Molecular Docking Studies of Cu-chitosan nanoparticles as Prospective Stem rust Inhibitor Candidates among some Egyptian wheat genotypes

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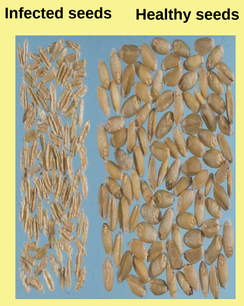
Conclusion

Wheat production can be enhanced by using tolerant and resistant genotypes. Therefore, evaluation and assessment of the stem and leaf rust resistance potential in wheat through marker analysis could potentially diminish the cost of breeding programs and be a powerful strategy for the selection of the most resistant stem and leaf rust genotype. SARAP markers were more efficient and had stronger discriminating power than SCoT markers for assessing the genetic diversity of the tested wheat genotypes , as shown by the number of specific bands, polymorphism percentage, and high values of the genetic diversity indices. Besides, spraying foliar wheat plants with the cu-chitosan nanoparticles composite plant reduced the form of host reaction to stem and leaf rust and increased incubation and latent periods. An insight into the interactions between cu-chitosan nanoparticles and MAPK *P. graminis tritici* or MAPK *P. triticina* protein is given in the computational analysis. Besides confirming the validity of virtual screening techniques, our predictive and experimental results could provide a valuable starting point for the design of MAPK *P. triticina I* and PGTG of *P. graminis f. sp. tritici* nhibitors are able to limit the transduction signal that controls numerous stem and leaf rust infection processes. It elucidates the way the fungal infection is inhibited by MAPK protein targeting. This is the first report on the use of (CuChNp) of foliar fertilizers for controlling rust disease in wheat. The results showed that (CuChNp) could be a useful biological pesticide for controlling stem and leaf rust diseases. We proposed that the CuChNp represents a safe and good opportunity for the development of commercial plant protection against rust disease. However, with regard to the antifungal activity of CuChNp, many experimental trials are needed to understand the particular mechanism of penetration and transportation of these treatments into plant cells and their interaction with fungal cells in these cells.



Introduction

Wheat (*Triticum aestivum*) is considered to be a major source of food grains in Egypt and worldwide. By 2050, wheat demand is expected to noticeably rise by 60 percent, particularly in the developing countries. This increased demand for wheat is very serious, especially in the context of climate change, resulting in a 29% decrease in final productivity. Furthermore, wheat is mainly targeted by three rust diseases, i.e. stripe, leaf, and stem rusts. The latter was caused by *Puccinia graminis* Pers. f. sp. *tritici* is a devastating disease in wheat crops around the world where it has become has epidemic under suitable environmental conditions. Stem rust fungus attacks wheat plants, especially those planted lately, leading to blockage of the vascular system, plant stunting, and finally causing up to approximately 100% yield losses due to damaged grains and tillers.

Molecular marker techniques are considered a useful tool for the determination, characterization, and assessment of genetic diversity among different organisms at the species-level (Hanaa, *et al*. 2020). SCoT and SRAP markers are effectively used in various species of microorganisms to test the genetic diversity, structure, identification of cultivars, quantitative trait loci (QTL) mapping and DNA fingerprinting .

Bioinformatics and molecular docking approaches are important to identify new sources of resistant genes and to decipher the role of natural compounds such as chitosan-copper nanoparticles as natural fungicides that could serve as an environmentally friendly alternative to synthetic fungicides. Recent studies [12] have shown that a single MAP kinase.

Chitosan mainly works on plant innate immunity that makes it resistant to most pathogen and plant diseases. This property could additionally be improved by utilizing it as nanoparticles. Moreover, chitosan can interact with several metals, establishing different chemical bonds that increase its stability when converted to nanoparticles. Metal-based chitosan nanomaterial plays a dual role as a plant growth promoter and a plant protection agent as well. Due to their dual activity, metal-based chitosan nanomaterial have attracted a lot of interest. Chitosan encapsulated metals are also less toxic due to the process of slow-release and have also shown a long-lasting impact on plants.

herefore, the main objectives of this study are to evaluate the disease response of 18 Egyptian wheat varieties against the bio-agent stem and leaf rust at the adult plant stage, under field conditions and at the seedling stage under greenhouse conditions. Moreover, to assess the genetic diversity among eighteen species of wheat plants using both SRAP and SCoT molecular markers. Also, to determine the relationship between the disease parameters and molecular markers linked to them. Additionally, a preliminary screening and evaluation process of the copper-chitosan nanoparticles antifungal on 18 Egyptian wheat varieties against stem rust was performed and their mode of action was studied by molecular docking analysis.

Results

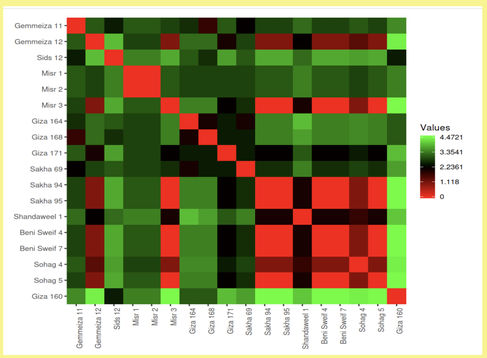


Fig.1. One-dimensional heat map showing the clustering of the 18 wheat varieties based on the presence of rust strains and varieties' performance revealed grouping into three distinct groups. Rows represent the 18 wheat genotypes and columns represent the 20 rust strains.

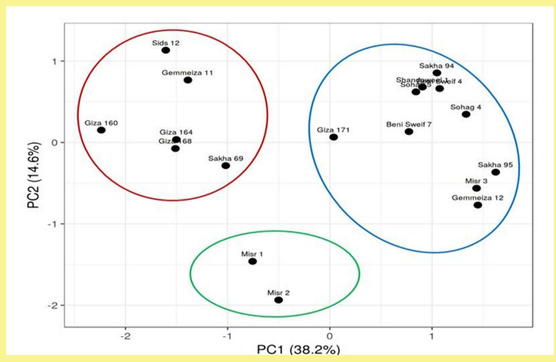


Fig.2. Principal component analysis (PCA) based on the rust race scoring data. The figure demonstrates a sharp clustering into three distinctive groups (blue: resistance to stem rust in the seedling stage; green: moderate susceptible to stem rust races in the seedling stage; genotypes; red: highly susceptible to stem rust races in seedling stage genotypes; wheat genotypes).

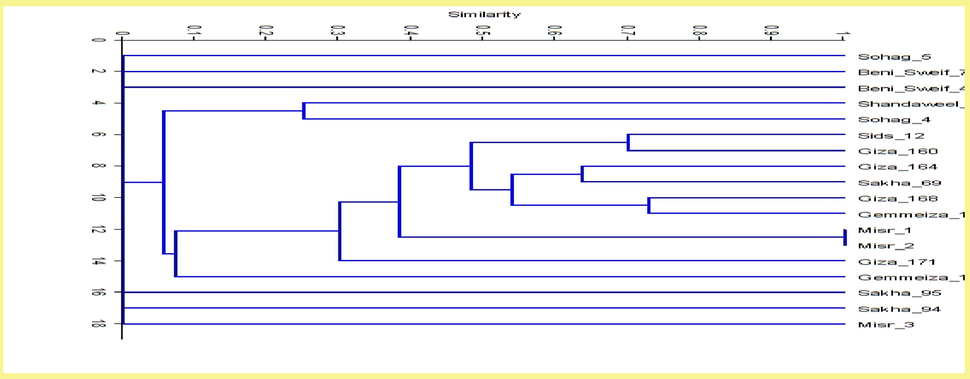


Fig. 3. A dendrogram based on UPGMA analysis using showing the clustering of the 18 wheat varieties based on the presence of rust strains and varieties' performance revealed grouping into three distinct groups. Rows represent the 18 wheat genotypes and columns represent the 20 rust strains.

Moreover, using the heat map to determine the relationship between the eighteen wheat genotypes and twenty stem rust races were performed. The results of Heat map and Principal component (PCA) as analyses revealed that one-dimensional heatmap visualization of the interaction between the presence of rust races and wheat varieties performance revealed grouping of the 18 wheat varieties into three distinct groups as presented in Fig. 1 and 2. Cluster I consist of almost resistance to stem rust in the seedling stage; cluster II included moderate susceptible to stem rust races in the seedling stage and cluster III included the highly susceptible to stem rust races in seedling wheat genotypes. In this respect, the results confirmed that there is a correlation between the genotypes and the stem rust at the seedling. The results were quite similar to those from the previous analysis, with the inclusion of the term "response to seedling stage , All the terms are related to the response of plants to stem rust races disease .

Table 1. Effect of Cu-chitosan NPs treatments and application methods on incubation, latent periods and infection type of the wheat stem rust disease at seedling stag.

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

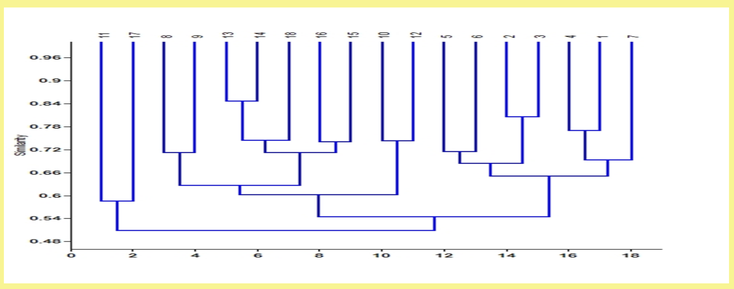


Fig. 4. Amplification profile generated by 14 SRAP primers in 18 wheat genotypes. M: molecular marker (100 bp); lanes 1–18,

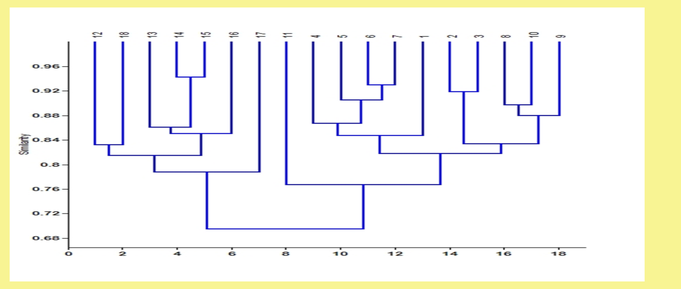


Fig. 5. Amplification profile obtained with eleven Scot primers in eighteen wheat genotypes. M: molecular marker (100 bp); lanes 1–18

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

Fig. 7. A dendrogram based on UPGMA analysis using SCoT marker

In the present investigation, 20 SCoT primers were selected to study the genetic diversity analysis among the eighteen Egyptian wheat genotypes, including stem and leaf resistant and susceptible species. Only eleven SCoT primers generated distinct polymorphic bands. However, a total of 216 bands were polymorphic (97 %), and their sizes ranged from 200 to 3000 bp. The number of polymorphic bands for each primer ranged from 6 (SCot71) to 24 (SCot13) and the number of monomorphic bands ranged from 1 (SCoT 14, 24, and 71) to 2 (SCoT 33). Moreover, the number of unique bands ranged from 1 (SCoT 70) to 23 (SCoT 13). The Jaccard's genetic similarity values of the eighteen Egyptian genotypes, including stem and leaf resistant and susceptible, depend on SCoT molecular markers, ranging from 38 to 84%. The highest genetic similarity revealed by the SCoT molecular markers analysis was 84 % recorded between Sakha 69 and Giza 164, which are categorized as susceptible to stem and leaf rust. Meanwhile, the lowest similarity percentage (38%) was recorded for Shandaweel 1 and Gemmeiza 11, given that Shandweel 1 is resistant to stem rust but susceptible to leaf rust. While Gemmeiza 11 is categorized as susceptible to both stem and leaf rust (Table S3). A dendrogram depends on UPGMA analysis using SCoT marker consisting of two main clusters. The first cluster grouped all plants from the phylogenetically similar susceptible to stem and leaf rust races, i.e. Beni Sweif 7 and Sohag 4. While the second cluster consists of two sub clusters. The second sub cluster I indicated that the most susceptible genotypes to leaf and stem rust (Giza 164, Sakha 69, Sohag 5, Bani Sweif 4, Giza 160 and Shandaweel 1) were genetically similar to three resistant genotypes to leaf rust races (Giza 171, Sakha 94 and Sakha 95). In addition, the second sub cluster II showed that all species belonging to the most resistant genotypes to leaf rust (Misr 1, Misr 2, Misr 3, Gemmiza 12, and Sids12 were similar to some species belonging to susceptible genotypes to stem and leaf rust (Giza 168 and Gemmiza11) races. These results indicate that SCoT primers have high amplification efficiency and are reliable in the discovery of polymorphisms between stem and leaf rust races.

3SRAP markers diversity pattern:

To investigate the genetic diversity and to evaluate the polymorphism degree among the eighteen Egyptian genotypes, including stem and leaf resistant and also susceptible species. A total of 20 SARP primer combinations were tested against these species genotypes. Only 14 out of 20 primer combinations revealed discernible polymorphism. However, a total of 230 bands were polymorphic (99%), and their sizes ranged from 100 to 2000 bps. The results showed that the total number of bands produced were distinct sharp bands. The number of polymorphic bands per primer combination ranged from 3 (Me1-Em6) to 16 (Me2-Em1), and the number of unique bands per primer ranged from 2 (Me4-Em4) to 19 (Me1-Em3) . The Jaccard's genetic similarity values of the eighteen wheat species depend on the SRAP profile molecular markers that ranged from 60 to 94%. The highest genetic similarity value revealed by the SRAP molecular markers analysis was 94 % between Sakha 69 and Giza 160, belonging to susceptible genotypes for the stem rust race. Meanwhile, the lowest similarity percentage (0.60%) was noticed for Sohag 5 and Gemmeiza 11 (Table S4). A dendrogram analysis generated from SRAP analysis based on UPGMA clustering grouped the 18 wheat genotypes into two main clusters as presented. 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 grouped the largest number of the most resistant genotypes to leaf rust (Misr 1, Misr 2, Misr 3, Gemmeiza 12, Giza genotypes 171, Sakha 95 and Sakha 94) beside some cultivars susceptible to stem and leaf rust (Beni Sweif 7, Giza 168, Gemmeiza 11, Sids 12). According to the cluster analysis, the SRAP data successfully clustered similar resistant and susceptible genotypes to leaf and stem rust into the same phenotypes and genotypic groups. These results show that the SRAP clusters exhibited a relatively direct connection with plant sequence taxonomy and assessment of stem and leaf rust races.

structure prediction, quality assessment and validation

BLASTp analysis provided PDB ids: 2VKN and 2FA2 putative templates, respectively showed high-level sequence identity with MAPK1 and PgMAPK sequences as presented in Table (1). Accordingly, the BLAST analysis PDB ID: 2vkn chain A with a resolution of 2.05 Å and 2FA2 chain A with a resolution of 2.85 Å reflected the best template structure for the comparative model building of MAPK1 and PgMAPK, respectively. The query coverage of protein sequences revealed MAP kinase 1 of *P. triticina* and PGTG of *P. graminis f. sp. tritici* (84 and 12%) query coverage's with 56.14 , 56.15 identity with the template proteins (2VKN , 2FA2) that used as template proteins for homology modeling of our target proteins. The SWISSMODLE server-generated 25 and 35 predictive models for MAPK1 of *P. triticina* and PgMAPK of *P. graminis* f. sp. *tritici* proteins with different (QMEAN) score values. The model with low values for QMEAN score (-1.53 and -0.69, respectively) was selected as a final model for *in silico* characterization and docking studies.

The structural analysis and verification server were used to analyze and validate the stability of the MAPK1 and PgMAPK models (https://servicesn.mbi.ucla.edu/SAVES/). The reliability of the backbone of torsion angles i.e. φ and Ψ was evaluated using the PROCHECK program, which measures amino acid residues falling in the existing regions of Ramachandran plot, as depicted in Fig. (8 and 9). Analysis of Ramachandran plot for MAPK1 and PgMAPK revealed that , respectively 91 and 93% residues were found in the most favored regions (A, B, and L) and only 8.7 and 6.1 % residues occupied the additionally, allowed regions (a, b, l, and p). 8.3% residues in additional acceptable regions, 0.8% residues in generously acceptable regions, and 1.4% residue in disallowed regions (Table S5). The quality of the MAPK1 model was further justified by a better ERRAT score of 71.6931 (a value of ~95% reveals high resolution), which recommended an acceptable environment for protein (Colovos and Yeates, 1993). This confirms that the predicted model quality MAPK1 of *P. triticina* and PgMAPK of *P. graminis* f. sp. *tritici* had a good stereochemical quality and was close to the template structure. The ProQ is a neural network-based predictor based on several of structural features as it predicts the quality of the protein model. The ERRAT score for the modeled structure was found to be 96.22 and 78.4314, respectively. The 3D revealed that the predicted proteins MAPK1 and PgMAPK have 96.22 and 81.36% of the residues have an average 3D-1D score >= 0.2 Pass At least 80% of the amino acids have scored >= 0.2 in the 3D/1D profile. Moreover, the ligand-binding sites identified in the target modeling proteins structure are presented.

Protein-ligand docking

In this investigation, the modeled protein MAPK1 and PgMAPK were docked with all the chitosan and chitosan- copper complex to generate their binding mode to refine the best the pose with an allowed conformational change in the tested proteins. The MPAK1 was bound to chitosan with ΔGbind were bound and docking score was -6.9 kcal/mol with the interactive binding site residues (ARG106 , ASP192 , GLU210 , GLY212 , LEU213 , GLU223, THR224 , GLY225 , MET227 , VAL231 , ALA232 and ARG234).While, the MPAK1 was bound to chitosan with CU the ΔGbind are -6.6kcal/ mol with the interactive binding site residues (ARG106, LYS194, GLU223, MET227, GLU229, VAL231, ALA232, GLN247 and GLY273). On the other hand, the PGAT protein for leaf rust was bound to chitosan with ΔGbind -4.6kcal/mol with the interactive binding site residues (TYR441, PRO448, ASN449, SER483, SER478, GLN470 and LYS467). Also, the PGAT was bound to chitosan with CU the ΔGbind are -5.6 kcal/mol with the interactive binding site residues (pro 448, Ala443, Glu450, Arg472, Arg 473 and Ph453).

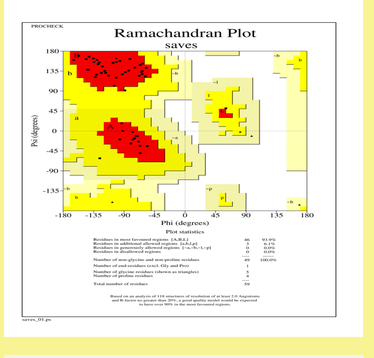


Figure 8. Ramachandran plot of the modeled structure of MAPK1, evaluated and checked by SAVES. For both residues, the plot demonstrates phi-psi torsion angles.

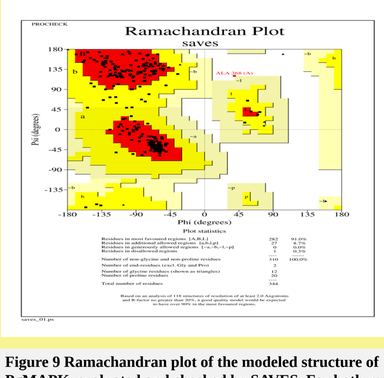


Figure 9 Ramachandran plot of the modeled structure of PgMAPK, evaluated and checked by SAVES. For both residues, the plot demonstrates phi-psi torsion angles.

Figure 10. The binding site of A. the MAKP1 of the leaf rust fungus and B. the PGTG protein of stem rust

Table 10. Binding free energy of chitosan alone or complexed with copper predicted by molecular docking with MAPK1 and PgMAPK proteins of P. triticina and P. graminis tritici, respectively.

Fungi	Grid box (x, y, z)	Protein/accession no.	Ligands	Binding energy
P. triticina	x = 8.6875 y = 7.9130 z = 26.0847	MAP kinase 1 P. triticina (AAY89655.1)	chitosan	-6.9
			cu-chitosan	-6.6
P. graminis f. sp. tritici	x = 11.3055 y =22.877 z = 1.3282	PGTG P. graminis f. sp. tritici (10532062)	chitosan	-4.6
			cu-chitosan	-5.6

Table 14. Sequence identity, Query coverage and QMEAN scores of templates structure for homology model building of MAPK1 and PgMAPK proteins of *P. triticina* and *P. graminis tritici* through BLAST against RCSB PDB

Fungi	Protein / accession number	Templates (PDB id)	Chain	Query coverage (%)	QMEAN	% of identity	Resolution (Å)
P. graminis tritici	PgMAPK 10532062	2FA2	A	0.12	-0.69	56.14	2.05
P. triticina	MAPK1 AAY89655.1	2FA2	A	0.84	-1.53	56.14	2.85

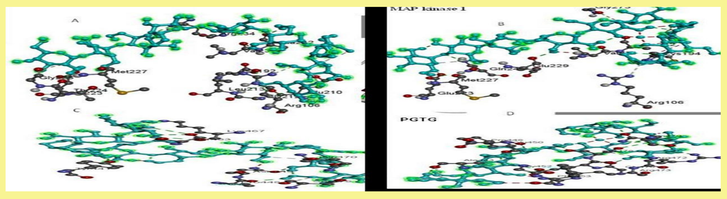


FIGURE 11. The overall 3D surface view of the modeled protein PtMAPK1 and PGTG with chitosan only and chitosan – Cu complex . (A) MAPK1 with chitosan only, (B) MAPK1 with chitosan – Cu complex, (C) PGTG with chitosan only and (D) PGTG with chitosan – Cu complex.

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