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Characterization of SlFSR Gene for Increasing the Shelf Life of Tomato by Some Bioinformatics Tools

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Abstract

Tomatoes are known as Solanum lycopersicum in scientific language around the world. These are predominantly consumed in the cooking of food and salads. It enhances the food's taste. Due to being temperature sensitive, it has a short life span on the kitchen shelf. To control the temperature sensitivity of tomatoes, first understand the mechanism of the genes that are involved in it. This study is mainly related to the SlFSR gene. It is involved in the ripening mechanism that's why it has a great impact in controlling the shelf life of tomatoes. The GRAS transcriptional factor controls many gene's process, it is also responsible for the proper functioning of SlFSR gene. The genes of the GRAS (TF) family contribute its role in the ripening procedure of fruits. To increase the shelf life of fruits, these genes will be helpful. According to this study, If SlFSR gene mute its function, or slow down the process of ripening it may enhance the shelf life of the fruit. This Insilico-based research, made the understanding of different computational analysis. It provides the deep information about the target gene, e.g., structure, function, and atomic composition of the gene. The Expasy translate tool is used to convert nucleotide sequence into protein sequence, Expasy Portparam identifies the domain and function of the gene, InterProScan tells about the protein family, Swiss model is used to generate the 3-D modeling structure, and the phylogeny tree is generated by ClustalW tool to understand the evolutionary history of the desired gene. The insilico tools and softwares predict that the SlFSR gene product has instability index of 48.52, 89.77 aliphatic indexes, and a -0.245 Grand average of hydropathicity. These values strongly predict that the SlFSR is unstable and belongs to the transcription GRAS family that may alter the expression of the genes for the desired product by using DNA binding protein.

Keywords: *SIFSR genes, GRAS family, Tomato, Shelf life*

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Introduction

In Pakistan, the tomato (*Solanum lycopersicum*) is a major and extensively grown vegetable. It is primarily grown as a summer crop and is a member of the Solanaceae family. Because they are high in vitamins A and C as well as vital minerals like calcium, phosphorus, and iron, tomatoes play a significant role in Pakistani diets. Additionally, they offer antioxidants that support health, such as lycopene, β-carotene, phenolics, and vitamin E (Todevska et al., 2024).

In 2023–2024, tomato cultivation in Pakistan covered approximately 67,130 hectares, producing a total of about 797,318 tonnes of tomatoes. During this time, the average yield per hectare was approximately 11.87 tonnes. With about 25,870 hectares, or 38.53% of the total cultivated area, Sindh led the provinces in tomato cultivation. Balochistan came next, accounting for 35.60% of the country's tomato production. Significant participation was also shown by Punjab and Khyber Pakhtunkhwa, each of which had different districts with different cultivation areas and yields (Ministry of National Food Security & Research, 2024).

The postharvest quality deterioration severely limits its shelf life and market value. In developing countries, losses of harvested tomato fruit can reach 25–42%, largely due to rapid softening and decay (Qasim et al., 2022). These losses occur because ripening in climacteric fruits like tomato involves dramatic texture changes: endogenous ethylene and other signals trigger cell-wall disassembly, leading to softening and susceptibility to pathogens (Zhang et al., 2018). Thus, extending shelf life by slowing softening and decay is a major goal for tomato breeders and biotechnologists.

Solanum lycopersicum fruit shelf regulator gene present in GRAS transcription factor. The genes from GRAS family are also involved in other fruit plants. The function of these genes includes the temperature regulation, droughness, and stress management. The fruits from all plants have their own benefit at nutritional level. The change occurs in the gene of the GRAS family may lead to perform the desired function of the gene by mutating the original function of the gene. This method helps to increase the shelf life of tomato by mutating the original function of the responsible gene. Different types of studies have been conducted to study about the molecular mechanism of tomatoes. As the fruits ripe early it will have more probability of having plant diseases, or decrease shelf life as compared to slow ripening fruits. The shelf life of any fruit will be increased by targeting the responsible enzymes (Wang & Seymour, 2022).

There are number of genes involved in ripening process e.g., *SlGRAS38* and *SlFSR* genes. Both genes are responsible for the development of tomatoes by using different enzymes. The developing process will be stopped if these enzymes are repressed properly. This can lead to maintain the longer shelf life of early ripened fruit. On the other way if the responsible genes are enhanced the production these enzymes it will lead to reduce the shelf life of the tomatoes. The main gene associated with development of fruits are *SlFSR* gene no other gene will be able for the developing process before it. The change occur in fruit shelf regulator will increase the shelf life of fruit and also maintained the temperature regulation. After this change, the shelf life of tomatoes will be increased up to 60 days at 25 to 30 °C (Zhang et al., 2018).

Today's the shelf life of tomatoes and any other fruit are crucial for market values. Due to low shelf life, tomatoes are rotten early and it is a great loss of economy. The major problem occurs in transportation of fruits and after successfully transported, reduced shelf life can cause the loss of tomatoes in a large amount. There are number of procedures are introduced to enhance the shelf life of tomatoes, recently the study investigated the ripening inhibitors, which involved in the ripening process. In this technique the inhibitors ceased the growth process which is ultimately increased the shelf life of fruit. It has a great importance at economical level (Sinha et al., 2019).

Research Design and Methodology

This research was conducted at Minhaj University Lahore. The *SlFSR* gene was targeted in tomato. In this research silent the function of gene to improve the tomato freshness.

Sequence repossession

The sequence of the gene *SlFSR* was gained by the National Center of Biotechnology Institue (NCBI). It is mainly present in the growth of cell wall of tomatoes. It was NM_001375439 accession number. The retrieved sequence was further analyzed in computational tools to predict the molecular function of gene (Bukhari et al., 2020).

ExPASy-Translate

The *SlFSR* gene sequence was put into the expasy tool to convert the DNA sequence into the amino acids sequence. Copied the sequence from NCBI and pasted into the box of expasy translate and gained the protein sequence (Gasteiger et al., 2003).

ExPASy-ProtParam

It is used to understand the qualities of a gene and analyze its protein functions, e.g., index rate, composition, half-life, molecular weight, number of amine group, etc. Copied the protein sequence from translate tool and pasted in to the box for consequences (Sahay et al., 2020).

InterProScan

To identify the protein family and domain, interproscan is used commonly. The copied protein sequence was pasted into the box and get the desired results about the protein domains, family and length of the sequence (Blum et al., 2021).

Swiss Model

This tool is famous for making 3-D protein structures. The copied sequence from the translate tool also pasted in the Swiss modeling website. After sometime the model of the required protein was formed and shown on the screen (Bashir et al., 2019).

Ramachandran plot

This tool verifies the reliability of the predicted structure of the protein as it may be useful or not after remodeling. The result can predict that the selected protein is worked after mutation according to the given changes.

Global alignment tool

The phylogeny report or tree is very important for linking the evolutionary history of the gene or genome. ClustalW is the alignment tool used for the formation of phylogenetic tree. The selected sequence files of blast analysis were used to form historical tree. The alignment of different genes was shown in the form of branches. It is also called evolutionary tree (Zaal & Nota, 2016).

Results and Discussion

Sequence Retrieve

The nucleotide and protein sequences of the *SlFSR* (Fruit Softening Related) gene from *Solanum lycopersicum* were retrieved from the National Center for Biotechnology Information (NCBI) database (Bukhari et al., 2020). The *SlFSR* gene is a protein-coding gene known to play a vital role in tomato fruit ripening and softening. The retrieved sequence data included several key identifiers: Gene identity: 101268586, Symbol of the gene FSR, name of the gene *SlFSR*, and it is classified as protein-coding. The scientific term of the source organism was *Solanum lycopersicum*. The transcript and protein accession numbers were NM_001375439 and NP_001362368.1, respectively, with *SlFSR* used as the primary query. This retrieved information provided the basis for further in silico analyses, including sequence alignment, structural prediction, and functional characterization.

ExPASy Translate and ExPASy PortParam

The ExPASy Translate Tool was used to convert the nucleotide sequence of SlFSR into its matching amino acid sequence. ExPASy ProtParam was then used to analyze the translated sequence and as certain its physicochemical characteristics. It was discovered that the *SlFSR* protein had 429 amino acids, an isoelectric point (pI) of 5.34, sand 48,611.24 Da molecular weight of gene. The empirical chemical formula was determined to be $C_{2158}H_{3360}N_{590}O_{648}S_{21}$. The protein's expected half-life in mammalian reticulocytes was 30 hours, suggesting that it would be moderately stable in vivo. The aliphatic index of 89.77 and the grand average of hydropathicity (GRAVY) score of -0.245 indicated that the protein is relatively hydrophilic in nature, whereas the instability index value of 48.52 indicated that the protein is unstable. These findings indicate that *SlFSR* functions as a soluble regulatory protein rather than a membrane-associated protein.

Figure 1: *The Protein Sequence of SlFSR Gene in the form of ORFs*

Results of translation

- Open reading frames are highlighted in red
- Select your initiator on one of the following frames to retrieve your amino acid sequence

5'3' Frame 1

```
MNSTTIPLQNPSFFNNSQSSLAGALQGCIGSLDGACLEKLLLHCASALENNDVTLAQQVMWVLNNLASSNGDPNQRLTSWFLRALISRASRVYPNATNLHGSSNLERRLMSVTELAGYVDLIPWH
RFGFCASNSVIYKAIERQTKVHILDFSITHCMQWPTLIDAIAKRPEGPPSLRISVPSWRPQVPPLLNVSSEEVGQRLANFAKFRDVPFEFHVIEDLNYDMFLSQLNPSTLQIRDDEALVVNCQNW
LRYLHDDEQIKGALSSRDIFLDRVKDLNPCIVTIVDEDCDLGNPTTLTSRIATCFNYLWIPFDALETFLPKDSKQRLDYEAEIGHKIENIIGFEGNQRIERLESCNKFSQRMENSGYMSVPFSEE
TIKEVKSLLDEHASGWGMKKEEHDMLVLTWKGHNSVYATSWVIVSPPHMDMSSL-
```

5'3' Frame 2

```
-TLQPFPSKTLVFSTTLKVL-QEHYKDALGALMEHVWRNYYFIVQVP-RTMM-LWLNKSCGCSTIWLLLMEIQIKDSHHGSLGH-FQGLLGFILMPQIYMEVVTLKGD--V-LS-QGMWISSHGI
DLDFVHQIVLFIRPLKGKQKFTY-ILVSLIVCNGQLSLMQLLRGLRVLLLSEYRCLHGDHKFLHCSMCQVKKLANVWQISRNLEMFLLNSM-LKT-TMTCS-AN-ILQLFKLEMMKLWL-IAKIG
-GTYMMMNKLRVHFRLATYFLIGLRI-TLVL-LLLMKIVIWETQLL-HQE-PLVLTIYGYLLMH-RHFCLRIVSKG-IMKLRLATKLKTLLDLKGTKG-RD-RVATSSHKGWKIVVT-VYLLVKK
Q-RKSSHCWMNMQVDGA-KKRNMTCLY-HGKVITLSMQHLGS-SHHPIWT-VAY
```

5'3' Frame 3

```
ELYNHSPPKP-FFQQLSKFSSRSTTRMHWEP-WSMFGETITSLCKCLREQ-CNFGSTSHVGAQQFGFF-WRSKSKTHIMVP-GINFKGF-GLS-CHKFTWK--P-KEINECD-ASRVCGSHPMA-
IWILCIK-CYL-GH-KANKSSHIRF-YHSLYAMANSH-CNC-EA-GSSFSPNIGAFMETTSSSIAQCVK-RSWPTFGKFREI-RCSF-IPCD-RLEL-HVLEPIESFNSSN-R--SFGCELPKLV
KVLT---TN-GCTFVSRHIS--G-GFKPLYCDYC--RL-FGKPNYFNIKNSHLF-LSMDTF-CIRDIFA-G--AKARL-S-DWPQN-KHYWI-REPKDREIRELQQVLTKDGK-WLHECTF--RN
NKGSQVIVG-TCKWMGHEKRGT-HACINMERS-LCLCNILGHSLTTPYGHE-LI
```

3'5' Frame 1

```
L-ATHVHMGW-DYDPRCCIDRVMTFPC-YKHVMFLFFHAPSTCMFIQQ-LDFLYCFFTKRYTHVTTIFHPL-ELVATL-SLYPLVPFKSNNVFNFVANLSFII-PLLTILRQKCL-CIKRYP-IV
KTSGYS-C-SSWVSQITIFINNSHNTRV-ILNPIKKYVARRKCTLNLFIIM-VP-PILAIHNQSFIISNLKS-RIQLAQEHVIVQVFNHMEFKRNISKFREICQTLANFFT-HIEQWRNLWSP-R
HRYSERRRTLRPLSNCINESWPLHTMSDTKI-YVNFCLPFNGLINNTI-CTKSKSMPWDEIHIPC-LSHTH-SPFKVTTSM-ICGIRINPRSP-N-CPKEP-CESLIWISIRRSQIVEHPHDLLS
QSYIIVL-GTCTMK--FLQTCSIKAPNASL-CSC-RTLRVVEKTRVLEGNGCRVH
```

3'5' Frame 2

```
YKLLMSIWGGETMTQDVA-TEL-PFHVNTSMSCSSFFMPHPLACSSNNDLTSFIVSSLKGTLM-PLFSILCENLLQLSNLSILWFPSNPIMFSILWPISAS-SSLCLLSLGKNVSNASKGIHR-L
KQVAILDVKVVGFPKSQSSSTIVTIQGFKSLTLSRNMSRDESAPLICSSSCKYLNQFWQFTTKASSSLI-RVEGFNWLKNMS-FKSSITWNSKGTSLNFAKFAKRWPTSSLDTLSNGGTCGLHEG
TDIRREGGPSGLLAIASMRVGHCIQ-VILKSNM-TFVCLSMAL-ITLFDAQNPNLCHGMRSTYPASSVTLINLLSRLLLPCKFVALG-TLEALEINALRNHDVSL-FGSPLEEAKLLSTHMTC-A
KVTSLFSKALAQ-SNSFSKHAPSRLPMHPCSAPAREL-ELLKKLGFWRGMVVEF
```

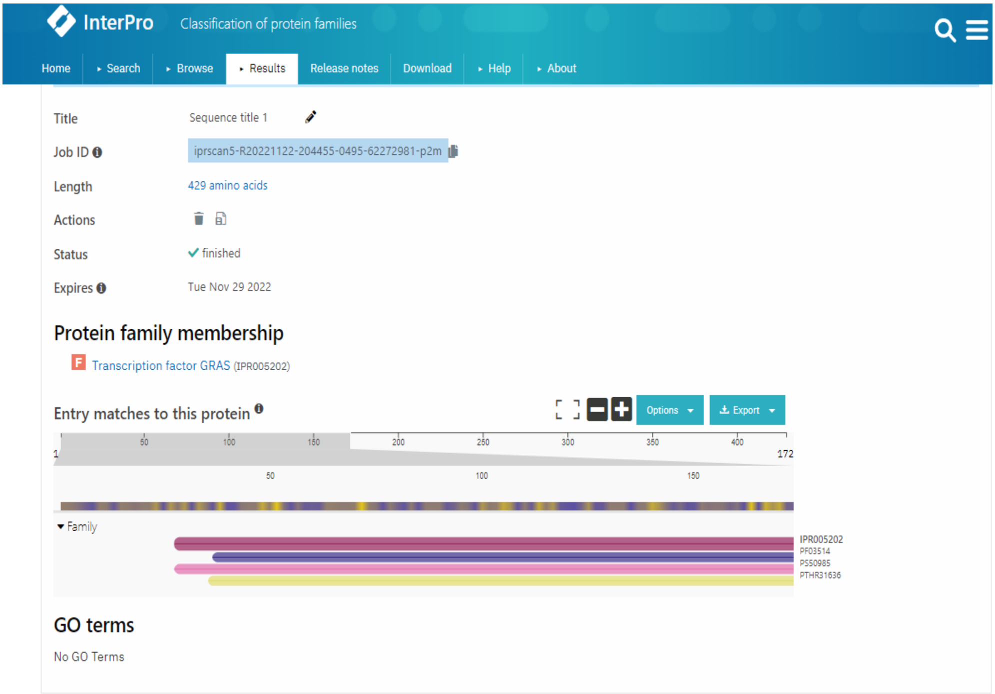
3'5' Frame 3

```
ISYSCPYGVVRL-PKMLHRQSYDLSMLIQACHVPLFSCPIHLHVHPTMT-LPLLFLH-KVHSCNHYFPSFVRTCCNSLISLSFGSLQIQ-CFQFCGQSQLHNLAFAYYP-AKMSLMHQKVSIDS-
NKWLFLMLK-LGFPNHNLHQQ-SQYKGLNP-PYQEICRETKVHP-FVHHHVSTLTNFGNSQPKLHHL-FEELKDSIGSRTCHSSSLQSHGIQKEHL-ISRNLPNVGQLLHLTH-AMEELVVSMKA
PIFGEKEDPQAS-QLHQ-ELAIAYNE-Y-NLICELLFAFQWPYK-HYLMHKIQIYAMG-DPHTLLAQSHSLISFQGYYFHVNLWH-DKP-KPLKLMP-GTMM-VFDLDLH-KKPNC-APT-LVEP
KLHHCSLRHLHNEVIVSPNMLHQGSQCILVVLLLENFESC-KN-GFGGEWL-SS
```

InterProScan

InterProScan functional and domain analysis showed that SlFSR is a member of the GRAS transcription factor superfamily, which controls plant development, including fruit ripening and stress responses, and is important in gibberellin signaling pathways (Wang et al., 2020). It was anticipated that the SlFSR protein would have several domains, the most notable of which would be a cytoplasmic domain involved in transcriptional regulation and signal perception. These findings are in line with earlier research that suggested that SlFSR mutation or silencing prolongs the tomato's shelf life by delaying the ripening process (Blum et al., 2021).

Figure 2: *Domains and Family of Protein.*



Title: Sequence title 1

Job ID: iprscan5-R20221122-204455-0495-62272981-p2m

Length: 429 amino acids

Actions

Status: ✓ finished

Expires: Tue Nov 29 2022

Protein family membership

F Transcription factor GRAS (IPR005202)

Entry matches to this protein

GO terms

No GO Terms

Swiss Model

The Swiss-Model server was used for homology modeling, producing a three-dimensional coiled structure of the SlFSR protein. A well-folded protein was indicated by the stable secondary structural elements in the final model. The Ramachandran plot was used to assess the predicted model's dependability. It verified that the majority of the amino acid residues occupied advantageous regions, confirming the predicted conformation's accuracy (Biasini et al., 2014).

Figure 3: *3D Structure of a Protein*

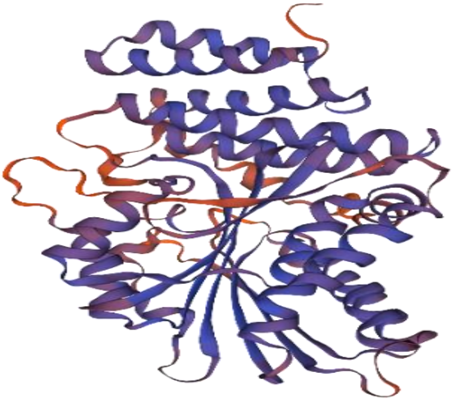
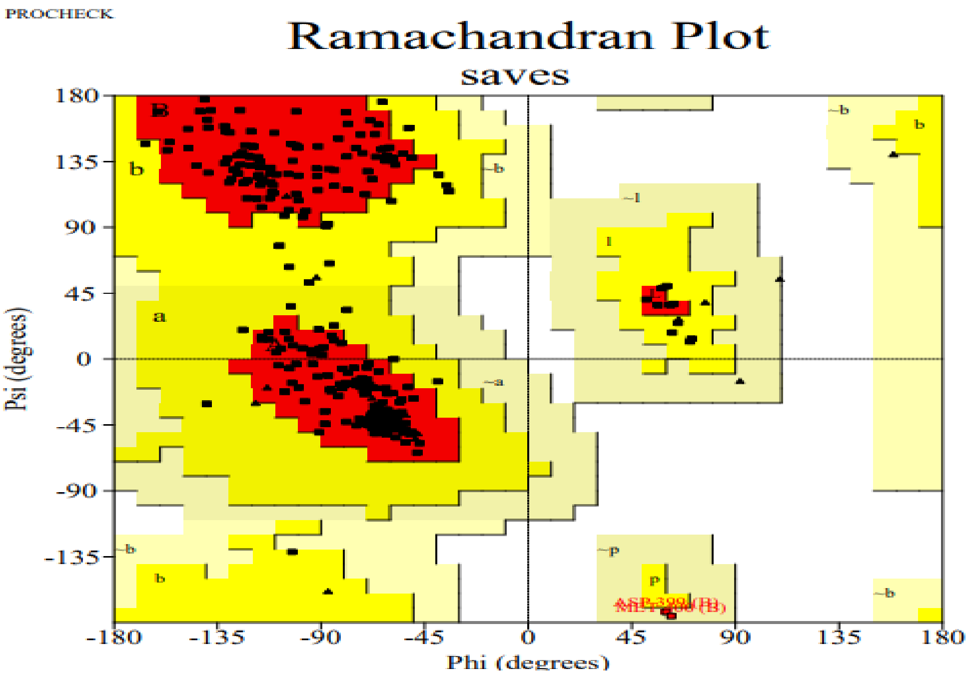


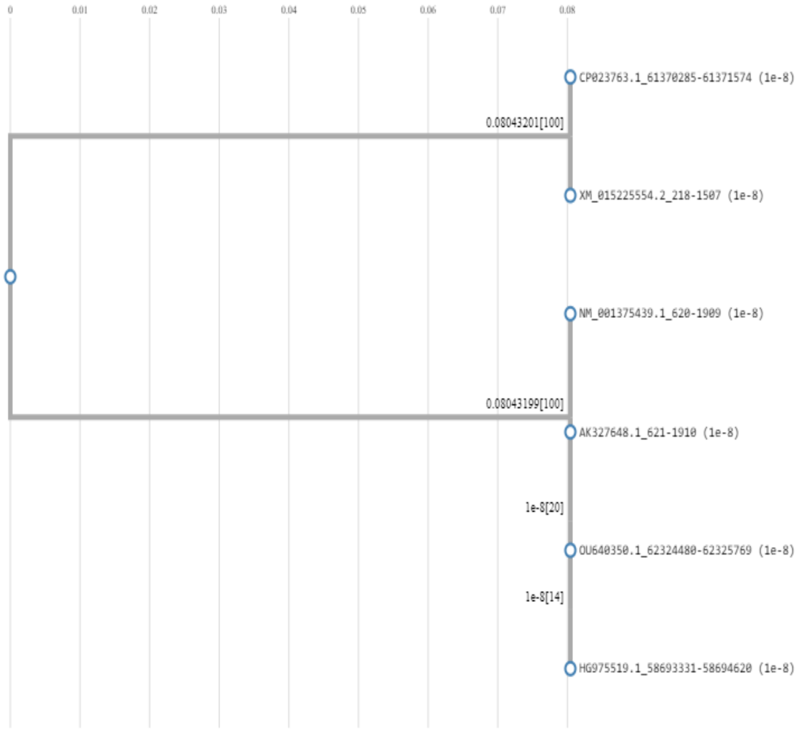
Figure 4: *Ramachandran Plot*



Clustal W

The evolutionary relationship between SlFSR and related GRAS family members was ascertained through phylogenetic analysis using the ClustalW tool. The phylogenetic tree showed that SlFSR has a conserved evolutionary role in ripening regulation and fruit development, sharing close homology with other GRAS transcription factors found in a variety of plant species (Bashir et al., 2019).

Figure 5: *Phylogenetic Tree*



Conclusion

The current in silico study successfully described the SlFSR gene of *Solanum lycopersicum* and its encoded protein utilizing a variety of bioinformatics methods. Sequence retrieval from the NCBI database (Accession No. NM_001375439), followed by translation and physicochemical investigation, revealed that SlFSR encodes a modestly large, unstable GRAS transcription factor protein. The instability index, aliphatic index, and GRAVY score all indicated that the protein is hydrophilic and functions as a soluble regulatory molecule.

Functional annotation with InterProScan revealed the existence of GRAS-specific domains, notably those implicated in gibberellin signaling and transcriptional control. The Swiss-Model tool produced a reliable 3D structure of the SlFSR protein, which was confirmed using a Ramachandran plot to confirm the model's structural quality and stability. Phylogenetic study revealed a close evolutionary link between SlFSR and other GRAS family members from other plant species, confirming its conserved regulatory role.

References

Bashir, S., Bukhari, S. A., & Mahmood-ur-Rahman, M. A. (2019). 57. Homology modeling, structure and active site prediction of stem rust resistant gene Sr22 in wheat cultivars. *Pure and Applied Biology (PAB), 8*(1), 553-558.

Biasini, M., Bienert, S., Waterhouse, A., Arnold, K., Studer, G., Schmidt, T., Kiefer, F., Cassarino, T. G., Bertoni, M., & Bordoli, L. (2014). SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. *Nucleic Acids Research*, *42*(W1), W252-W258.

Blum, M., Chang, H.-Y., Chuguransky, S., Grego, T., Kandasaamy, S., Mitchell, A., Nuka, G., Paysan-Lafosse, T., Qureshi, M., & Raj, S. (2021). The InterPro protein families and domains database: 20 years on. *Nucleic Acids Research*, *49*(D1), D344-D354.

Bukhari, S. A., Mustafa, G., Bashir, S., Akram, N. A., Rahman, M.-u., Sadia, B., Alyemeni, M., & Ahmad, P. (2020). Genetic transformation of Sr22 gene in a high yielding susceptible cultivar of commercial wheat (Triticum aestivum L.). *3 Biotech*, *10*(5), 1-9.

Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R. D., & Bairoch, A. (2003). ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Research*, *31*(13), 3784-3788.

Ministry of National Food Security & Research. (2024). *Agricultural statistics of Pakistan 2023–24* [PDF]. Islamabad, Pakistan: Author. Retrieved from https://mnfsr.gov.pk/SiteImage/Misc/files/ASP%20for%20Web%202024.pdf

Qasim, M., Samman, L., Atta, U., Khan, H., Nasir, H., Awan, M. S., & Akbar, K. (2022). Postharvest factors affecting shelf life and quality of harvested tomatoes; a comprehensive review. *Sch. J. Agric. Vet. Sci*, *9*(6), 65-69.

Sahay, A., Piprodhe, A., & Pise, M. (2020). In silico analysis and homology modeling of strictosidine synthase involved in alkaloid biosynthesis in catharanthus roseus. *Journal of Genetic Engineering and Biotechnology*, *18*(1), 1-6.

Sinha, S. R., Singha, A., Faruquee, M., Jiku, M., Sayem, A., Rahaman, M., Alam, M., & Kader, M. A. (2019). Post-harvest assessment of fruit quality and shelf life of two elite tomato varieties cultivated in Bangladesh. *Bulletin of the National Research Centre*, *43*(1), 1-12.

Todevska, D., Velickovska, S. K., Iljovski, I., Kovacevik, B., Ilieva, F., & Crvenkovski, M. (2024). Introduction and determination of new tomato hybrids. *Journal of Agriculture and Plant Sciences*, *22*(1), 41-45.

Wang, D., & Seymour, G. B. (2022). Molecular and biochemical basis of softening in tomato. *Molecular Horticulture*, *2*(1), 1-10.

Wang, T.-T., Yu, T.-F., Fu, J.-D., Su, H.-G., Chen, J., Zhou, Y.-B., Chen, M., Guo, J., Ma, Y.-Z., & Wei, W.-L. (2020). Genome-wide analysis of the GRAS gene family and functional identification of GmGRAS37 in drought and salt tolerance. *Frontiers in Plant Science, 11*, 2024.

Zaal, D., & Nota, B. (2016). ADOMA: A Command Line Tool to Modify ClustalW Multiple Alignment Output. *Molecular Informatics, 35*(1), 42-44.

Zhang, L., Zhu, M., Ren, L., Li, A., Chen, G., & Hu, Z. (2018). The SlFSR gene controls fruit shelf-life in tomato. *Journal of Experimental Botany*, *69*(12), 2897-2909.