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Characterization of Pollen Specific Proteins SF3 and SF21 from Sunflower (*Helianthus annuus* L.) for the Allergenicity Potential

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Abstract Pollen from different type of plants carry different proteins that are more likely to become allergenic than others. In this study pollen specific proteins SF3 and SF21 from Sunflower (*Helianthus annuus*) were *in-silico* characterized for allergenicity potential. Bioinformatics allergen prediction tools were employed for prediction of SF3 and SF21 as candidate allergens. The phylogenetic relationship between the pollen specific proteins and protein allergens of Asteraceae family was also analyzed to unveil their similarity. The results by Bepipred Linear Epitope prediction demonstrated a good number of epitopes in pollen specific protein SF3 and SF21 signifying that both are potential allergens. However, further analysis through ConSurf revealed the presence of allergen-specific patches with remarkably higher proportion of surface-exposed hydrophobic residue in SF3 than SF21. Further prediction by AlgPred and ProAp methods with Support Vector Machines (SVMs) revealed that only SF3 contained IgE epitope thus confirming it as a potential allergen. The phylogenetic analysis revealed a close identity of SF3 with the major allergen of *Ambrosia artemisiifolia* pollen, Amb a 3. The study has demonstrated high allergenic activity of SF3 protein with shared similarity with the pollen protein allergens of Asteraceae family. This study forms a basis in predicting cross-reactivity of pollen specific proteins, designing of therapeutic procedures and evaluating the allergenic potential of novel proteins.

Keywords: *Helianthus annuus, Pollen allergens, Pollen specific proteins, phylogenetic analysis*

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1. Introduction

Allergens are proteins that may result in stimulation of a specific immune response of the body giving rise to over activation of antibody resulting in high inflammatory response leading to varied allergic reactions such as asthma, hay fever and eczema [1,2]. Pollen allergens are prompted mostly by pollen of various plant species which may cause hypersensitivity (allergy) after being in contact with the immune system [3]. Pollen is very fine powder sizes (12 to 300mm diameter) from flowers with a role of fertilizing the female gametophyte. People can be allergic to different types of pollen through inhaling the pollen. For instance, some are allergic to pollen from only sunflowers; others are allergic to pollen from only certain kinds of grasses. Pollen grains are the primary carriers of pollen allergens and allergenic proteins are usually located within the pollen protoplast and readily released during the rehydration process [4].

The consequences of allergic disorders caused by pollen are so great that can vary from seasonal allergic rhinitis to bronchial asthma thus leading to significant illness, employment absenteeism and in some cases fatal outcomes [5,6]. It is estimated that pollen allergies affect approximately 40% of allergic individuals [6,7]. Pollen specific proteins SF3 and SF21 are proteins present in mature pollen grains specifically in pollen and pistils of the common sunflower (*Helianthus annuus*). The species belongs to the Asteraceae or Compositae family which has about 13,000 species in 900 genera, forming one of the largest of flowering plant families [8,9,10]. These pollen specific proteins SF3 and SF21 are also expressed in the bi-cellular and tri-cellular stages of pollen development and could possibly be involved in controlling pollen-specific processes such as male gamete maturation, pollen tube formation and fertilization.

Sunflower is usually grown as a crop for its edible oil and edible fruits (sunflower seeds), also used as livestock forage and in some industrial applications. Although pollen allergy is recognized as a problem in many parts of the world causing allergic disease, the allergenic potential of pollen specific proteins SF3 and SF21 from the sunflower has not been elucidated. The aim this study was to characterize sunflower pollen specific proteins SF3 and SF21 through *in silico* for their allergenicity potential. With consideration of an economic importance of the sunflower and its current pace in agronomy, the search for its potential aeroallergens is important and may provide valuable information for therapeutics and bio-pharmaceuticals.

2. Material and Methods

Protein sequences of pollen-specific proteins SF3 and SF21 for sunflower (*Helianthus annuus)* were obtained from the Universal Protein Resource (UniProtKB). Further search was done in Swiss-Prot database for sequences evidence of the protein existence. Physicochemical analysis of the sequences in terms of molecular weight, theoretical pI, amino acid composition, instability index, aliphatic index and grand average of hydropathicity (GRAVY) were also done through PROTPARAM tool available on the ExPASy Server [11].

Different methods for *in silico* characterization of protein for allergenicity potential were employed. Pollen specific protein SF3 and SF21 were screened for their B-cell epitopes using Bepipred Linear Epitope Prediction method [12]. The graphical presentation was used to indicate the number of scored epitopes per pollen specific protein. In order to identify the conservation patterns of amino acids in pollen-specific proteins SF3 and SF21 and search for exposed and buried residues the ConSurf methods was used for identification of functionally and structural important residues in protein sequences [13]. AlgPred method [14] was used for prediction allergens through application of SVM (support vector machine), a method based classification of allergens and non-allergens by single amino acid composition [AlgPred(SVM_single_aa)] and by dipeptide composition [AlgPred(SVM_dipeptide). The prediction of allergens based on mapping of IgE epitope in a region of protein was also incorporated.

Furthermore prediction of potential allergenicity of proteins was done through a ProAp method [15]. A method based on an application that provided three main kinds of prediction, namely Motif-based method, SVM-AAC method (take amino acid composition as protein features) and the FAO/WHO criteria. The methods employed herein for allergenicity prediction are those previously reported as useful techniques for determination of cross-reactivity between potential allergens and known allergens.

In order to elucidate the phylogenetic relationship between pollen specific proteins (SF3 and SF21) and the known protein allergens from the family Asteraceae in which *Helianthus annuus* belongs, the proteins allergens sequences for Asteraceae family were extracted from the Allergome database. The protein allergens sequences and pollen specific protein sequences for SF3 and SF21 were aligned using Multiple Sequence Alignment by CLUSTALW and a phylogenetic tree built.

3. Results

3.1. Physicochemical Parameters

The sequences of pollen specific proteins SF3 and SF21 obtained from the Universal Protein Resource (UniProtKB - P29675 and UniProtKB - O23969) revealed that the number of amino acids residues in protein sequences for SF3 and SF21 were 219 and 352 respectively. Results for physicochemical parameters for pollen specific proteins SF3 and SF21 are indicated in Table 1. The results indicated that that pollen specific protein SF21 with average molecular weight of 39191.3 had higher number of negatively charged residues (38), instability index (53.86) and aliphatic index (93.32) than SF3 which had an average molecular weight of 24831.7. Both proteins were characterized with good number of positively charged residues, being 38 in SF3 and 33 in SF21. The overall theoretical isoelectric point (pI) of SF3 was 8.52 while for SF21 was 6.18.

Table 1. Physicochemical parameters of pollen specific protein SF3 and SF21

Sno.	Protein type	Physicochemical parameters						
		AMW	NCR	PCR	TP	II	AI	G
1	SF3	24831.7	23	38	8.52	28.96	50.32	-0.751
2	SF21	39191.3	38	33	6.18	53.86	93.32	-0.035

AMW = Average molecular weight; NCR = negatively charged residues; PCR = positively charged residues; TP = Theoretical pI; II =Instability index; AI = Aliphatic index; G = Gravity.

3.2. B-cell Epitopes

Pollen specific protein SF3 and SF21 screened for their B-cell epitopes through Bepipred Linear Epitope prediction method revealed a good number of epitopes in each pollen specific protein. A significant variation in B cell epitopes existed between the two proteins with higher number in SF21. Figure 1 shows the number of B cell epitopes for pollen specific protein SF3. From the figure the the protein had a number of 5 B cell epitopes marked (A, B, C, D and E). The B cell epitopes in SF3 were detected at an average threshold 0.214 along residue positions. The epitopes and their predicted peptide sequences are as follows; epitope marked A (*SFTGTTQKC*) from residue position 3 to 11, epitope marked B *(SLEKSFDGTPKFKPERTFSQETQSAN)* from residue position 72 to 97 and epitope marked C *(EGTRDKC)* from residue position 104 to 110. Other epitopes were mark D *(VKVDGTAY)* from residue position 123 to 130 and mark E *(TISPSNYIA)* from residue position 143 to 150.

The number of detected B cell epitopes from pollen specific protein SF21 is shown in Figure 2. A total number of 17 B cell epitopes were revealed as marked (from A to R). The B cell epitopes were detected at an average threshold -0.145 along the residue position. The epitopes major predicted peptide sequences were as follows; epitope marked G (*INPPGHELGAASIGIDDPVPSIED)* from residue position 80 to 103, epitope marked Q *(PCRYSNSPRSPLGPSSIDPELLYP)* from residue position 306 to 329 followed with epitope marked E *(CGSVSVTVCGDQEKPPLITY)* from residue position 28 to 47. Except for B cell epitopes marked with letters D, H and R at residue position 50, 140 and 133 which had peptide sequences ranging from one residue to three residues, the rest of B cell epitopes revealed in SF21 had peptide sequences ranging from five residues to twelve residues.

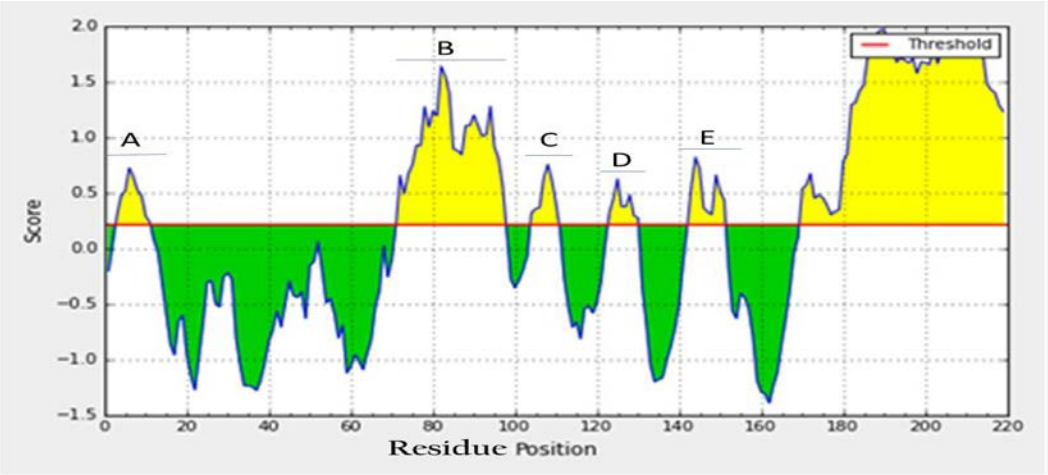

Figure 1. B cell epitopes predicted for pollen-specific protein SF3. Letters presented (A-E) are the scored numbers of B cell epitopes for the protein

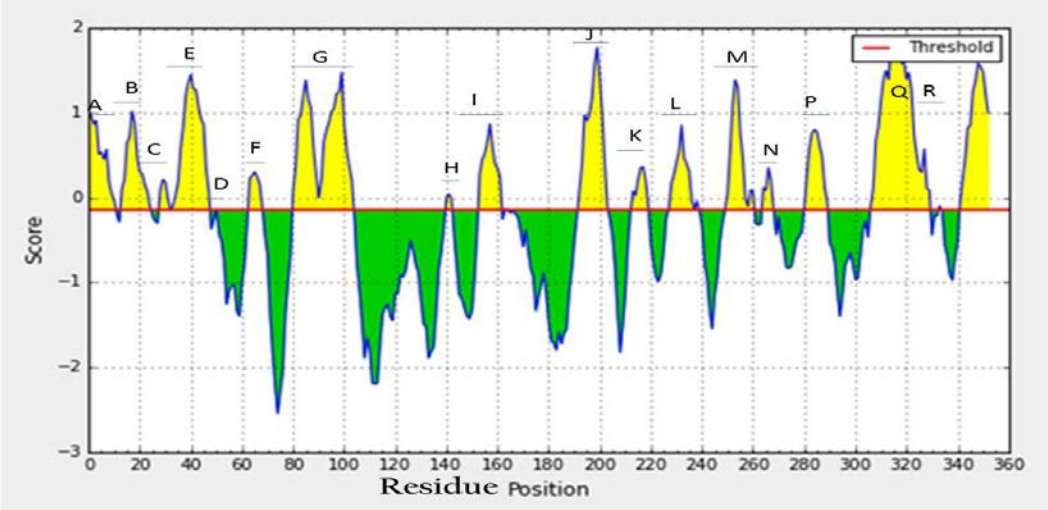

Figure 2. B cell epitopes predicted for pollen-specific protein SF21. Letters presented (A-R) are the scored number of B cell epitopes for the protein

The functionally and structural important residues identified in protein sequences of SF3 and SF21 are shown in Figure 3 and Figure 4 respectively. The results through Consurf demonstrated the presence of high proportion of surface-exposed hydrophobic residues. Pollen specific protein SF3 had higher score of patterns for the predicted functional residues (highly conserved and exposed) and structural residues (highly conserved and buried) in its protein sequence compared to the sequence of pollen specific protein SF21. In terms of residues proportions for the occurrence conserved and exposed residues, the occurrence of Lys (K) was relatively high compared to other residues in both SF3 and SF21 and when compared to sequence length the presence of K was higher in protein sequence of SF3 than SF21. The conservation scale is indicated by different colour from levels 1-9 whereby the highly variable are in blue colour (levels 1–2), the average scores (in white) and the highly conserved in red colour, (levels 8–9). The overall conservation patterns score was high for protein sequence of pollen specific protein SF3 compared to the protein sequence of SF21.

Figure 3. Residue conservation pattern scored through ConSurf for pollen-specific protein SF3. A letter ***f*** represents predicted functional residues (highly conserved and exposed) and letter ***s*** represents predicted structural residues (highly conserved and buried)

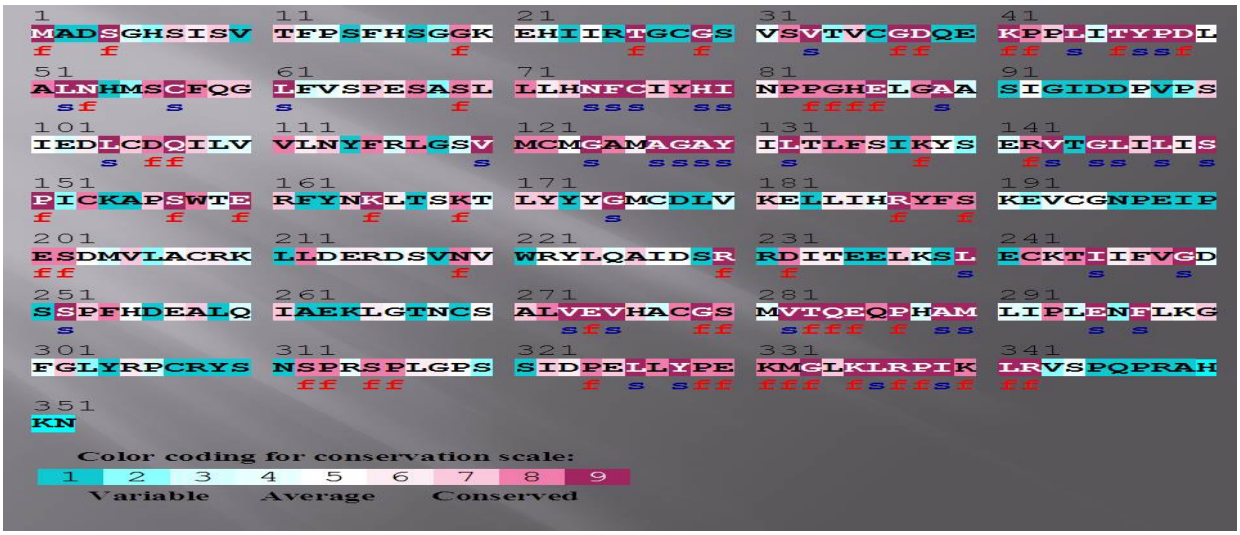

Figure 4. Residue conservation pattern scored through ConSurf for pollen-specific protein SF3. A letter ***f*** represents predicted functional residues (highly conserved and exposed) and letter ***s*** represents predicted structural residues (highly conserved and buried)

The result for allergen prediction in pollen specific protein SF3 and SF21 computed through AlgPred method is shown in Table 3. The application of the three different prediction methods under AlgPred revealed different results revealed for SF3 and SF21. The prediction through SVM (support vector machine) by single amino acid composition method revealed a score of 0.76 with percentage positive predictive value of 87.05% for SF3 while the score for SF21 was -1.62 with percentage positive predictive value of 15.19%. Also the prediction through SVM based by dipeptide composition method indicated score of 0.67 with percentage positive predictive value of 82.97% being higher than the scores and percentage predictive values achieved for SF21. The overall results for both SVM (aa) and SVM (dipeptide) revealed that pollen specific protein SF3 is potential allergen while SF21 is non-allergen. Further results for prediction of allergenity based on mapping of IgE epitope in a region of protein indicated that FS3 contain epitope*'EEPTAAPAEP'* at position 178 thus being a potential allergen while SF21 is non-allergen (Table 3).

Table 2. AlgPred Method with scores of allergens prediction for pollen specific protein SF3 and SF21

Sno.	Protein type	AlgPred methods for allergens prediction					Protein status for allergenicity
		SVM (aa)		SVM (dipeptide)		Mapping of IgE Epitopes	
		Score	Positive Predictive Value (%)	Score	Positive Predictive Value (%)		
1	SF3	0.76	87.05	0.67	82.97	Contains IgE epitope ***'EEPTAAPAEP'*** at position 178	Potential Allergen
2	SF21	-1.62	15.19	-1.57	8.69	Does not contain IgE epitope	Non- Allergen

Further evaluation of allergenicity potential of pollen specific proteins SF3 and SF21 done through employing ProAp methods revealed variation is scores and the probability of a protein being an allergen (Table 3). The results for motif-based approach and SVM-AAC (support vector machine-amino acid composition) further confirmed higher scores and probability for allergenicity in SF3 than SF2 (Table 3). The status for overall score through motif based and prediction through SVM-AAC revealed that SF3 is a potential allergen while SF21 is non-allergen. However, further analysis done through the FAO/WHO evaluation scheme for sequence identity cutoff >= 35% indicated that both pollen specific protein SF3 and SF21 were non-allergen (Table 3).

Table 3. ProAp Method with scores of allergens prediction for pollen specific protein SF3 and SF21

Sno.	Protein type	ProAp methods for allergens prediction				
		Motif-based		SVM-AAC		FAO/WHO: Sequence identity cutoff >= 35%
		Score E-value	Status	Prediction for allergenicity	Status	
1	SF3	0.0017	Allergen	Allergen probability (0.75)	Allergen	Non-Allergen
2	SF21	0.001	Allergen	Non-allergen probability (0.98).	Non Allergen	Non-Allergen

The phylogenetic tree constructed with amino acid sequences of pollen specific proteins (SF3 and SF21) and the sequences of proteins allergens from the family Asteraceae revealed three clusters (Figure 3). The phylogenetic tree shows that pollen specific proteins (SF3 and SF21) share a set of amino acid sequences with proteins allergens from the family Asteraceae. The first cluster consisted mainly protein allergens Hel a 2, Art v 4, Amb a 8, Amb a 1 Amb a 2 and Ole e 1 from the following species *Helianthus annuus, Ambrosia artemisiifolia, Artemisia vulgaris* and *Artemisia_annua*. Pollen specific protein SF21 was in the second cluster which consisted protein allergens Amb a 4 and Art v 1 for Art*emisia vulgaris* and *Ambrosia artemisiifolia* species

respectively. Pollen specific protein SF3 formed the third cluster with close identity mainly with protein allergen Amb a 3 which had from the species *Ambrosia artemisiifolia.*

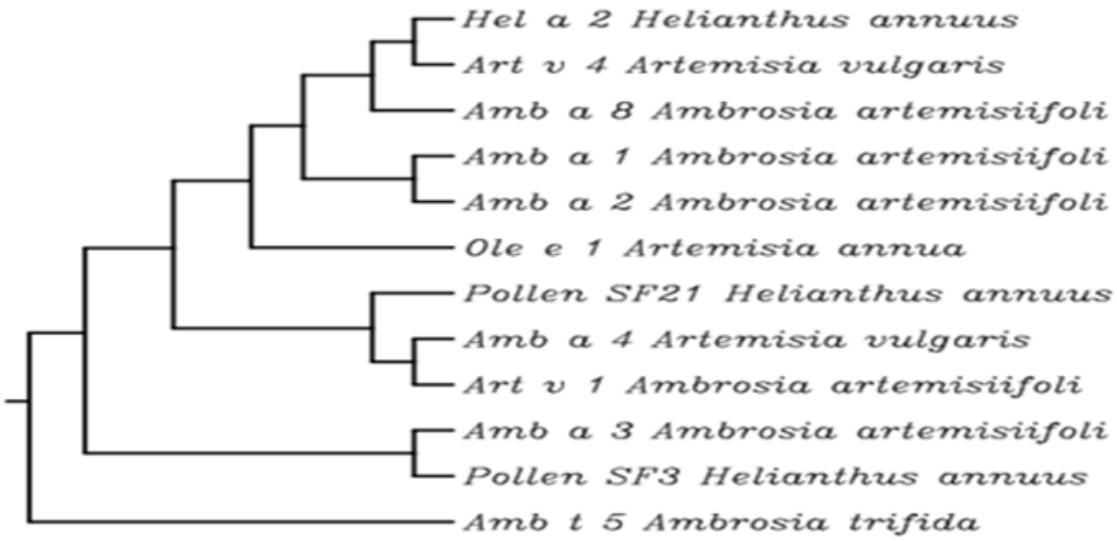

Figure 5. Phylogenetic tree constructed for the amino acid sequences of pollen specific proteins (SF3 and SF21) and the sequences of proteins allergens with species from the family Asteraceae

4. Discussion

The presence of charged residues in proteins particularly the number of negatively charged acidic residues plays an important role in determination of allergemicity potential of a protein. Although both pollen specific protein SF3 and SF21 revealed good number of negatively charged residues in their sequences but they had an overall basic theoretical isoelectric point (pI) not conforming to possibility of eliciting allegenicity potential. In characterizing allergens based on their charges it has previously been reported that non-allergens were characterized by neutral to basic pI while the possible allergens were mostly negatively charged [16]. The importance of charge in protein for allergenicity has also previously been reported in connection with the population of T-cell that recognizes immunogens based on their charge [17].

The presence of B-cell epitopes on a protein sequence is another consideration of probable allergens or cross reactive of a protein considered as candidate for allergenicity potential [18, 19, 20]. A B-cell epitope is a collection of separate amino acid residues on the surface of protein antigens that are recognized by antibodies and specifically bind to, thus triggering a protective immune response. Both pollen specific proteins SF3 and SF21 revealed a number of B cell epitopes in their sequences. Although the number of B cell epitopes was higher in pollen specific protein SF21 than SF3, the presence of B cell epitopes alone is not adequate to identify a protein with allergenic potential, thus the consideration of other computed features and characteristics of proteins revealed the variation for allergenicity potential between pollen specific protein SF3 and SF21.

The Consurf analysis demonstrated the presence of allergen-specific portions containing remarkably high proportion of conserved and surface-exposed hydrophobic residues in pollen specific protein SF3 compared to S pollen specific protein F21 which signify as determinants apparently responsible for allergenic epitopes. The portions identified may represent molecular patterns identifiable by cells of the innate immune system. Variation in functionally and structural important residues in protein sequences of SF3 and SF21 revealed their differences for allergenicity potential. The findings of this study are in agreement with Furmonaviciene et al., [1] for the approach used to define allergen-specific molecular surface features. The hydrophobicity of a protein has previously been reported to play important role for a protein to be identifiable as an allergen [21].

The high proportions in Lys pollen specific protein SF3 revealed the possibility of high propensity to occur in immunoglobulin E (IgE) binding sites. These findings are in agreement with other studies reported that conserved parts of the protein with surface-exposed amino acids are reckoned with high proportions of Lys accessible thus increasing the potential for high propensity to occur in IgE binding sites [22,23]. Findings from several studies have also reported that conserved residues are responsible for allergenic epitopes and lay a central role in allergy by initiating cross-linking of specific IgE on basophils/mast cells [24,25].

The AlgPred method for allergens prediction by incorporating support vector machine (SVM) revealed that only SF3 contained IgE epitope indicating that it is a potential allergen. Generally the differentiating feature between an allergens and non-allergens is the formers ability to induce a specific IgE response via a sequence of complex interactions with the immune system comprising uptake, processing and recognition [16]. Allergic sensitization to proteins involves the allergen-specific Th2 cells to drive the B cells to produce IgE in appropriate magnitude to enable the elicitation of an inflammatory reaction after the successive exposure to the same or a cross-reactive allergen [26,27].

The prediction of allegenicity potential for pollen specific protein SF3 and SF21 done by ProAp Method through Motif-based and support vector machine-amino acid composition further confirmed that the SF3 was the only potential allergen. The ProAp method has previously

been reported to show highest sensitivity in evaluating allergenicity potential when compared with other different methods applicable, it has the ability to recognize 94% of the allergens in the set f as the second followed by AlgPred method which has 89% [28]. However, on further confirmation through the FAO/WHO evaluation scheme for sequence identity cutoff >= 35% revealed that both pollen specific protein SF3 and SF21 were non-allergen. The FAO/WHO scheme requires exact match a stretch of six or more consecutive identical amino acids or more than 35% identity within any window of 80 amino acids in comparison with any known allergen [29].

In the phylogenetic relationship the pollen specific protein SF3 displayed sequence similarity with the major allergen of *Ambrosia artemisiifolia* pollen, Amb a 3 while pollen specific protein SF21 with allergens Amb a 4 and Art v 1 of Art*emisia vulgaris* and *Ambrosia artemisiifolia* respectively. The close identity in phylogenetic relationship might be an indication that the proteins share common epitopes with the pollen protein allergens. Due to their wide distribution, the family Asteraceae has previously been reported as one of the most potent elicitors of pollen allergy in many regions in the world [30]. Such phylogenetic relationship has previously been reported to occur in pollens of several plants species with proteins allergens demonstrating that cross-reactive features existing in pollen allergens responsible for allergenicity potential could also be shared in pollen specific proteins [31,32].

5. Conclusion

The allergenicity potential prediction in pollen specific protein SF3 and SF21 done through varied prediction approaches based on sequence analysis revealed that pollen specific protein SF3 is potential allergen. Based on the prediction it can be asserted SF3 protein has high potential of eliciting cross reactivity at some level of the immune response compared to SF21. Although not confirmed with the FAO/WHO scheme requirement of a protein being an allergen, further studies in characterization of SF3 protein through clinical and epidemiological aspects is proposed. The information obtained for *in -silico* characterization of pollen specific protein SF3 and SF21 may be useful in assessing the possible hazards and risks of allergic disease associated with the proteins but also can be useful in designing of potential synthetic vaccines for the allergens.

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