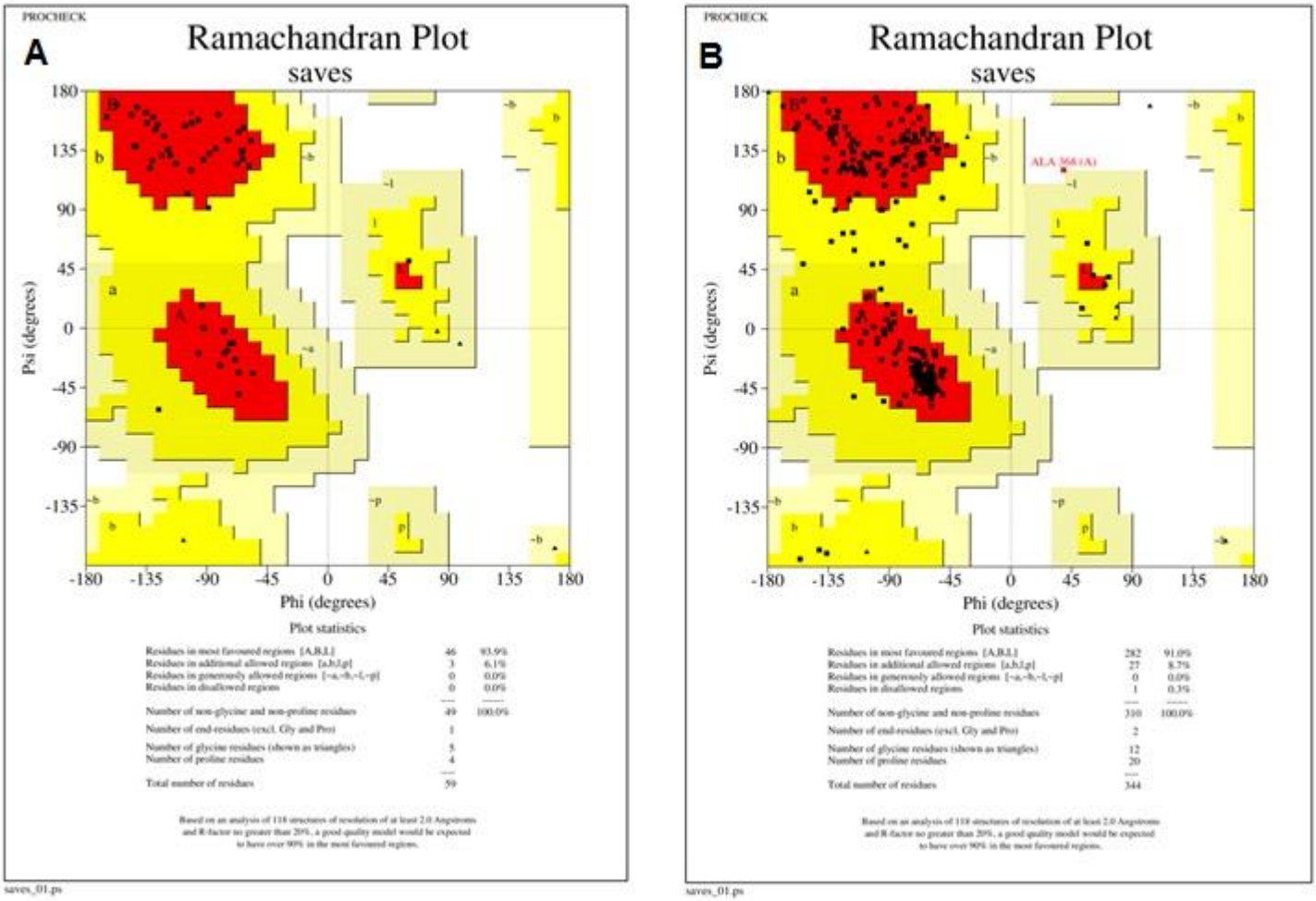

Fig .8. A Ramachandran plot based on The PROCHECK module of the PDBSum server for proteins models of MAP kinase 1 [Puccinia triticina] **and PGTG** *Puccinia graminis f. sp. Tritici*

The PROCHECK module of the PDBSum server, further justified the stereochemical goodness of the predicted models MAP kinase 1 [Puccinia triticina] **and PGTG** *Puccinia graminis f. sp. Tritici* , with 91% , 93% residues accommodating in the most favored regions (A, B, and L) and only 8.7% , 6.1 % residues occupied in the additionally allowed regions (a, b, l, and p) representatively .This confirms the predicted models quality MAP kinase 1 [Puccinia triticina] **and PGTG** *Puccinia graminis f. sp. tritici* had good stereochemical quality and was close to the template structure. the ProQ is a neural network-based predictor based on a number of structural features predicts the quality of protein model. The ERRAT score for the modeled structure was found to be 96.22% ,

78.4314 representetevely The Verify 3D evaluated that the predicted proteins MAP kinase 1 [Puccinia triticina] **and PGTG** *Puccinia graminis f. sp. tritici* have 96.22% , 81.36% of the residues have averaged 3D-1D score >= 0.2 **Pass** At least 80% of the amino acids have scored >= 0.2 in the 3D/1D profil

Target	Ligands	Binding energy	X,Y , Z value
MAP kinase 1 [Puccinia triticina]	Chitosan	-6.6	center_x = 8.6875 center_y = 7.9130 center_z = 26.0847
	cu-chitosan	-6.9	
PGTG *Puccinia graminis f. sp. tritici*	Chitosan	-4.6	center_x = 11.3055 center_y =22.877 center_z = 1.3282
	cu-chitosan	-5.6	

Binding site prediction, Protein-ligand docking

The predicted model was visualized through the visualization module of the Discovery Studio 3.0 (Figure 5). The ligand binding sites identified in the target proteins structure .

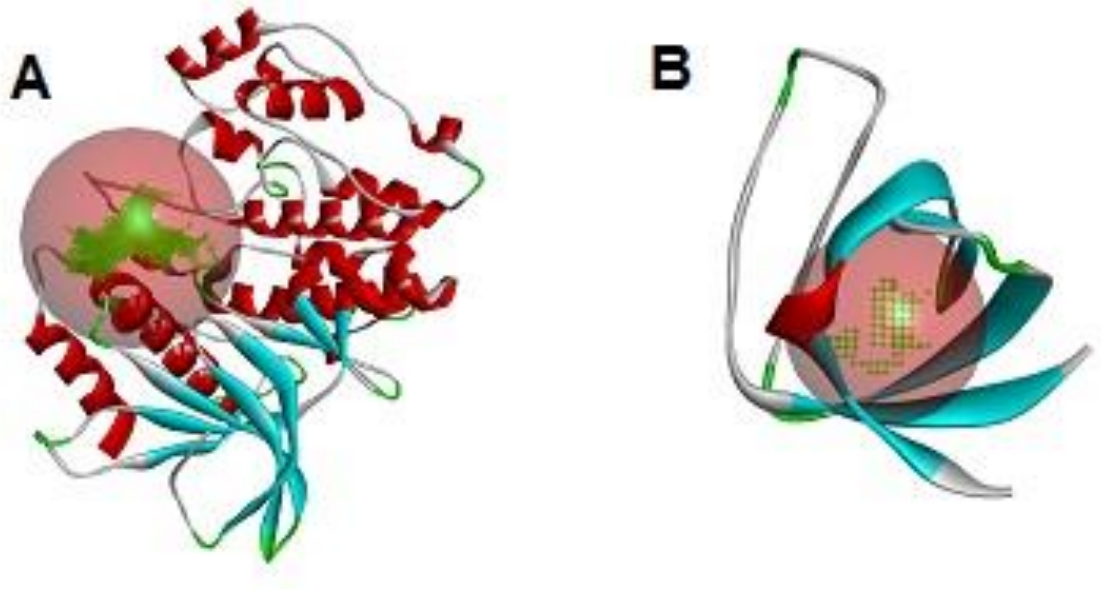

Fig 9 represent the binding site of the MAKP1 of the fungus and B its the PGTG protein The binding sites inside the proteins, the protein with red balls showing active sites

Active Site Prediction and Protein–Fungicide Interaction

The putative ligand binding sites (both major and minor) for the predicted protein were identified through ***Discovery studio software***.

The modeled proteins MAPK1 and PGAT was docked with all the chitosan and chitosan with cu to generate their binding mode which done to refine the best pose with allowed conformational change in the MAPK1 and PGAT . We have evaluated the protein–fungicide interaction through ***Discovery studio software***. It was found that the tool have discrepancies in results for accurate pose prediction among the

various putative docking poses, revealed through scoring functions, which might leads into conclusion that, docking scores are not sufficiently precise to represent the protein ligand binding affinity (Suenaga et al., 2012).

for the MPAK1 its bind to chitosan with ΔG_{bind} and docking score are -6.9 ….. kcal/mol with the interactive binding site residues (ARG106 , ASP192 , GLU210 , GLY212 , LEU213 , GLU223 , THR224 , GLY225 , MET227 , VAL231 , ALA232 and ARG234)

for the MPAK1 its bind to chitosan with CU the ΔG_{bind} are -6.6kcal/mol with the interactive binding site residues (ARG106, LYS194, GLU223, MET227, GLU229, VAL231, ALA232, GLN247 and GLY273)

for the PGAT its bind to chitosan with ΔG_{bind} -4.6kcal/mol with the interactive binding site residues (TYR441 , PRO448 , ASN449 , SER483,SER478,GLN470,LYS467)

for the PGAT its bind to chitosan with CU the ΔG_{bind} are -5.6 kcal/mol with the interactive binding site residues (ALA443 , PRO448 , ASN449 , GLY452 , PHE453 , GLN470 , ARG472 , GLY476 , GLU477 , SER478)

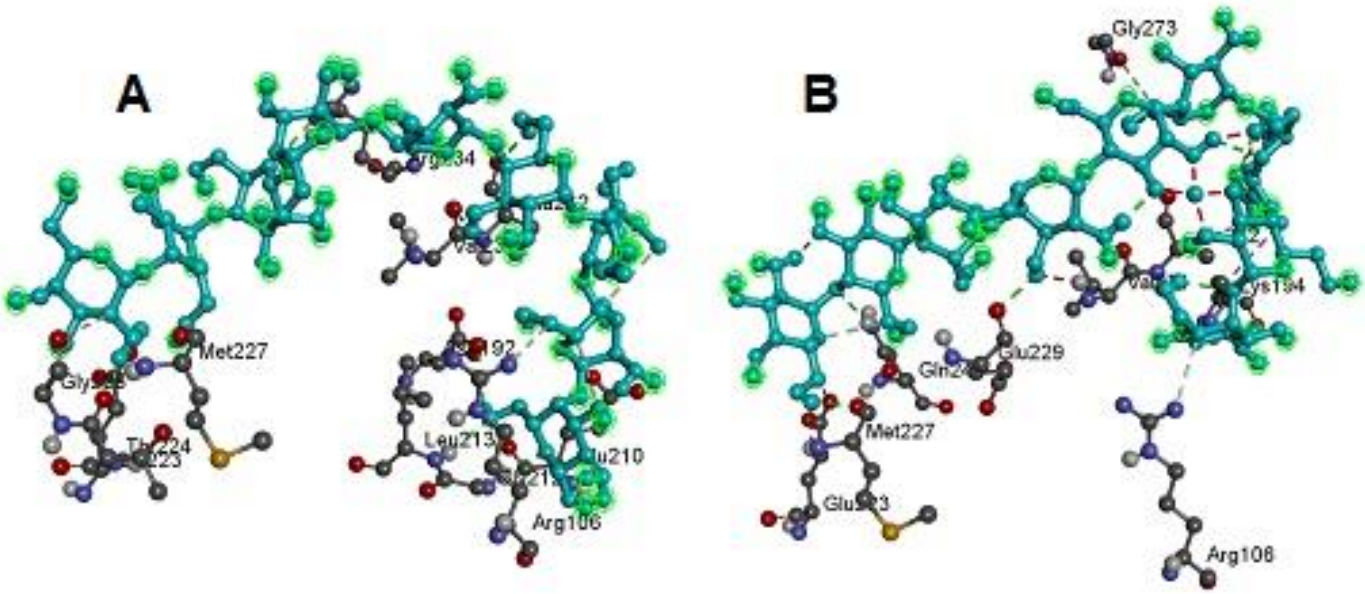

FIGURE 10. (A) The 3D surface view of MAPK1 modeled protein with chitosan reveals only the interactive residues on the binding site of a protein. (B) chitosan MAPK1 –Cu was the interactive residue of binding sites of the protein binding sites (active sites).

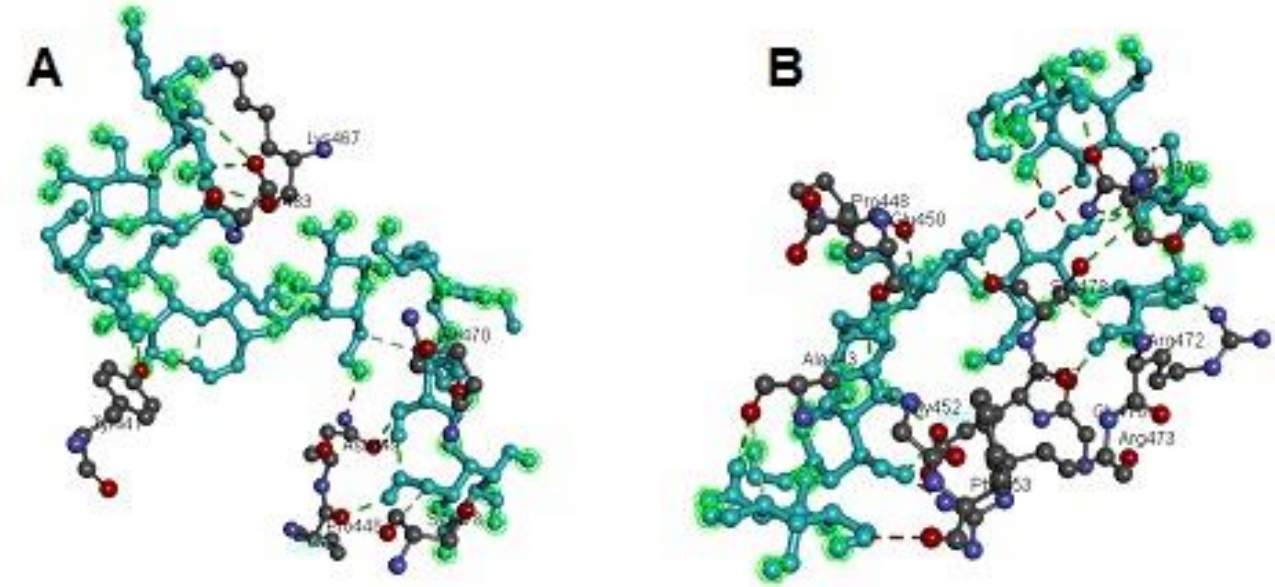

FIGURE 11 (A) The 3D surface view of PGAT modeled protein with chitosan reveals only the interactive residues on the binding site of a protein. (B) PGAT chitosan –Cu was the interactive residue of binding sites of the protein binding sites (active sites).

Discussion

Gain of wheat lines tolerance to stem rust caused by *P. graminis f. sp. tritici* and leaf rust caused by *P. triticina* are urgent because of the rareness of resistance genes against these global wheat destructive diseases. Thus, the world needs to identify new resistance gene resources. Egyptian wheat varieties collected from different regions have a diverse resistant genes , thus it may be exploited as a new source of rust resistance genes for gaining and developing novel resistant wheat genotypes (Sthapit et al., 2014 and olivera et al. 2018). Knowledge of genetic diversity and population structure is a crucial step for an efficient use of available material in a plant breeding program and for germplasm conservation strategies (Mansi et al. 2019). In this work, eighteen Egyptian wheat genotypes were evaluated in their seedling stage against 20 different *pgt* races under greenhouse conditions as well as in their adult stage in the fields located in two different locations during 2019-20 growing season. The values of host interaction type on wheat seedlings demonstrated that, among the 18 tested wheat seedling genotypes, 10 genotypes (Misr 3, Sakha 94, Sakha 95, Beni Sweif 4, Beni Sweif 7, Sohag 4 and Sohag 5, Gemmeiza 12, Shandaweel 1 and Giza 171) were resistant with different levels and 8 genotypes (Gemmeiza 11, Sids 12, Misr 1, Misr 2, Sakha 69, Giza 164, Giza 168 and Giza 160) were susceptible with different levels to the tested stem rust races. Misr 3, Sakha 94, Sakha 95, Beni Sweif 4, Beni Sweif 7, Sohag 4 and Sohag 5 genotypes were resistant to all races, but Giza 160 was susceptible to all races. Therefore, the tested genotypes have a diverse genetic backgrounds owing to the obvious difference among them in their susceptibility to stem rust races. The high frequency of MS to S genotypes among the 18 genotypes suggests presence of ineffective stem rust resistance genes in their backgrounds. similarly, Chaves et al (2011) reported high frequency of moderately susceptible to

susceptible infection types within Brazilian genotypes with aluminium tolerance backgrounds. Furthermore, 9 and two genotypes displayed resistant to moderately resistant (RMR) to leaf rust and stem rust, respectively. This suggests that these lines could be carrying diverse resistance genes which are effective against leaf rust and stem rust and could be efficiently used as parents to improve resistance to leaf rust and stem rust in breeding programs (Mahmoud *et al*., 2015).

On the other hand, the AUDPC values were differed among the tested genotypes, the disease and locations. The AUDPC more than 250 classified as highly susceptible to rusts but those with values less than 250 classified as highly resistant genotypes. Therefore, the genotypes, Giza 164, Sakha 69 and Giza 160 were highly susceptible to stem rust and leaf rust in Giza and sids stations with highly AUDPC values. Misr 1 and Misr 2 genotypes were highly susceptible to stem rust but not leaf rust. Beni Sweif 4 and Gemmeiza 11 were highly susceptible to leaf rust but not stem rust. Sids 12 and Sakha 95 were highly susceptible to stem rust in sids station only. The remaining genotypes displayed the lowest AUDPC values (less than 250) either for stem rust or leaf rust in both locations. These results are in harmony with those obtained by (Hasan *et al.* 2016, El-Orabey *et al*. 2017, Abou-Zeid *et al.* 2018, Hanaa *et al*. 2019 and Shehab-Eldeen and Abou-Zeid 2020).

The study of genetic relationship among different plant species and genera is very important where the plant evolution requires the discover of new relationships among plants (Savolainen and Chase, 2003). In this study, we assess the genetic diversity among 18 Egyptian wheat genotypes by using two different gene-targeted molecular markers, *i.e*. SCoT and SRAP. These markers have been mentioned in different references by their powerful in determining the genetic diversity among different crops such as wheat (Deif et al., 2013, Etminan et al., 2016 and El-Moneim, 2019). The generated results of this work exhibited the potentiality of the two markers in distinguished resistant from susceptible to stem rust and leaf rust in some wheat genotypes. In this respect, out of 20 scot and SRAP primers evaluated, eleven and fourteen primers, respectively exhibited polymorphism in 18 wheat genotypes. SCoT and SRAP primers generated 140 and 121 polymorphic band

with 97 and 99% polymorphism, respectively. Among them, 71 and 73 were unique loci for SCoT and SRAP, respectively. The highest similarity values among the 18 genotypes obtained by SCoT and SRAP primers were noticed between Sakha 69 and Giza 164 which characterized as susceptible to stem and leaf rust that could mean that the both genotypes may carry ineffective stem rust and leaf rust resistance genes in their background. Shandweel 1 (moderately resistant to stem rust) possess genetic background far from Gemmeiza 11 (susceptible to both stem and leaf rust) according to similarity value (38%) obtained by scot analysis, but according to SRAP analysis, Shandweel 1 was vary with Sohag 5 (susceptible to stem and leaf rust) with 0.95% similarity. In general, SRAP was more effective than SCoT in differentiate between resistant and susceptible wheat genotypes especially to leaf rust as it exhibited the greatest genetic polymorphism. SCoT13 primers gave the biggest number of polymorphic and unique bands. Furthermore, SRAP (Me1–Em2 and Me2–Em5) exhibited the biggest number of polymorphic band but Me4- Em2 and Me1- Em5 SRAP primers gave the biggest unique bands. Thus, they deemed to be the best primers for determining and differentiate the wheat genotypes. Furthermore, the similarity matrix revealed the close relation between Sakha 69 and Giza 160 genotypes both in SRAP and combined data analysis. The potentiality of the markers in differentiate and determine the genetic diversity among the genotypes considered the most important feature of the best marker (El-Moneim, 2019). Therefore, as the great genetic variation observed among the 18 wheat genotypes, SRAP and SCoT markers could be suitable for distinguishing the resistance and susceptible of wheat genotypes for stem rust and leaf rust. This result are in agreement with those obtained by Etminan et al., 2016 and El-Moneim, 2019.

Chitosan NPs have previously been reported as immune modulator through induction of antioxidant/defense enzymes activity in tea and finger millet plants (11, 19). In this work, sprayed of wheat seedlings with cu-chitosan composite nanoparticles either before or before and after inoculation with the uredospore s of stem rust successfully reduced the infection type compared to the control and increased the Incubation and latent

periods. The positive effects on both incubation and latent periods might be due to the induction of systemic resistance (ISR) as a main mechanism of plant response (Loeffler et al. 1986). The antimicrobial activity of chitosan nanoparticles (CS/NPs) has been recently studied and it has been demonstrated that CS/NPs has the ability to control of Fusarium head blight of wheat. Kheiri et al. (2016) demonstrated that CS/NPs have significant inhibitory effects on the fungal growth, colony formation and conidial germination of F. graminearum. Also, Taran et al. (2017) reported that colloidal solution of Cu, Zn-nanoparticles decreased the negative effect of drought stress on wheat. In particular, increased activity of antioxidative enzymes reduced the level of accumulation of thiobarbituric acid reactive substances (TBARS) and stabilized the content of photosynthetic pigments and increased relative water content of leaves. Moreover, the changes in plant morphometric indexes such as leaf area and relative water content in leaves are the result of the adaptation mechanism triggered by colloidal solution of Cu, Zn-nanoparticles under drought conditions. Similarly, another research finding of Yasmeen et al. (2017) indicated that Cu NPs improve the tolerance of wheat varieties to drought stress. Cu NPs improved the yield and drought stress tolerance of wheat by mediating starch degradation, glycolysis, and tricarboxylic acid cycle in wheat varieties (Yasmeen et al., 2017).

Our results indicate that SRAP (Me1–Em2 and Me2–Em5) and SCoT13 primers are suitable for determining genetic diversity in wheat genotypes. Thus, their sequences can be used to identify suitable parents of wheat genotypes to identify the resistance genes. Furthermore, spray foliar plant with cu-chitosan composite decreased the host reaction type to stem rust and increase the incubation and latent periods. In the silico analysis, the MAP kinase 1 [Puccinia triticina] and PGTG Puccinia graminis f. sp. tritici activity is suppressed and inhibited by the decrease of deferent gen in rust and stem of our spices on our signaling pathways.

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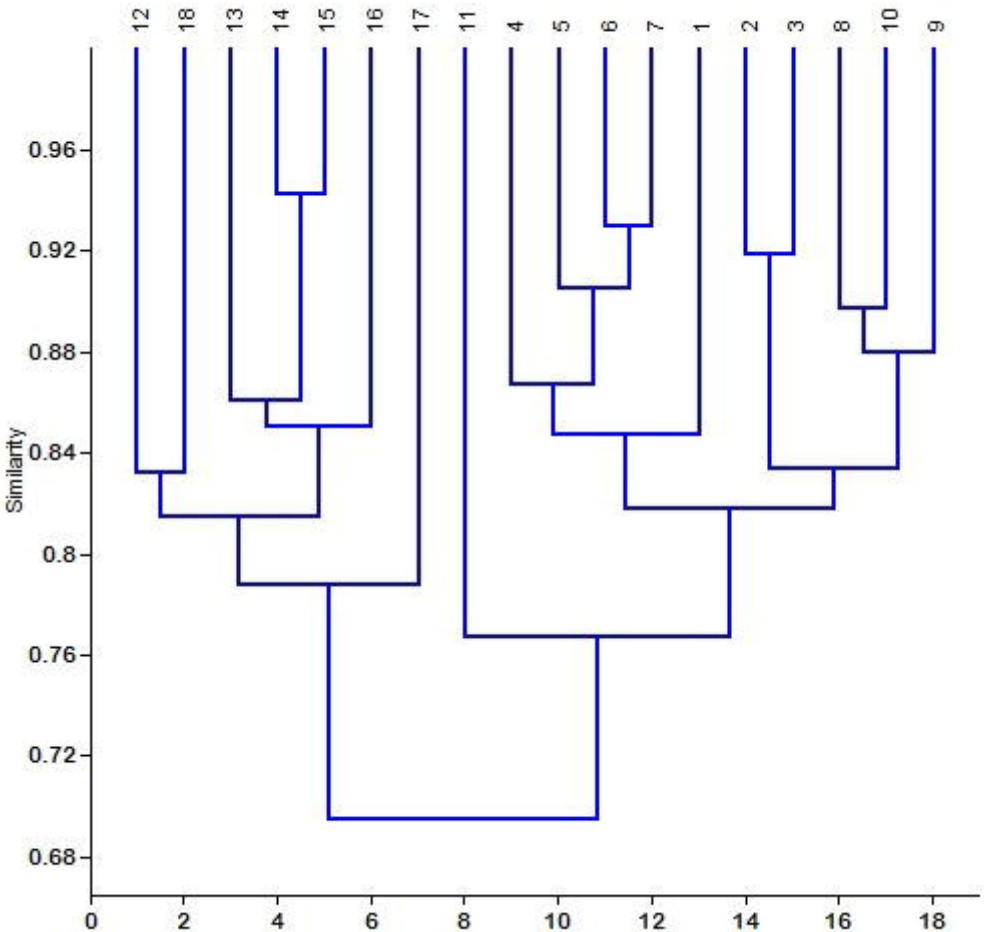
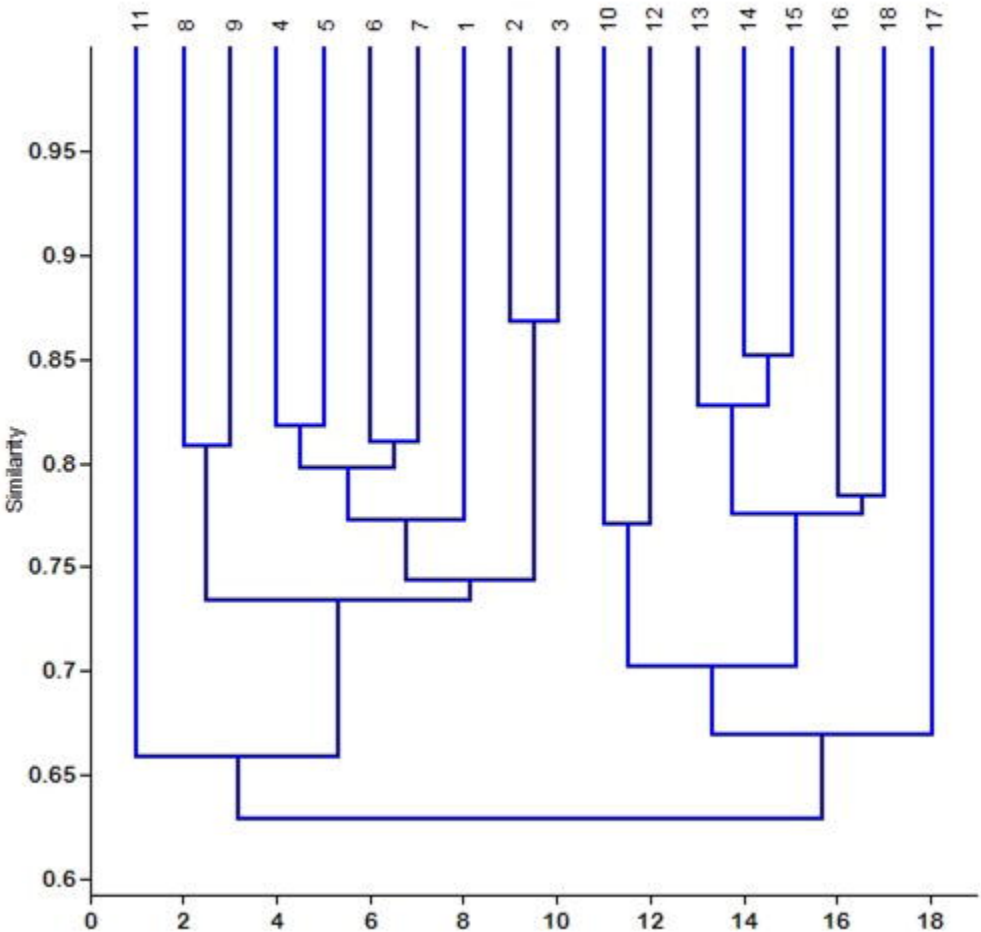

12
18
13
14
15
16
17
11
4
5
6
7
1
2
3
8
10
9
Similarity
0.96
0.92
0.88
0.84
0.8
0.76
0.72
0.68
0
2
4
6
8
10
12
14
16
18

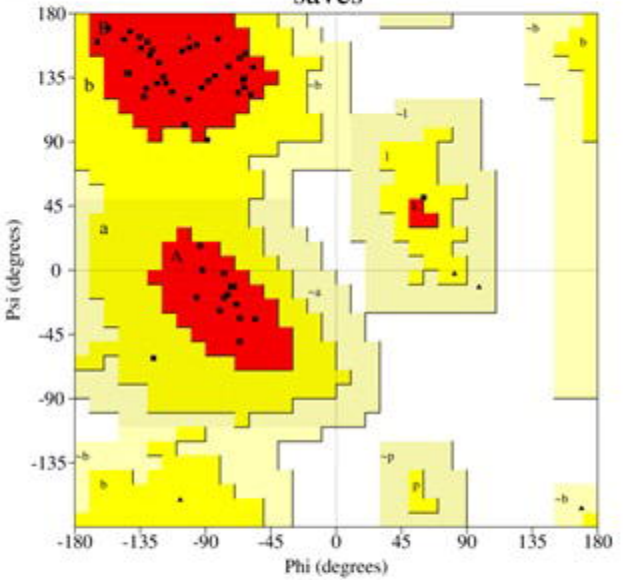

11
8
9
4
5
6
7
1
2
3
10
12
13
14
15
16
18
17
Similarity
0.95
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0
2
4
6
8
10
12
14
16
18

PROCHECK

A

Ramachandran Plot

saves



Plot statistics

Residues in most favoured regions [A,B,L]	46	93.9%
Residues in additional allowed regions [a,b,l,p]	3	6.1%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	49	100.0%
Number of end-residues (excl. Gly and Pro)	1	
Number of glycine residues (shown as triangles)	5	
Number of proline residues	4	
Total number of residues	59	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

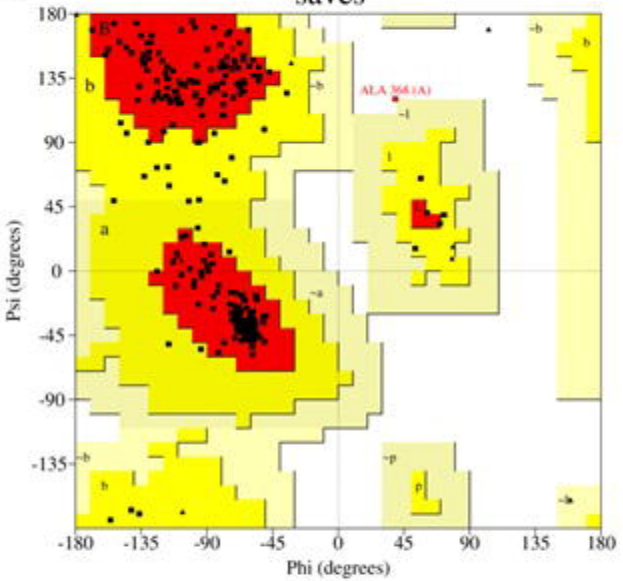
saves_01.ps

PROCHECK

B

Ramachandran Plot

saves

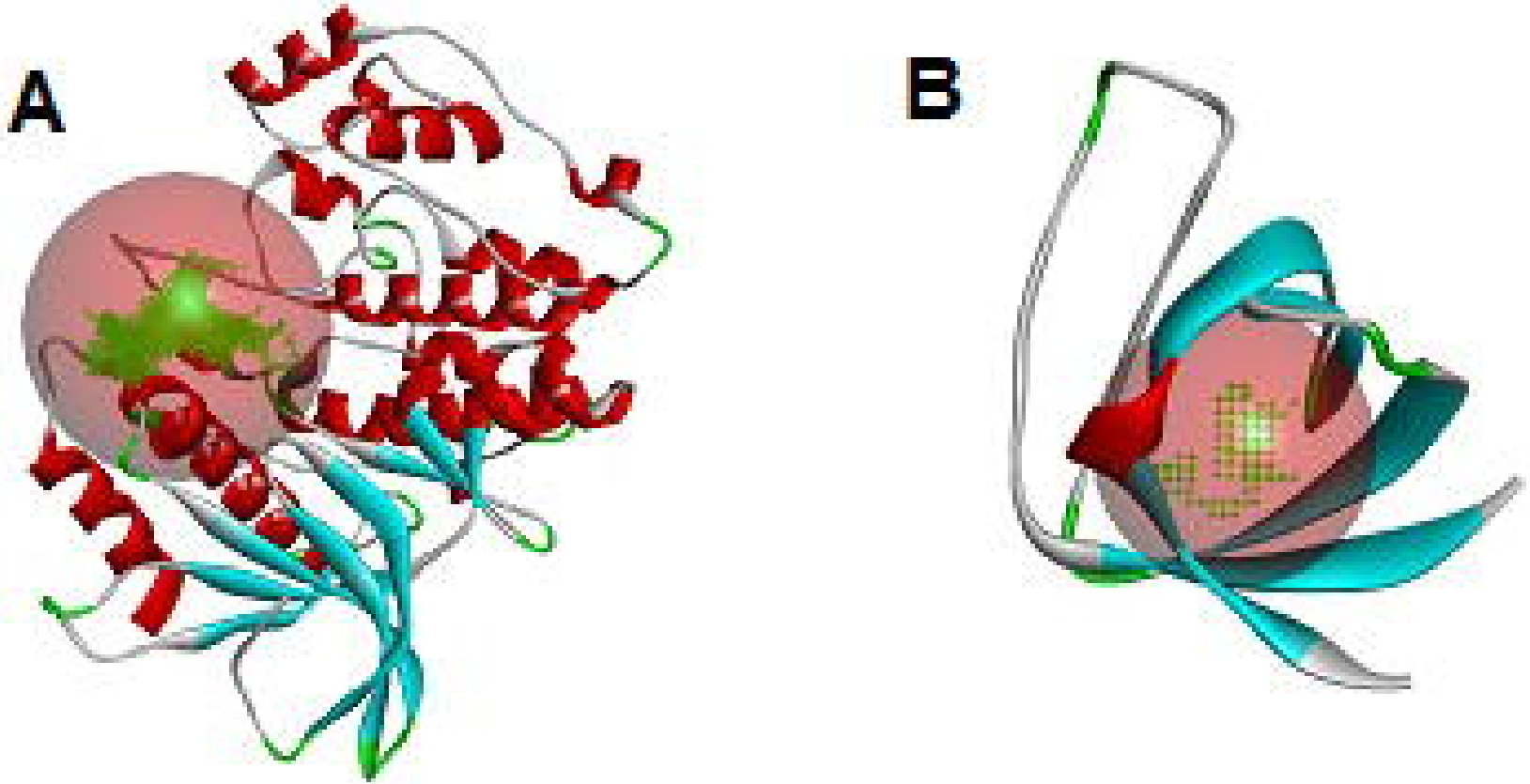


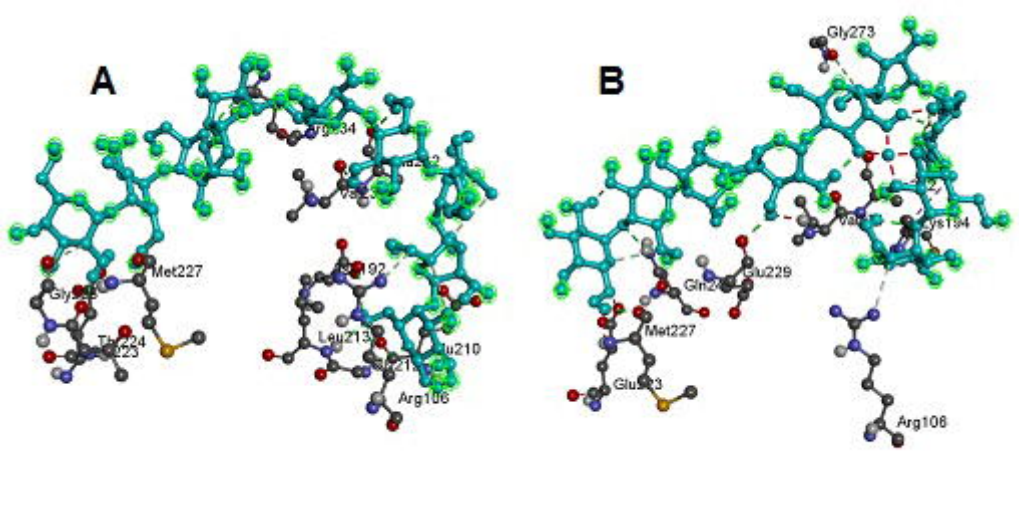
Plot statistics

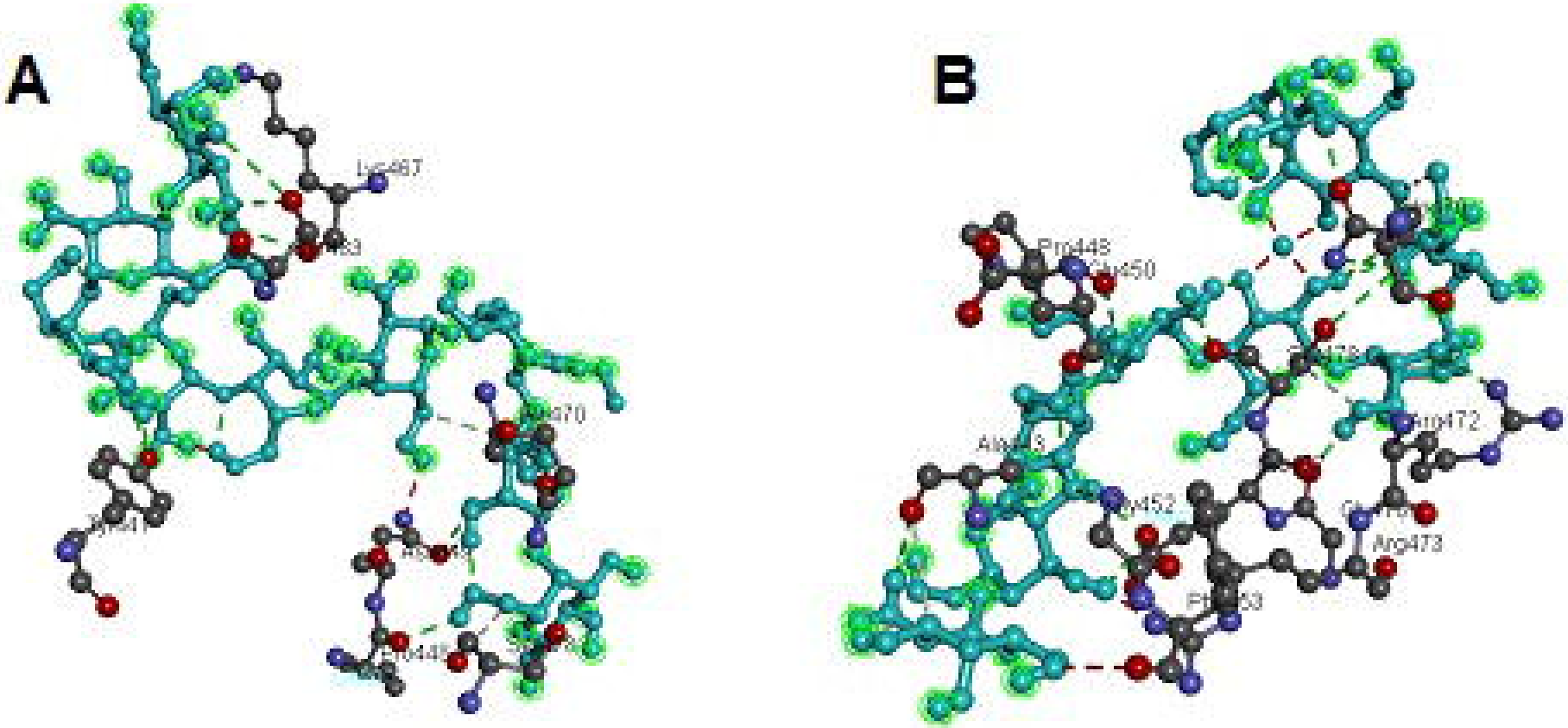
Residues in most favoured regions [A,B,L]	282	91.0%
Residues in additional allowed regions [a,b,l,p]	27	8.7%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	1	0.3%
Number of non-glycine and non-proline residues	310	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	12	
Number of proline residues	20	
Total number of residues	344	

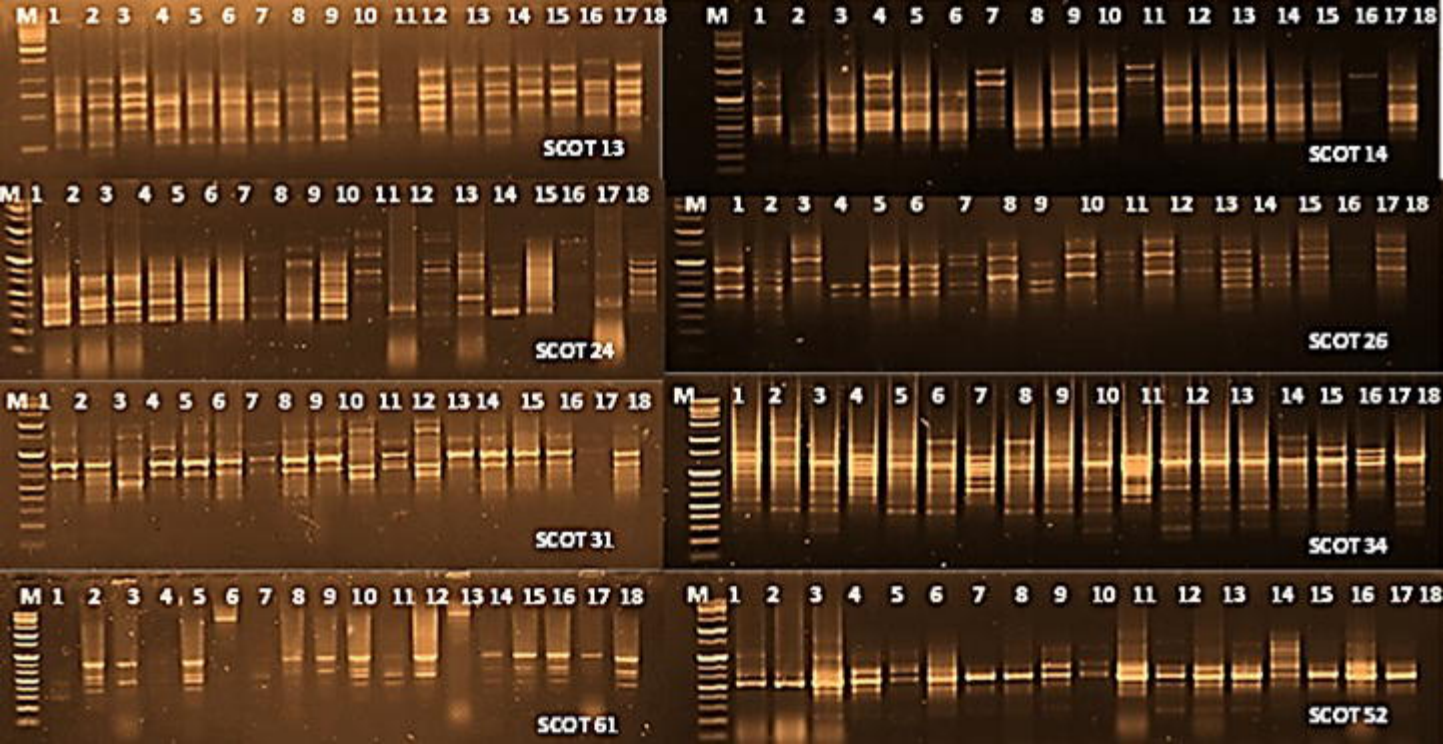
Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

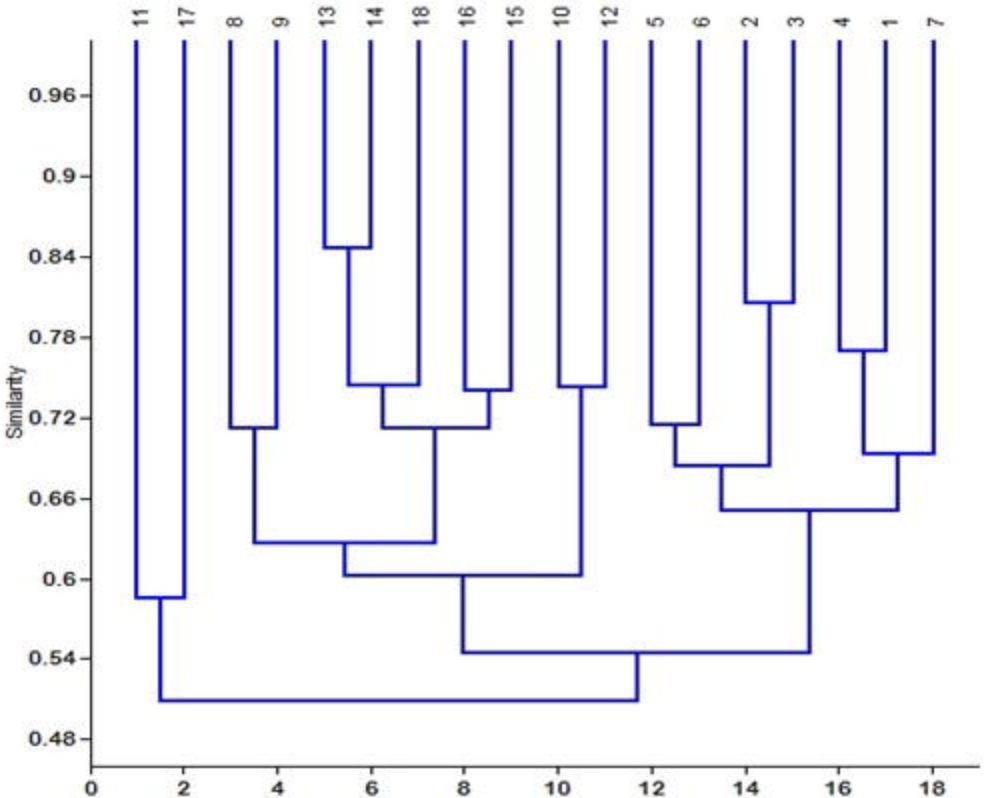
saves_01.ps


A
B


A
Met227
Leu213
Arg106
B
Gly273
Glu229
Met227
Arg106


A
B
Arg473


M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
SCOT 13
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
SCOT 14
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
SCOT 24
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
SCOT 26
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
SCOT 31
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
SCOT 34
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
SCOT 61
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
SCOT 52


11
17
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16
15
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12
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6
2
3
4
1
7
Similarity
0.96
0.9
0.84
0.78
0.72
0.66
0.6
0.54
0.48
0
2
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6
8
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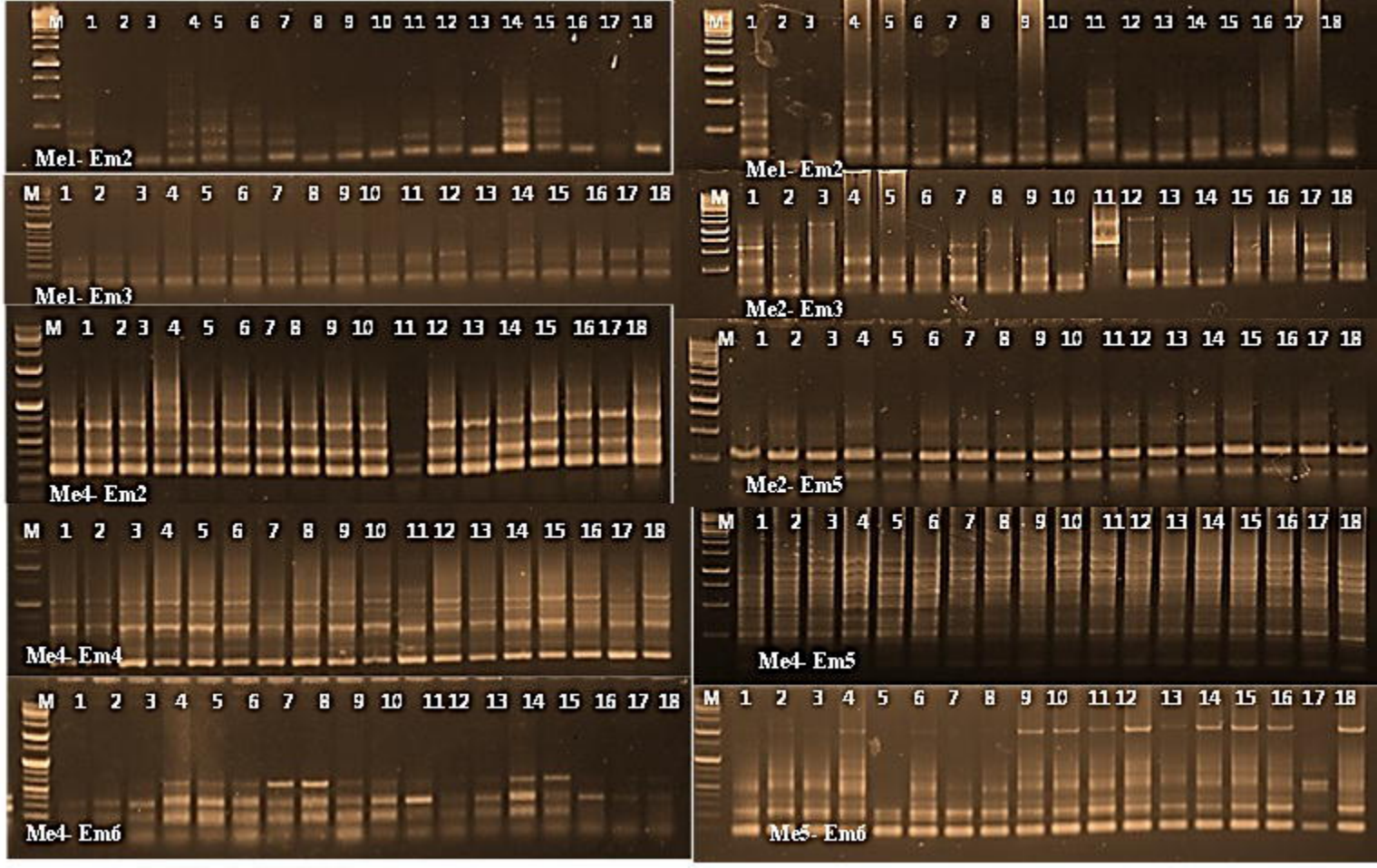
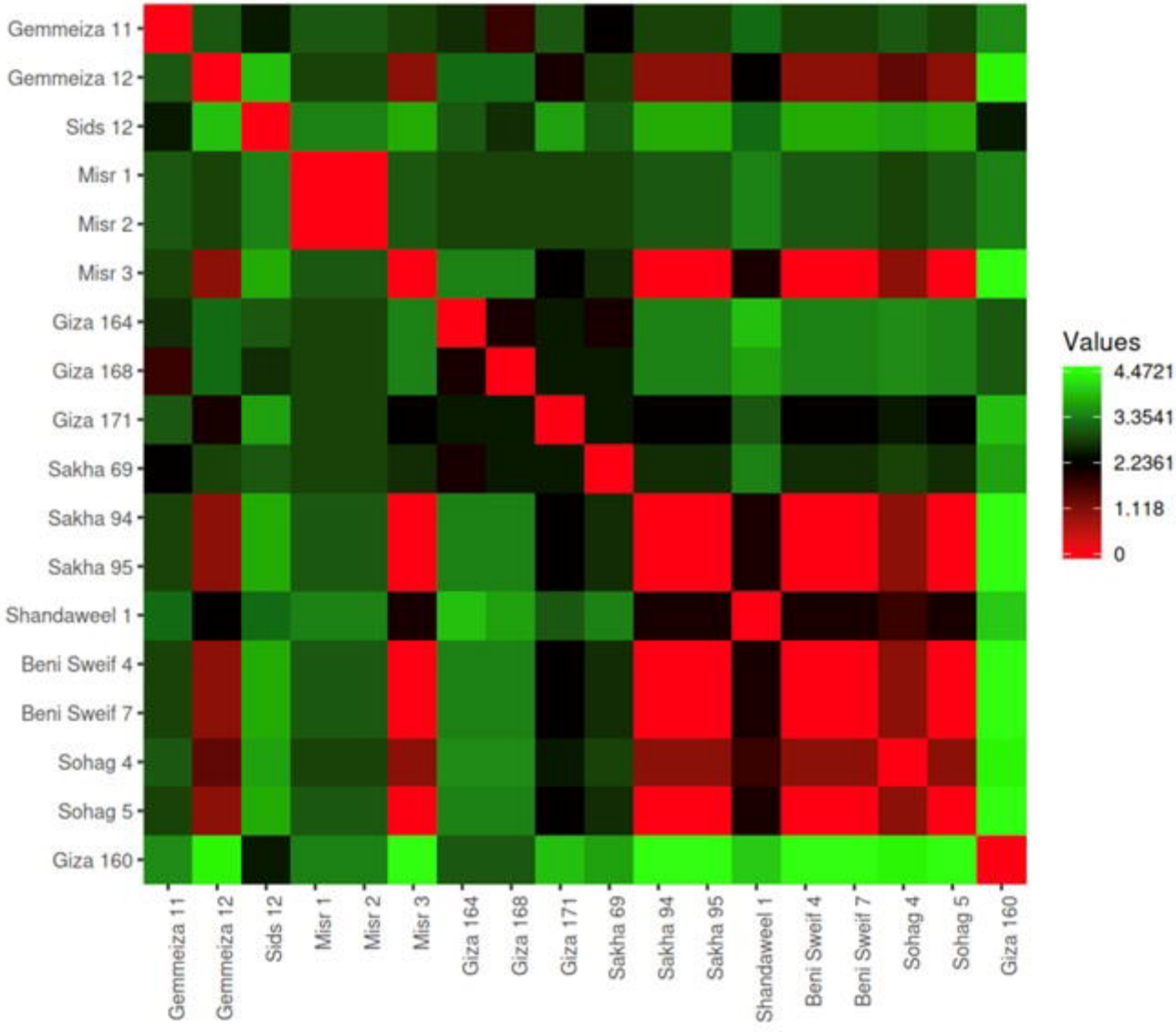
18


M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Me1- Em2
Me1- Em3
Me4- Em2
Me4- Em4
Me4- Em6
Me2- Em3
Me2- Em5
Me4- Em5
Me5- Em6


Gemmeiza 11
Gemmeiza 12
Sids 12
Misr 1
Misr 2
Misr 3
Giza 164
Giza 168
Giza 171
Sakha 69
Sakha 94
Sakha 95
Shandaweel 1
Beni Sweif 4
Beni Sweif 7
Sohag 4
Sohag 5
Giza 160
Values
4.4721
3.3541
2.2361
1.118
0