

The present invention relates to a polypeptide comprising a macadamia protein selected from the group comprising a protein running in a 2D SDS PAGE at pH 6.3 to 8.7 and 53 to 67 kDa and a protein running in a 2D SDS PAGE at pH 6.5 to 7.9 and 20 to 25 kDa or an antigenic variant thereof, preferably the protein running at pH 6.3 to 8.7 and 53 to 67 kDa or an antigenic variant thereof.

Allergic reactions to tree nuts can be serious and life-threatening. This holds true for common species such as hazelnut, peanut, walnut, but also for the macadamia nut, the fruit of an Australian evergreen tree (*Macadamia tetraphylla* and *M. integrifolia*).

Macadamia nut, which is, in terms of botany, a seed, is not as common as a food as many other tree nuts, but is more and more frequently consumed fresh or roasted and is often added to a variety of foods such as snack bars, cereals and mixed nuts. Ground macadamia meal is becoming an increasingly popular ingredient in baked goods, as is cold pressed macadamia oil. Not in the least, macadamia nut is often available as part of nut mixes and as such sold in countries which did not previously import it.

Allergic reactions caused by exposure to macadamia nuts in sensitized individuals may include serious symptoms (Pallares, D.E. (2000): Allergy to macadamia nut. Ann Allergy Asthma Immunol 2000; 85(5): 385-6). Therefore, it is crucial that subjects suffering from an allergy are aware of the source of allergens, since they need to avoid contact with foodstuffs, cosmetics and other products that contain or have been in contact with any of them.

Allergies may be diagnosed using conventional skin prick assays that involve exposure of the skin of a subject suspected of suffering from an allergy to the extract of a potentially allergenic fruit, complemented with suitable controls. However, false negative results occur frequently, since certain allergens may be present in such extracts at insufficiently low concentrations (Sicherer, S.H., Teuber, S.S. (2004): Current approach to the diagnosis and management of adverse reactions to foods. Journal of Allergy and Clinical Immunology 2004; 114.5, 1146–50). In addition, specific allergens may be subject to proteolysis in extracts (Rancé, F., Juchet, A., Brémont, F., Dutau, G. (1997): Correlation between skin prick tests

using commercial extracts and fresh foods, specific IgE and food challenges. Allergy 1997; 52:10, 1031–5). Furthermore, assays based on whole extracts are insufficiently specific owing to cross reactivities.

Therefore, it is desirable to devise assays based on the detection of antibodies such as IgE or IgG to specific allergens. Such assays involve the exposure of a patient sample comprising antibodies to an immobilized allergen followed by detection, optionally quantification of IgE antibodies binding to the immobilized allergen (Volcheck, G. W. (2009): Clinical Allergy, Diagnosis and Management).

Major allergens have been identified in a range of nuts. For example, the vast majority of cases of peanut allergy is associated with sensitivity to storage proteins Ara h 2, Ara h 7 and Ara h 6. Consequently, reasonably reliable assays based on the detection of IgE antibodies to these allergens have been devised (Codreanu, F., Collignon, O., Roitel, O., Thouvenot, B., Sauvage, C., Vilain, A.C., Cousin, M.O., Decoster, A., Renaudin, J.M., Astier, C., Monnez, J.M., Vallois, P., Morisset, M., Moneret-Vautrin, D.A., Brulliard, M., Ogier, V., Castelain, M.C., Kanny, G., Bihain, B.E., Jacquenet, S. (2011): A novel immunoassay using recombinant allergens simplifies peanut allergy diagnosis. Int Arch Allergy Immunol 2011; 154(3):216-26; EP15075033.9).

By contrast, very little information is available regarding the identities of macadamia nut allergens. Authors of state of the art documents describing immunoassays for the diagnosis of macadamia allergy so far had to rely on whole nut extracts or partially purified extracts. For example, Ghorbani *et al.* performed an immunoassay based on the detection of residual macadamia nut proteins in foodstuffs (Ghorbani, M., Morgan, M.R.A. (2010): Protein characterisation and immunochemical measurements of residual macadamia nut proteins in foodstuffs. Food Agr Immunol 2010; 21:347-360).

Herbst *et al.* used a whole nut extract from a single batch of macadamia nuts which caused a patient to show allergic symptoms (Herbst, R.A., Wahl, R., Frosch, P.J. (2010): Specific IgE reactivity and identification of potential allergens in macadamia allergy. J Eur Acad Dermatol Venereol 2010; 24(11):1361-3).

Sathe *et al.* reported the solubilisation and use in a Western blot of full extracts made from various tree nuts including macadamia nut (Sathe, S.K., Venkatachalam, M., Sharma, G.M., Kshirsagar, H.H., Teuber, S.S., Roux, K.H. (2009): Solubilization and electrophoretic

characterization of select edible nut seed proteins. J Agric Food Chem 2009; 57(17):7846-56).

In the absence of specific allergens, it is not feasible to provide a sufficiently reliable diagnosis. Whole nut extracts or partially purified extracts are associated with limitations similar to the shortcomings of skin prick assays such as instability and insufficient specificity, more specifically false negative results.

Therefore, the problem underlying the present invention is to provide a method for the diagnosis of an allergy, preferably a nut allergy, more preferably a macadamia allergy, and reagents for such a method, which method has an improved diagnostic reliability, in particular sensitivity, when compared to state of the art assays.

Another problem underlying the present invention is to provide an assay for the differential diagnosis of allergies, preferably nut allergies such as tree nut allergies, more preferably for distinguishing an allergy to a macadamia nut from an allergy to a nut other than macadamia, and/or for monitoring the therapy of an individual suffering from such an allergy.

Another problem underlying the present invention is to provide reagents useful for such an assay, methods for making such reagents and methods for such diagnosis. Another problem underlying the present invention is to provide a method of treatment and a pharmaceutical composition for treating patients suffering from an allergy to a macadamia nut.

The problem underlying the present invention is solved by the subject-matter of the attached independent and dependent claims.

Summary

In one aspect, the present disclosure refers to a diagnostically useful carrier comprising a polypeptide comprising a macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2 or a variant thereof having the ability to bind to a macadamia nut allergy-specific IgE antibody, wherein the variant has at least 80 % sequence identity with amino acid residues 21-645 of SEQ ID NO2, wherein the variant may in addition comprise a covalent chemical modification selected from the group comprising glycosylation, phosphorylation, acetylation, decarboxylation, citrullination and hydroxylation, wherein the polypeptide is a purified, isolated or recombinant polypeptide, wherein the diagnostically useful carrier is selected from the group comprising a bead, a test strip, a microtiter plate, a

blot, preferably from the group comprising western blot, line blot and dot blot, a glass surface, a slide, a biochip, a membrane, a microarray, an electrophoresis gel and a microtiter plate.

In another aspect, the present disclosure refers to an *in vitro* method for the diagnosis of a macadamia nut allergy comprising the step detecting in a sample obtained, preferably from a mammal, more preferably a human, an antibody binding to the macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2.

In yet another aspect, the present disclosure refers to a test kit for the diagnosis of a disease, comprising the diagnostically useful carrier as described herein, one or more solutions from the group comprising sample dilution buffer, washing buffer and a buffer comprising a means for specifically detecting any specifically detected antibody, preferably a secondary antibody and optionally a means for detecting a complex comprising the polypeptide and an antibody binding to a macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2.

In yet another aspect, the present disclosure refers to a polypeptide comprising a macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2 or a variant thereof having the ability to bind to a macadamia nut allergy-specific IgE antibody, wherein the variant has at least 80 % sequence identity with amino acid residues 21-645 of SEQ ID NO2, wherein the variant may in addition comprise a covalent chemical modification selected from the group comprising glycosylation, phosphorylation, acetylation, decarboxylation, citrullination and hydroxylation, wherein the polypeptide is a purified isolated or recombinant polypeptide, for use in therapy in the diagnosis of a macadamia nut allergy.

Detailed Description

In a first aspect, the problem underlying the present invention is solved by a polypeptide comprising a macadamia protein selected from the group comprising a protein running in a 2D SDS PAGE at pH 6.3 to 8.7 and 53 to 67 kDa and a protein running in a 2D SDS PAGE at pH 6.5 to 7.9 and 20 to 25 kDa or a variant thereof, preferably the protein running at pH 6.3 to 8.7 and 53 to 67 kDa or a variant thereof. The polypeptide is a polypeptide from *Macadamia tetraphylla* or *M. integrifolia*, preferably *M. integrifolia*.

In a preferred embodiment, the polypeptide is an isolated and/or recombinant polypeptide.

In another preferred embodiment, the polypeptide is a fusion polypeptide, preferably comprising a tag for detection or purification.

In a second aspect, the problem is solved by a nucleic acid encoding the polypeptide according to the present invention, preferably in a vector, more preferably in a vector in a cell.

In another preferred embodiment, the polypeptide is immobilized.

In a 3rd aspect, the problem is solved by a medical device comprising the polypeptide according to the present invention, wherein the medical device is preferably a diagnostically useful carrier, more preferably selected from the group comprising a bead, a test strip, a microtiter plate, a blot, preferably from the group comprising western blot, line blot and dot blot, a glass surface, a slide, a biochip, a membrane, a microarray, an electrophoresis gel and a microtiter plate.

In a 4th aspect, the problem is solved by a method comprising the step detecting in a sample, preferably from a mammal, more preferably a human, an antibody binding to the polypeptide according to the present invention, which method is preferably for the diagnosis of a nut allergy, more preferably for the diagnosis of a macadamia allergy.

In a 5th aspect, the problem is solved by a method for the treatment of a disease comprising the step administering to a subject or contacting with a sample from a subject the polypeptide according to the present invention,

wherein the disease is preferably a nut allergy, more preferably a tree nut allergy, most preferably a macadamia allergy.

In a 6th aspect, the problem is solved by an antibody binding to a macadamia protein selected from the group comprising a protein running in a 2D SDS PAGE at pH 6.3 to 8.7 and 53 to 67 kDa and a protein running in a 2D SDS PAGE at pH 6.5 to 7.9 and 20 to 25 kDa,

preferably the protein running at pH 6.3 to 8.7 and 53 to 67 kDa,

wherein optionally the antibody is in complex with said macadamia protein or a variant thereof. In a preferred embodiment, the antibody binding to a macadamia protein is an isolated antibody.

In a 7th aspect, the problem is solved by a test kit for the diagnosis of a disease, comprising the polypeptide according to the present invention and optionally a means for detecting a complex comprising the polypeptide and the antibody according to the present invention.

In an 8th aspect, the problem is solved by a method for isolating an antibody binding to a macadamia protein selected from the group comprising a protein running in a 2D SDS PAGE at pH 6.3 to 8.7 and 53 to 67 kDa and a protein running in a 2D SDS PAGE at pH 6.5 to 7.9 and 20 to 25 kDa or an antigenic fragment thereof, comprising the steps

- a) contacting a sample comprising the antibody with the macadamia protein or a variant thereof under conditions compatible with formation of a complex,
- b) isolating the complex formed in step a)
- c) dissociating the complex isolated in step b), and
- d) separating the antibody from the macadamia protein.

In a 9th aspect, the problem is solved by a pharmaceutical composition or vaccine comprising the polypeptide according to the present invention, optionally comprising, in addition, a pharmaceutically acceptable carrier.

In a preferred embodiment, the sample is a bodily fluid comprising antibodies, preferably selected from the group comprising whole-blood, serum, cerebrospinal fluid and saliva, more preferably whole blood.

In a preferred embodiment, the antibody is an IgE or IgG such as IgG4, preferably an IgE antibody.

In a 10th aspect, the problem is solved by a use of the polypeptide according to the present invention for the diagnosis of an allergy, preferably a nut allergy, more preferably a tree nut allergy, most preferably for a macadamia allergy.

The present invention is based on the inventors' surprising finding that a range of specific allergens exist in macadamia nuts that display reactivity towards the sera from patients suffering from nut allergy and may be used to diagnose and treat a nut allergy, in particular a

macadamia allergy. Moreover, these allergens may be used to differentiate nut allergies, in particular to distinguish an allergy to macadamia to an allergy to a nut other than macadamia. As far as the inventors are aware, these antigens have not yet been described as such in the state of the art.

In a preferred embodiment, the term “tree nut”, as used herein, is a nut selected from the group comprising macadamia nut, hazel nut, peanut, walnut, almond, pecan nut, cashew nut, brazil nut, chestnut, pine nut, pistachio, Mongongo Yeheb nut, Candlenut and Chilean hazelnut.

The invention relates to a diagnostically useful carrier, which is preferably a solid carrier for contacting a means for specifically capturing an antibody, which means is associated with said carrier, with a bodily fluid sample from a subject, preferably a mammalian subject, more preferably a human subject. The carrier may directly be contacted with a sample and allows exposure of the means for specifically capturing the antibody to any antibodies in the sample. In a preferred embodiment, the solid carrier is a diagnostic device, more preferably selected from the group comprising a bead, a test strip, a microtiter plate, a blot, preferably from the group comprising western blot, line blot and dot blot, a glass surface, a slide, a biochip, a membrane, a microarray, an electrophoresis gel and a microtiter plate.

In a most preferred embodiment, the diagnostically useful carrier is a line blot (Raoult, D., and Dasch, G. A. (1989), The line blot: an immunoassay for monoclonal and other antibodies. Its application to the serotyping of gram-negative bacteria. J. Immunol. Methods, 125 (1-2), 57-65; WO2013041540). In a preferred embodiment, the term “line blot”, as used herein, refers to a test strip, more preferably membrane-based, that has been coated with one or more means for capturing an antibody, preferably a polypeptide each. If two or more means are used, they are preferably spatially separated on the carrier. Preferably, the width of the bands is at least 30, more preferably 40, 50, 60, 70 or 80 % the width of the test strip. The test strip may comprise one or more control bands for confirming that it has been contacted with sample sufficiently long and under adequate conditions, in particular in the presence of human serum, antibody conjugate, or both. A multitude of line blots are commercially available, for example from EUROIMMUN AG, Lübeck, Germany.

It is essential that the sample used to practice the present invention comprises antibodies, also referred to as immunoglobulins. Typically the sample is a bodily fluid comprising a representative set of the entirety of the subject's immunoglobulins. However, the sample, once provided, may be subjected to further processing which may include fractionation,

centrifugation, enriching or isolating the entirety of immunoglobulins or any immunoglobulin class of the subject, preferably IgE and/or IgG4, which may affect the relative distribution of immunoglobulins of the various classes. The sample may be selected from the group comprising whole-blood, serum, cerebrospinal fluid and saliva and is preferably serum. In a most preferred embodiment, the sample comprises IgE class antibodies.

It is essential that the diagnostically useful carrier comprises a means for specifically capturing an antibody to a macadamia protein running in a 2D SDS PAGE at pH 6.3 to 8.7 and 53 to 67 kDa or a macadamia protein running in a 2D SDS PAGE at pH 6.5 to 7.9 and 20 to 25 kDa, preferably the macadamia protein running at pH 6.3 to 8.7 and 53 to 67 kDa, which has been identified by the inventors as macadamia vicilin-like protein (data base accession code of the polypeptide: Q9SPL3; data base accession code of the nucleic acid encoding said protein: AF161885). Any data base codes referred to throughout this application refers to the polypeptide sequence available via the NCBI data bases as online at the priority date of this application.

Preferably such means for specifically capturing such an antibody is a polypeptide comprising a macadamia protein selected from the group comprising the protein running in a 2D SDS PAGE at pH 6.3 to 8.7 and 53 to 67 kDa or the protein running in a 2D SDS PAGE at pH 6.5 to 7.9 and 20 to 25 kDa or an variant thereof, preferably the protein running at pH 6.3 to 8.7 and 53 to 67 kDa or a variant thereof.

According to the present invention, the carrier comprises one or more means for specifically capturing an antibody, preferably one or more, more preferably two or more, more preferably three or more, more preferably four or more such means, each of them capable of specifically capturing a different antibody. In addition to a means for capturing an macadamia protein, preferably macadamia Vicilin-like protein (Q9SLP3), the carrier may comprise a means for capturing an antibody to one or more than one protein from the group comprising SEQ ID NO3 (Cor a11 from hazelnut), SEQ ID NO4 (Ses i3 from Sesam), SEQ ID NO5 (Ara h1 from peanut), SEQ ID NO6 (Pis v3 from pistachio), SEQ ID NO7 (Ana o1 from cashew), SEQ ID NO8 (Car i2 from pecan), SEQ ID NO9 (Jug r2 from walnut), for example a means for capturing an antibody to SEQ ID NO3 and a means for capturing an antibody to SED ID NO4. Said means is preferably immobilized on said carrier. In a preferred embodiment, the means for specifically capturing an antibody is the polypeptide to which the antibody to be captured binds such as the polypeptide according to the present invention or SEQ ID NO3 (Cor a11 from hazelnut), SEQ ID NO4 (Ses i3 from Sesam), SEQ ID NO5 (Ara h1 from peanut), SEQ ID NO6 (Pis v3 from pistachio), SEQ ID NO7 (Ana o1 from cashew), SEQ ID

NO8 (Car i2 from pecan), SEQ ID NO9 (Jug r2 from walnut), for example a means for capturing an antibody to SEQ ID NO3 and a means for capturing an antibody to SED ID NO4 or a variant thereof. Said means, together with the insoluble carrier to which it is attached, may be separated from a reaction mixture in a straightforward manner, for example by filtration, centrifugation or decanting. Said means may be immobilized in a reversible or irreversible manner. For example, the immobilization is reversible if the molecule interacts with the carrier via ionic interactions which may be masked by addition of a high concentration of salt or if the molecule is bound via a cleavable covalent bond. By contrast, the immobilization is irreversible if the molecule is tethered to the carrier via a covalent bond that cannot be cleaved in aqueous solution. The means, preferably the polypeptide, may be indirectly immobilized, for example by immobilizing an antibody or other entity having affinity to the polypeptide, followed by addition of the polypeptide and formation of a polypeptide-antibody complex.

However, the teachings of the present invention may not only be carried out using polypeptides having the exact sequences referred to in this application explicitly, for example by function, name, sequence or accession number, or implicitly, or wild type sequences, but also using variants of such polypeptides.

In a preferred embodiment, the term “variant”, as used herein, may refer to at least one fragment of the full length sequence referred to, more specifically an amino acid or nucleic acid sequence which is, relative to the full-length sequence, truncated at one or both termini by one or more amino acids. Such a fragment comprises or encodes for a peptide having at least 10, 15, 25, 50, 75, 100, 150, 200, 300, 400 or 500 successive amino acids of the original sequence or a variant thereof. The total length of the variant may be at 25, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids. A preferred fragment is SEQ ID No.1 or a variant thereof.

The variant may be at least 40, 50, 60, 70, 75, 80, 85, 90, 92, 94, 95, 96, 97, 98 or 99 % identical to the reference amino acid sequence referred to or the fragment thereof, wherein amino acids other than those essential for the biological activity are deleted or substituted and/or one or more such essential amino acids are replaced in a conservative manner and/or amino acids are added or deleted such that the biological activity of the polypeptide is at least partially preserved. The state of the art comprises various methods that may be used to align two given nucleic acid or amino acid sequences and to calculate the degree of identity, see for example Arthur Lesk (2008), Introduction to bioinformatics, Oxford University Press, 2008, 3rd edition. In a preferred embodiment, the ClustalW software (Larkin, M. A.,

Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., Higgins, D. G. (2007): Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947-2948) is used applying default settings.

In a preferred embodiment, variants may, in addition, comprise chemical modifications, for example isotopic labels or covalent modifications such as glycosylation, phosphorylation, acetylation, decarboxylation, citrullination, hydroxylation and the like. The person skilled in the art is familiar with methods for the modification of polypeptides. Moreover, variants may also be generated by way of fusion with other known polypeptides or variants thereof.

It is essential that the variant of the polypeptide has biological activity. In a preferred embodiment, such biological activity is the ability to bind to an allergy-specific IgE antibody from a patient, preferably a patient suffering from an allergy, more preferably an allergy to a nut, most preferably an allergy to a macadamia. In another preferred embodiment, such biological activity is the ability to elicit an allergic response in a subject allergic to a nut, preferably a tree nut, more preferably a macadamia nut.

The person skilled in the art is capable of designing variants by starting from the original macadamia protein, introducing modifications point mutations, truncations and the like and subsequently confirming that the variant still has biological activity by testing whether said variant binds to an antibody in a sample obtained from a subject allergic to a macadamia nut.

Variants may be made using genetic engineering approaches or chemical synthesis.

The binding activity may be determined by Western blot, using as a source of antibody a serum from a patient allergic to macadamia, preferably as described in the examples.

The polypeptide according to the present invention or variant thereof may be a fusion protein, i.e. a polypeptide fused, preferably N-terminally or C-terminally, to another sequence, optionally via a linker. The other sequence may be a tag for the detection, for example a FLAG tag, or a tag for the purification, for example selected from the group comprising a GST, His or MBP tag. An exemplary fusion polypeptide according to the invention is shown in SEQ ID NO 2.

The polypeptide according to the present invention may be provided in any form and at any degree of purification, from tissues, fruits or cells comprising said polypeptide in an

endogenous form, more preferably cells overexpressing the polypeptide, crude or enriched lysates of such cells, to purified and/or isolated polypeptide which is essentially pure. In a preferred embodiment, the polypeptide is a native polypeptide, wherein the term “native polypeptide”, as used herein, refers to a folded polypeptide, more preferably to a folded polypeptide purified from tissues or cells, more preferably from mammalian cells or tissues, optionally from non-recombinant tissues or cell. If a native polypeptide is used, it is preferably enriched compared to its natural state.

According to the present invention, the polypeptide may be a recombinant protein, wherein the term “recombinant”, as used herein, refers to a polypeptide produced using genetic engineering approaches at any stage of the production process, for example by fusing a nucleic acid encoding the polypeptide to a strong promoter for overexpression in cells or tissues or by engineering the sequence of the polypeptide itself and/or using for the expression a cell modified by genetic engineering. The person skilled in the art is familiar with methods for engineering nucleic acids and polypeptides encoded (for example, described in Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989), *Molecular Cloning*, CSH or in Brown T. A. (1986), *Gene Cloning – an introduction*, Chapman & Hall) and for producing and purifying native or recombinant polypeptides (for example *Handbooks „Strategies for Protein Purification“, „Antibody Purification“, published by GE Healthcare Life Sciences, and in Burgess, R. R., Deutscher, M. P. (2009): Guide to Protein Purification).*

In a preferred embodiment, the term “isolated” means that the polypeptide has been enriched relative to the entirety of the macadamia proteins, preferably macadamia allergens, in a whole nut or whole nut extract obtained from a whole nut. In another more preferred embodiment, the polypeptide is an isolated polypeptide, wherein the term “isolated” means that the polypeptide has been enriched compared to its state upon production using a biotechnological or synthetic approach and is preferably pure, *i.e.* at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 95 or 99 % of the polypeptide used consists of said polypeptide as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie blue staining and visual inspection.

The subject according to the present invention is an animal including a human producing antibodies, preferably IgE class or equivalent allergy-related antibodies, more preferably from a mammal, most preferably a human.

A method according to the present invention contemplates detecting in a sample an antibody binding to the polypeptide according to the invention, which method is preferably for the

diagnosis of a nut allergy, more preferably for the diagnosis of a macadamia allergy. The person skilled in the art is familiar with various methods that may be used to detect an antibody in a sample, for example selected from the group comprising immunodiffusion techniques, basophil activation by IgE antibody, immunoelectrophoretic techniques, light scattering immunoassays, agglutination techniques, labeled immunoassays such as those from the group comprising radiolabeled immunoassay, enzyme immunoassays such as colourimetric assays, chemiluminescence immunoassays and immunofluorescence techniques.

In many cases detecting the absence or presence of an antibody, optionally meaning determining whether the concentration of the antibody is beyond a certain threshold, often suggested by the detection limit, in the sample, is sufficient for the diagnosis. If the antibody can be detected, this will be information instrumental for the clinician's diagnosis and indicates an increased likelihood that the patient suffers from an allergy. In a preferred embodiment, the relative concentration of the antibody in the serum, compared to the level that may be found in the average healthy subject, may be determined. In a preferred embodiment, the term "detecting the presence or absence", as used herein, means that it is sufficient to check whether a signal sufficiently beyond any background level may be detected using a suitable complex detection method that indicates that the antibody of interest is present or more antibody of interest is present than would be in a healthy subject. In a more preferred embodiment, this may involve determining whether the concentration is beyond the concentration in a control or beyond a threshold, preferably at least 0.1, preferably 0.2, 0.5, 1, 2, 5, 10, 20, 25, 50, 100, 200, 500, 1000, 10000 or 100000 times higher than the concentration of the antibody of interest found in the average healthy subject.

Briefly, detecting the antibody may involve as step a) contacting a sample comprising the antibody with the polypeptide according to the present invention or a variant thereof under conditions compatible with formation of a complex. This may be achieved by contacting the liquid sample with the polypeptide in an immobilized form, for example immobilized on a membrane such as used in a Western blot or a line blot. As the sample and the polypeptide are in contact, any antibody present in the sample which is capable of binding specifically to the polypeptide will do so to the effect that a complex comprising the polypeptide and the antibody is present.

Subsequently, step b) is carried out which involves isolating said complex formed in step a), for example by removing the sample and, optionally, washing the immobilized polypeptide using a washing buffer.

A final step c) involves detecting the complex, for example by adding a secondary antibody which may bind to the antibody from the sample. The secondary antibody may comprise a label, for example an enzyme or a radioactive isotope. Alternatively, the complex may be detected directly using methods such as chemical crosslinking followed by SDS PAGE or using mass spectrometry.

Detection of an antibody to the polypeptide according to the invention suggests that the subject donating the sample is allergic to macadamia. In case a subject shows allergic responses, but such a sample does not contain antibodies to the inventive subject, it is likely that the subject suffers from an allergy, for example a nut allergy, but is not allergic to macadamia nut.

Within the scope of the present invention is a diagnostically useful carrier comprising a means for specifically capturing an antibody to the inventive polypeptide. In a preferred embodiment, the term “specifically capturing an antibody”, as used herein, refers to the ability to bind specifically to the antibody of interest to the effect that it is bound and removed from the sample, whereas other antibodies are essentially not bound and remain in the sample.

According to the present invention, a means for specifically detecting a captured antibody is provided, optionally as part of a kit. In a preferred embodiment, the term “specifically detecting a captured antibody”, as used herein, means that the antibody binding specifically to the means for specifically capturing the antibody binding to the polypeptide according to the invention, following capture, is detected rather than any other antibody present in the sample. In a preferred embodiment, the term “binding specifically”, as used herein, means that the binding is stronger than a binding reaction characterized by a dissociation constant of 1×10^{-5} M, more preferably 1×10^{-7} M, more preferably 1×10^{-8} M, more preferably 1×10^{-9} M, more preferably 1×10^{-10} M, more preferably 1×10^{-11} M, more preferably 1×10^{-12} M, as determined by surface plasmon resonance using Biacore equipment at 25 °C in PBS buffer at pH 7.

The inventive teachings provide a kit, preferably for diagnosing an allergy, more preferably for diagnosing a macadamia allergy. Such a kit is a container that comprises specific reagents required to practice the inventive method, in particular the diagnostically useful carrier according to the present invention, optionally in addition to one or more solutions

required to practice the inventive method, preferably selected from or all from the group comprising sample dilution buffer, washing buffer and buffer comprising a means for detecting any specifically captured antibody, such as a secondary antibody and optionally a means for detecting the latter. Furthermore, it may comprise instructions detailing how to use the kit and the inventive diagnostically useful carrier for contacting the inventive polypeptide with a bodily fluid sample from a subject, preferably a human subject, for example a line blot wherein means for specifically capturing an antibody to the polypeptide according to the present invention is immobilized on the line blot. Furthermore, the kit may comprise a positive control, for example a recombinant antibody known to bind to the polypeptide according to the present invention, and a negative control, for example a protein having no detectable affinity to the polypeptide according to the present invention. Finally, such a kit may comprise a standard solution comprising an antibody for preparing a calibration curve.

According to the invention, a means for detecting the one or more captured antibodies is required. The person skilled in the art is aware of many methods that may be used, which are also described in the state of the art, for example in Zane, H. D. (2001), Immunology – Theoretical & Practical Concepts in Laboratory Medicine, W. B. Saunders Company, in particular in Chapter 14. In a preferred embodiment, a secondary antibody binding to the constant region of the one or more captured antibodies, which is the corresponding primary antibody, is used, which secondary antibody may be associated with a label that is straightforward to detect, for example a fluorescent, radioactive or enzymatically active label, the latter of which may catalyze a chemiluminescent reaction or the generation of a molecule detectable using colourimetry or spectroscopy or another analytical method.

Alternatively, a biological functional assay may be used as a means for detecting the one or more captured antibody under the proviso that it is an IgE class antibody, preferably an assay based on basophil activation by IgE antibody. Such assays have been described in the state of the art, for example Hausmann, O. V., Gentinetta, T., Bridts, C. H., and Ebo, E. G. (2009): The Basophil Activation Test in Immediate-Type Drug Allergy, Immunol. Allergy Clin. N. Am. 29, 555-566.

In a preferred embodiment, the term „diagnosis“, as used herein, refers to any kind of procedure aiming to obtain information instrumental in the assessment whether a patient suffers or is likely or more likely than the average or a comparative subject, the latter preferably having similar symptoms, to suffer from a certain disease or disorder in the past,

at the time of the diagnosis or in the future, to find out how the disease is progressing or is likely to progress in the future or to evaluate the responsiveness of a patient with regard to a certain treatment, for example the administration of suitable drugs such as drugs for the desensitization of allergic patients. In other words, the term “diagnosis” comprises not only diagnosing, but also prognosticating and/or monitoring the course of a disease or disorder or the success of a therapy. It is preferred that IgG, more preferably IgG 4, class autoantibodies detected for the purpose of monitoring the progression of the disease and/or the success of a therapy.

Therefore, the term “diagnosis” does preferably not imply that the diagnostic methods or agents according to the present invention will be definitive and sufficient to finalize the diagnosis on the basis of a single test, let alone parameter, but may refer to a contribution to what is referred to as a “differential diagnosis”, *i.e.* a systematic diagnostic procedure considering the likelihood of a range of possible conditions on the basis of a range of diagnostic parameters. The term “diagnosis” may also refer to a method or agent used to choose the most promising treatment regime for a patient. In other words, the method or agent may relate to selecting a treatment regimen for a subject.

The invention provides a pharmaceutical composition, preferably a vaccine, comprising the polypeptide according to the invention, which composition is preferably suitable for administration to a subject, preferably a mammalian subject, more preferably to a human. Such a pharmaceutical composition may comprise a pharmaceutically acceptable carrier. The pharmaceutical composition may, for example, be administered orally, parenterally, by inhalation spray, topically, by eyedrops, rectally, nasally, buccally, vaginally or via an implanted reservoir, wherein the term “parenterally”, as used herein, comprises subcutaneous, intracutaneous, intravenous, intramuscular, intra-articular, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques. The pharmaceutical composition may be provided in suitable dosage forms, for example capsules, tablets and aqueous suspensions and solutions, preferably in sterile form. It may be used in a method of treatment of a disease, preferably an allergy, which method comprises administering an effective amount of the inventive polypeptide to a subject. A hypoallergenic variant of the polypeptide according to the invention may be used. The person skilled in the art is familiar with methods for the generation of hypoallergenic variants of known allergens.

The invention provides a method for treating, preventing or ameliorating an allergy, preferably a nut allergy, more preferably a macadamia allergy, but administering to a subject the inventive pharmaceutical composition.

The present invention is further illustrated by the following figures and non-limiting sequences and examples from which further features, embodiments, aspects and advantages of the present invention may be taken.

Fig. 1 shows the results of 2D gel electrophoretic separation of macadamia nut extract. Proteins were separated in the first dimension on a pH 3-10 non-linear IPG strip and subsequently by SDS PAGE under reducing conditions, stained by Coomassie staining and cut out from the gel. Numbers mark the spots subsequently subjected to MALDI-TOF analysis. The arrow marks the band representing the protein subsequently identified as Macadamia vicilin-like protein.

Fig. 2 shows the results of Western blot analysis of macadamia nut extract and Western blotting of proteins separated by 2D gel electrophoresis. Molecular weight markers are the same as shown in **Fig. 1**.

Fig. 3 shows the analysis of human sera from macadamia-sensitized patient (n = 20) and healthy blood donors (n = 17) using the EUROLINE line assay coated with vicilin-like protein from macadamia. The evaluation of the bound antibodies from the human sera was carried out based on the EAST reference system.

The present application comprises a number of amino acid sequences, more specifically:

SEQ ID NO1 (preferred fragment of macadamia vicillin-like protein (Q9SPL3))

SEQ ID NO2 (fusion protein comprising macadamia vicillin-like protein)

SEQ ID NO3 (Cor a11 from hazelnut)

SEQ ID NO4 (Ses i3 from Sesam)

SEQ ID NO5 (Ara h1 from peanut)

SEQ ID NO6 (Pis v3 from pistachio)

SEQ ID NO7 (Ana o1 from cashew)

SEQ ID NO8 (Car i2 from pecan)

SEQ ID NO9 (Jug r2 from walnut)

SEQ ID NO10 (Mac I vicillin from Macadamia)

The present invention is further illustrated by the following non-limiting examples from which further features, embodiments, aspects and advantages of the present invention may be taken.

Example 1:

Preparation of macadamia nut extract

Roasted and salted commercially available macadamia nut kernels were ground, followed by defatting 3 times with acetone. The defatted nut debris was dried on a folded filter. The dried material was resuspended in a buffer such as Phosphate buffer saline (PBS, 50 mM sodium phosphate, 150 mM NaCl, pH 7.4) incubated at 4 °C for 3 hours in an incubation shaker, followed by filtration and centrifugation in a benchtop centrifuge for 30 minutes with 4000 rpm at 4°C.

The supernatant was precipitated using 100 % ammonium sulfate and the resulting pellet dialysed against PBS. Estimation of protein concentration was performed by Bio-Rad Protein Assay (Bio-Rad Laboratories, Inc., Hercules, USA) as described in the manufacturer's instructions.

Preparative gel electrophoresis

The crude macadamia extract was precipitated with cooled acetone and after incubation for 2 hours at -20 °C and centrifugation for 30 minutes at 20000 x g at 4 °C, the pellet was resuspended in a sample buffer for isoelectric focusing (2.5 % sulfobetaine 3-10, 4 M urea, 2 M thiourea, 0.5 % (v/v) Carrier Ampholyte pH 3-10, 40 mM DTT and 0.003 % bromphenol blue). Overnight the IPG-strip (ReadyStrip IPG Strips 7 cm pH 3 to 10 (Bio-Rad), immobilized pH-gradient) was rehydrated with the sample at room temperature.

Subsequently the proteins were focused using a PROTEAN i12 IEF System (Bio-Rad) and separated in the second dimension on the basis of their apparent molecular mass by polyacrylamide gel electrophoresis (4 to 12 % Bis-Tris Protein Gels, 1.0 mm x 2D well, Thermo Fisher Scientific) using to yield pure protein spots on the gel. For size estimation of the spots in the 2D gel, Spectra Multicolor Broad Range (Thermo Fisher Scientific) was used as described in the manufacturer's instructions.

Proteins of the resulting 2D gel were stained by Blue-silver staining (Candiano et al, Electrophoresis. 2004 May; 25(9):1327-33. Blue silver: a very sensitive colloidal Coomassie G-250 staining for proteome analysis). The stained gel is shown in **Fig. 1**.

Western blot

The 2D gel of the nut extract was transferred to a nitrocellulose membrane by TE22 Miniblotter (Hoefer Inc., Holliston, MA, USA) as described in the manufacturer's instructions. The transferred proteins were detected by staining with Ponceau S. The resulting Western blot was incubated for 15 minutes in wash buffer (EUROIMMUN AG, Lübeck, Germany) and subsequently with serum from a macadamia sensitized patient diluted 1:10 in wash buffer for 1 hour.

After washing 3 times for 5 minutes each with wash buffer (Article number ZD1100, as supplied by EUROIMMUN AG for EUROLINE line blots, Lübeck, Germany), the membrane was incubated with anti-IgE-AP conjugate (EUROIMMUN AG, Lübeck, Germany) and again washed 3 times for 5 minutes each with wash buffer. Bound antibodies were detected by incubation with BCIP/NBT substrate (EUROIMMUN AG, Lübeck, Germany) as recommended in the manufacturer's instructions.

The blot obtained is shown in **Fig. 2**. Major proteins of interest include a protein running in the 2D gel electrophoresis at pH 6.3 to 8.7 and 53 to 67 kDa and a protein running in the 2D gel electrophoresis at pH 6.5 to 7.9 and 20 to 25 kDa.

MALDI

The chosen spots of the 2D gel stained with Blue Silver stain were cut out and digested with Trypsin. A peptide mass fingerprint (PMF) and a MSMS were performed with the MALDI-TOF/TOF Autoflex III (Bruker Corporation, Billerica, MA, USA) as described in the manufacturer's instructions. The NCBI-Database for green plants was screened for convenient sequences, leading to the identification of macadamia vicilin-like protein (Data base accession code: Q9SPL3) as the protein running at pH 6.3 to 8.7 and 53 to 67 kDa.

Cloning, recombinant expression and purification of macadamia vicilin-like protein

The coding region of the macadamia vicilin-like protein, more specifically the coding DNA equivalent to the nucleotide sequence in AF161885 fused via a linker to a His tag as shown in SEQ ID NO 2, was generated by gene synthesis (Eurofins Genomics GmbH), amplified by polymerase chain reaction and subcloned into the recombinant vector using pBAD/TOPO®

ThioFusion™ Expression Kits (Invitrogen). The fusion protein was expressed in *E. coli* as described by the manufacturer and isolated by affinity chromatography on nickel matrix (IMAC).

Preparation of a line blot (EUROLINE)

The EUROLINE line blot was produced by coating the fusion protein using a precision dispenser (commercially available from Zeta Corporation) onto a nitrocellulose membrane (commercially available from Schleicher & Schüll). After blocking and washing as recommended in the manufacturer's instructions the membrane was cut in 3 mm strips.

Evaluation of samples using the EUROLINE line assay

The EUROLINE strips were incubated as recommended in the manufacturer's (EUROIMMUN AG, Lübeck, Germany) instructions. In detail the strips were incubated with 1ml 1/11 (v/v) diluted patient sample (20 macadamia sensitized patients and 17 healthy blood donors) in washing buffer for 16 h at room temperature. After discarding the patient samples the strips were washed 3 times for 5 min with 1 ml washing buffer per strip and subsequently incubated for 1 h with 1 ml anti-human-IgE antibody conjugated with AP. Afterwards the strips were again washed 5 times with washing buffer and then incubated for 10 min with BCIP/NBT substrate.

The evaluation of the results occurred by means of a scanner and EUROLINEScan® program (EUROIMMUN AG, Lübeck, Germany). A semiquantitative analysis can be realized by using an IgE calibration curve of WHO standard (75/502). The IgE concentration is expressed in international units kU/l (kilo IgE units per liter). The results can be interpreted for example using the RAST system (Radio-allergo-sorbent test) developed by the company Pharmacia (to date Thermo Scientific) as reference system (Volcheck, Clinical Allergy, Diagnosis and Management, 2009, ISBN 978-1-59745-315-8). The EUROLINEScan® program calculates automatically the intensities of the bands of the EUROLINE into EAST classes (enzyme-allergo-sorbent test), which correlates to the concentration levels of the RAST system. The classification of the EAST classes is shown in Tab. 1.

EAST class	Concentration [kU/l]
0	< 0.35
1	0.35 - 0.7
2	0.7 - 3.5
3	3.5 - 17.5
4	17.5 - 50
5	50 - 100

6	> 100
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Tab. 1: EAST classes

Example 2:

Vicillin-like proteins from hazelnut (SEQ ID NO3), sesame (SEQ ID NO4), peanut (SEQ ID NO5), pistachio (SEQ ID NO6), cashew nut (SEQ ID NO7), pecan nut (SEQ ID NO8), walnut (SEQ ID NO9) and macadamia nut (SEQ ID NO8) were expressed as His tag fusion proteins according to standard methods and purified as described in example 1.

Line blots were made, contacted with patient samples and evaluated as described in Examples 2 and 3 of EP15001277. Sera from 29 patients allergic to tree nuts and nine healthy blood donors were tested.

The control sera from the healthy blood donors were tested and all found negative (EAST class 0).

Figs. 4a to 4g show comparative data indicating the IgE concentration expressed in the form of EAST classes in sera from said patients as determined using the line blots. The figures show the correlation, if any, between IgE to one tree nut or kernel 7S vicillin-like protein from a tree nut other than Macadamia and the IgE to Mac I Vicilin from Macadamia.

For example, **Fig. 4g** shows that one patient showed a EAST class 1, patients an East class 2 and one patient an East class 5 IgE response to Mac I Vicilin, but no response to Ana o1 (cashew nut) as indicated by the fact that they are shown in the line corresponding to 0 EAST class for Ana o1.

These results show that a significant number of allergic patients (dark fields in Figs. **4a** to **4g**), in each comparative experiment, react to Mac I Vicilin, but not to the corresponding Vicilin-like protein from the other tree nut, more specifically 13 out of 29 patients (Cor a11 compared to Mac I), 9 out of 29 patients (Ses i3), 10 out of 29 patients (Jug r2), 9 out of 29 patients (Pis v3), 8 out of 29 patients (Car i2), four out of 29 patients (Ara h1) and four out of 29 patients (Ana o1). One of these patients could not be diagnosed by detecting IgE class antibodies to vicilins from other tree nuts rather than Mac I vicilin, since IgE antibodies in his serum reacted to Mac I vicilin only.

There is no EAST class correlation for the Mac I vicilin and the 7S vicilin-like proteins from other nuts and kernels. The reaction of some patients to Mac I vicillin is much stronger than to the respective other tree nut allergen, showing that the respective patient is primarily allergic to *Macamamia* rather than to another tree nut. For example, Fig. 4a shows that 4 patients have an EAST class of 6 (strong reaction) to Mac I vicillin, but no or only a mild reaction (EAST classes 0 or 1) with regard to Cor a11. This shows that Mac I Vicilin possesses unique IgE epitopes not shared with any of the other vicilins.

Claims

1. A diagnostically useful carrier comprising a polypeptide comprising a macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2

or a variant thereof having the ability to bind to a macadamia nut allergy-specific IgE antibody, wherein the variant has at least 80 % sequence identity with amino acid residues 21-645 of SEQ ID NO2, wherein the variant may in addition comprise a covalent chemical modification selected from the group comprising glycosylation, phosphorylation, acetylation, decarboxylation, citrullination and hydroxylation,

wherein the polypeptide is a purified, isolated or recombinant polypeptide,

wherein the diagnostically useful carrier is selected from the group comprising a bead, a test strip, a microtiter plate, a blot, preferably from the group comprising western blot, line blot and dot blot, a glass surface, a slide, a biochip, a membrane, a microarray, an electrophoresis gel and a microtiter plate.

2. An *in vitro* method for the diagnosis of a macadamia nut allergy comprising the step detecting in a sample obtained, preferably from a mammal, more preferably a human, an antibody binding to the macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2.
3. A test kit for the diagnosis of a disease, comprising the diagnostically useful carrier according to claim 1, one or more solutions from the group comprising sample dilution buffer, washing buffer and a buffer comprising a means for specifically detecting any specifically detected antibody, preferably a secondary antibody and optionally a means for detecting a complex comprising the polypeptide and an antibody binding to a macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2.

4. The method according to claim 2, wherein the sample is a bodily fluid comprising antibodies, preferably selected from the group comprising whole-blood, serum, cerebrospinal fluid and saliva, more preferably whole blood.
5. The method according to any one of claims 2 or 4 and the test kit according to claim 3, wherein the antibody is an IgE antibody.
6. A polypeptide comprising a macadamia vicilin-like protein consisting of amino acid residues 21-645 of SEQ ID NO2

or a variant thereof having the ability to bind to a macadamia nut allergy-specific IgE antibody, wherein the variant has at least 80 % sequence identity with amino acid residues 21-645 of SEQ ID NO2, wherein the variant may in addition comprise a covalent chemical modification selected from the group comprising glycosylation, phosphorylation, acetylation, decarboxylation, citrullination and hydroxylation,

wherein the polypeptide is a purified isolated or recombinant polypeptide,

for use in therapy in the diagnosis of a macadamia nut allergy.

7. The diagnostically useful carrier, method, kit or polypeptide according to any of claims 1 to 6, wherein the polypeptide is a fusion polypeptide, preferably comprising a tag for detection or purification.
8. The diagnostically useful carrier, method, kit or polypeptide according to any of claims 1 to 7, wherein the polypeptide is immobilized.

A novel macadamia allergen

Abstract

The present invention relates to a polypeptide comprising a macadamia protein selected from the group comprising a protein running in a 2D SDS PAGE at pH 6.3 to 8.7 and 53 to 67 kDa and a protein running in a 2D SDS PAGE at pH 6.5 to 7.9 and 20 to 25 kDa or an antigenic variant thereof, preferably the protein running at pH 6.3 to 8.7 and 53 to 67 kDa or an antigenic variant thereof.

[No Suitable Figure]

161102_Sequence listing Nachanmeldung Macadamia_ST25
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35 40 45

Arg Gln Gln Glu Ser Asp Pro Arg Gln Gln Gln Tyr Cys Gln Arg Arg
50 55 60

Cys Lys Glu Ile Cys Glu Glu Glu Glu Glu Tyr Asn Arg Gln Arg Asp
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Pro Gln Gln Gln Tyr Glu Gln Cys Gln Lys Arg Cys Gln Arg Arg Glu
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Thr Glu Pro Arg His Met Gln Ile Cys Gln Gln Arg Cys Glu Arg Arg
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Tyr Glu Lys Glu Lys Arg Lys Gln Gln Lys Arg Tyr Glu Glu Gln Gln
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Arg Glu Asp Glu Glu Lys Tyr Glu Glu Arg Met Lys Glu Gly Asp Asn
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Lys Arg Asp Pro Gln Gln Arg Glu Tyr Glu Asp Cys Arg Arg His Cys
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Cys Gln Arg Arg Cys Lys Glu Ile Cys Glu Glu Glu Glu Glu Tyr Asn
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Gln Arg Arg Glu Thr Glu Pro Arg His Met Gln Ile Cys Gln Gln Arg
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Arg Arg Cys Gln Glu Gln Gln Arg Gln His Gly Arg Gly Gly Asp Leu
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161102_Sequence listing Nachanmeldung Macadamia_ST25

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 Gly Arg Ser Lys Leu Leu Arg Ala Leu Lys Asn Tyr Arg Leu Val Leu
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 275 280 285
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 His Arg Asp Asn Arg Glu Ser Tyr Asn Leu Glu Cys Gly Asp Val Ile
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 325 330 335
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 Gln Tyr Lys Glu Phe Phe Pro Ala Gly Gly Gln Asn Pro Glu Pro Tyr
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 370 375 380
 Thr Glu Arg Leu Arg Gly Val Leu Gly Gln Gln Arg Glu Gly Val Ile
 385 390 395 400
 Ile Arg Ala Ser Gln Glu Gln Ile Arg Glu Leu Thr Arg Asp Asp Ser
 405 410 415
 Glu Ser Arg Arg Trp His Ile Arg Arg Gly Gly Glu Ser Ser Arg Gly
 420 425 430
 Pro Tyr Asn Leu Phe Asn Lys Arg Pro Leu Tyr Ser Asn Lys Tyr Gly
 435 440 445
 Gln Ala Tyr Glu Val Lys Pro Glu Asp Tyr Arg Gln Leu Gln Asp Met
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161102_Sequence listing Nachanmeldung Macadamia_ST25

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35 40 45

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50 55 60

Gln Asp Glu His Phe Glu Ser Arg Val Lys Thr Glu Glu Gly Arg Val
65 70 75 80

Gln Val Leu Glu Asn Phe Thr Lys Arg Ser Arg Leu Leu Ser Gly Ile

Glu Asn Phe Arg Leu Ala Ile Leu Glu Ala Asn Pro His Thr Phe Ile
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Ser Pro Ala His Phe Asp Ala Glu Leu Val Leu Phe Val Ala Lys Gly
115 120 125

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130 135 140

Val Glu His Gly Asp Ile Ile Arg Ile Pro Ala Gly Thr Pro Val Tyr
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Met Ile Asn Arg Asp Glu Asn Glu Lys Leu Phe Ile Val Lys Ile Leu
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Gln Pro Val Ser Ala Pro Gly His Phe Glu Ala Phe Tyr Gly Ala Gly
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260 265 270

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275 280 285

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290 295 300

Gly Ser Met Ala Gly Pro Tyr Tyr Asn Ser Arg Ala Thr Lys Ile Ser
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Val Val Val Glu Gly Glu Gly Phe Phe Glu Met Ala Cys Pro His Leu
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Gly Val Val Phe Val Ala Pro Ala Gly His Pro Val Ala Val Ile Ala

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35 40 45

Gly Asp Ile Leu Glu Glu Glu Val Trp Asn Arg Lys Ser Pro Ile Glu
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Arg Leu Arg Glu Cys Ser Arg Gly Cys Glu Gln Gln His Gly Glu Gln
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Arg Glu Glu Cys Leu Arg Arg Cys Gln Glu Glu Tyr Gln Arg Glu Lys
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Gly Arg Gln Asp Asp Asp Asn Pro Thr Asp Pro Glu Lys Gln Tyr Gln
100 105 110

Gln Cys Arg Leu Gln Cys Arg Arg Gln Gly Glu Gly Gly Phe Ser
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Arg Glu His Cys Glu Arg Arg Arg Glu Glu Lys Tyr Arg Glu Gln Gln
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161102_sequence listing Nachanmeldung Macadamia_ST25

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180 185 190

Val Leu Gln Lys Phe Thr Asp Arg Ser Glu Leu Leu Arg Gly Ile Glu
195 200 205

Asn Tyr Arg Val Ala Ile Leu Glu Ala Glu Pro Gln Thr Phe Ile Val
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Pro Asn His Trp Asp Ala Glu Ser Val Val Phe Val Ala Lys Gly Arg
225 230 235 240

Gly Thr Ile Ser Leu Val Arg Gln Asp Arg Arg Glu Ser Leu Asn Ile
245 250 255

Lys Gln Gly Asp Ile Leu Lys Ile Asn Ala Gly Thr Thr Ala Tyr Leu
260 265 270

Ile Asn Arg Asp Asn Asn Glu Arg Leu Val Leu Ala Lys Leu Leu Gln
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Pro Val Ser Thr Pro Gly Glu Phe Glu Leu Phe Phe Gly Ala Gly Gly
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Glu Asn Pro Glu Ser Phe Phe Lys Ser Phe Ser Asp Glu Ile Leu Glu
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Ala Ala Phe Asn Thr Arg Arg Asp Arg Leu Gln Arg Ile Phe Gly Gln
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370 375 380

Tyr Gly Gln Leu His Glu Val Asp Ala Ser Gln Tyr Arg Gln Leu Arg
385 390 395 400

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405 410 415

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161102_Sequence listing Nachanmeldung Macadamia_ST25

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465 470 475 480

His Pro Phe Val Ala Val Ala Ser Ser Asn Gln Asn Leu Gln Val Leu
485 490 495

Cys Phe Glu Val Asn Ala Asn Asn Asn Glu Lys Phe Pro Leu Ala Gly
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Arg Arg Asn Val Met Asn Gln Leu Glu Arg Glu Ala Lys Glu Leu Ala
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Leu Glu Tyr Asp Pro Arg Cys Val Tyr Asp Pro Arg Gly His Thr Gly
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Thr Thr Asn Gln Arg Ser Pro Pro Gly Glu Arg Thr Arg Gly Arg Gln
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161102_Sequence listing Nachanmeldung Macadamia_ST25

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Gly Arg Trp Gly Pro Ala Gly Pro Arg Glu Arg Glu Arg Glu Glu Asp
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130 135 140
Arg Lys Ile Arg Pro Glu Gly Arg Glu Gly Glu Gln Glu Trp Gly Thr
145 150 155 160
Pro Gly Ser His Val Arg Glu Glu Thr Ser Arg Asn Asn Pro Phe Tyr
165 170 175
Phe Pro Ser Arg Arg Phe Ser Thr Arg Tyr Gly Asn Gln Asn Gly Arg
180 185 190
Ile Arg Val Leu Gln Arg Phe Asp Gln Arg Ser Arg Gln Phe Gln Asn
195 200 205
Leu Gln Asn His Arg Ile Val Gln Ile Glu Ala Lys Pro Asn Thr Leu
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225 230 235 240
Gly Gln Ala Thr Val Thr Val Ala Asn Gly Asn Asn Arg Lys Ser Phe
245 250 255
Asn Leu Asp Glu Gly His Ala Leu Arg Ile Pro Ser Gly Phe Ile Ser
260 265 270
Tyr Ile Leu Asn Arg His Asp Asn Gln Asn Leu Arg Val Ala Lys Ile
275 280 285
Ser Met Pro Val Asn Thr Pro Gly Gln Phe Glu Asp Phe Phe Pro Ala
290 295 300
Ser Ser Arg Asp Gln Ser Ser Tyr Leu Gln Gly Phe Ser Arg Asn Thr
305 310 315 320
Leu Glu Ala Ala Phe Asn Ala Glu Phe Asn Glu Ile Arg Arg Val Leu
325 330 335
Leu Glu Glu Asn Ala Gly Gly Glu Gln Glu Glu Arg Gly Gln Arg Arg
340 345 350
Trp Ser Thr Arg Ser Ser Glu Asn Asn Glu Gly Val Ile Val Lys Val
355 360 365

161102_Sequence listing Nachanmeldung Macadamia_ST25

Ser Lys Glu His Val Glu Glu Leu Thr Lys His Ala Lys Ser Val Ser
370 375 380

Lys Lys Gly Ser Glu Glu Glu Gly Asp Ile Thr Asn Pro Ile Asn Leu
385 390 395 400

Arg Glu Gly Glu Pro Asp Leu Ser Asn Asn Phe Gly Lys Leu Phe Glu
405 410 415

Val Lys Pro Asp Lys Lys Asn Pro Gln Leu Gln Asp Leu Asp Met Met
420 425 430

Leu Thr Cys Val Glu Ile Lys Glu Gly Ala Leu Met Leu Pro His Phe
435 440 445

Asn Ser Lys Ala Met Val Ile Val Val Val Asn Lys Gly Thr Gly Asn
450 455 460

Leu Glu Leu Val Ala Val Arg Lys Glu Gln Gln Gln Arg Gly Arg Arg
465 470 475 480

Glu Glu Glu Glu Asp Glu Asp Glu Glu Glu Glu Gly Ser Asn Arg Glu
485 490 495

Val Arg Arg Tyr Thr Ala Arg Leu Lys Glu Gly Asp Val Phe Ile Met
500 505 510

Pro Ala Ala His Pro Val Ala Ile Asn Ala Ser Ser Glu Leu His Leu
515 520 525

Leu Gly Phe Gly Ile Asn Ala Glu Asn Asn His Arg Ile Phe Leu Ala
530 535 540

Gly Asp Lys Asp Asn Val Ile Asp Gln Ile Glu Lys Gln Ala Lys Asp
545 550 555 560

Leu Ala Phe Pro Gly Ser Gly Glu Gln Val Glu Lys Leu Ile Lys Asn
565 570 575

Gln Lys Glu Ser His Phe Val Ser Ala Arg Pro Gln Ser Gln Ser Gln
580 585 590

Ser Pro Ser Ser Pro Glu Lys Glu Ser Pro Glu Lys Glu Asp Gln Glu
595 600 605

Glu Glu Asn Gln Gly Gly Lys Gly Pro Leu Leu Ser Ile Leu Lys Ala
610 615 620

Phe Asn
625

161102_Sequence listing Nachanmeldung Macadamia_ST25

<210> 6
 <211> 519
 <212> PRT
 <213> pistachio

<400> 6

Lys Thr Asp Pro Glu Leu Lys Gln Cys Lys His Gln Cys Lys Val Gln
 1 5 10 15

Arg Gln Tyr Asp Glu Glu Gln Lys Glu Gln Cys Ala Lys Gly Cys Glu
 20 25 30

Lys Tyr Tyr Lys Glu Lys Lys Gly Arg Glu Gln Glu Glu Glu Glu
 35 40 45

Glu Glu Trp Gly Ser Gly Arg Gly Arg Gly Asp Glu Phe Ser Thr His
 50 55 60

Glu Pro Gly Glu Lys Arg Leu Ser Gln Cys Met Lys Gln Cys Glu Arg
 65 70 75 80

Gln Asp Gly Gly Gln Gln Lys Gln Leu Cys Arg Phe Arg Cys Gln Glu
 85 90 95

Lys Tyr Lys Lys Glu Arg Arg Glu His Ser Tyr Ser Arg Asp Glu Glu
 100 105 110

Glu Glu Glu Glu Gly Asp Glu Glu Gln Glu Glu Glu Asp Glu Asn Pro
 115 120 125

Tyr Val Phe Glu Asp Glu His Phe Thr Thr Arg Val Lys Thr Glu Gln
 130 135 140

Gly Lys Val Val Val Leu Pro Lys Phe Thr Lys Arg Ser Lys Leu Leu
 145 150 155 160

Arg Gly Leu Glu Lys Tyr Arg Leu Ala Phe Leu Val Ala Asn Pro Gln
 165 170 175

Ala Phe Val Val Pro Asn His Met Asp Ala Asp Ser Ile Phe Phe Val
 180 185 190

Ser Trp Gly Arg Gly Thr Ile Thr Lys Ile Arg Glu Asn Lys Arg Glu
 195 200 205

Ser Met Asn Val Lys Gln Gly Asp Ile Ile Arg Ile Arg Ala Gly Thr
 210 215 220

Pro Phe Tyr Ile Val Asn Thr Asp Glu Asn Glu Lys Leu Tyr Ile Val
 225 230 235 240

161102_Sequence listing Nachanmeldung Macadamia_ST25

Lys Leu Leu Gln Pro Val Asn Leu Pro Gly His Tyr Glu Val Phe His
245 250 255

Gly Pro Gly Gly Glu Asn Pro Glu Ser Phe Tyr Arg Ala Phe Ser Arg
260 265 270

Glu Val Leu Glu Ala Ala Leu Lys Thr Pro Arg Asp Lys Leu Glu Lys
275 280 285

Leu Phe Glu Lys Gln Asp Glu Gly Ala Ile Val Lys Ala Ser Lys Glu
290 295 300

Gln Ile Arg Ala Met Ser Arg Arg Gly Glu Gly Pro Ser Ile Trp Pro
305 310 315 320

Phe Thr Gly Lys Ser Thr Gly Thr Phe Asn Leu Phe Lys Lys Asp Pro
325 330 335

Ser Gln Ser Asn Asn Tyr Gly Gln Leu Phe Glu Ser Glu Phe Lys Asp
340 345 350

Tyr Pro Pro Leu Gln Glu Leu Asp Ile Met Val Ser Tyr Val Asn Ile
355 360 365

Thr Lys Gly Gly Met Ser Gly Pro Phe Tyr Asn Ser Arg Ala Thr Lys
370 375 380

Ile Ala Ile Val Val Ser Gly Glu Gly Arg Leu Glu Ile Ala Cys Pro
385 390 395 400

His Leu Ser Ser Ser Lys Asn Ser Gly Gln Glu Lys Ser Gly Pro Ser
405 410 415

Tyr Lys Lys Leu Ser Ser Ser Ile Arg Thr Asp Ser Val Phe Val Val
420 425 430

Pro Ala Gly His Pro Phe Val Thr Val Ala Ser Gly Asn Gln Asn Leu
435 440 445

Glu Ile Leu Cys Phe Glu Val Asn Ala Glu Gly Asn Ile Arg Tyr Thr
450 455 460

Leu Ala Gly Lys Lys Asn Ile Ile Glu Val Met Glu Lys Glu Ala Lys
465 470 475 480

Glu Leu Ala Phe Lys Thr Lys Gly Glu Glu Val Asp Lys Val Phe Gly
485 490 495

Lys Gln Asp Glu Glu Phe Phe Phe Gln Gly Pro Lys Trp Arg Gln His
500 505 510

161102_Sequence listing Nachanmeldung Macadamia_ST25
 Gln Gln Gly Arg Ala Asp Glu
 515

<210> 7
 <211> 512
 <212> PRT
 <213> cashew

<400> 7

Lys Ile Asp Pro Glu Leu Lys Gln Cys Lys His Gln Cys Lys Val Gln
 1 5 10 15

Arg Gln Tyr Asp Glu Gln Gln Lys Glu Gln Cys Val Lys Glu Cys Glu
 20 25 30

Lys Tyr Tyr Lys Glu Lys Lys Gly Arg Glu Arg Glu His Glu Glu Glu
 35 40 45

Glu Glu Glu Trp Gly Thr Gly Gly Val Asp Glu Pro Ser Thr His Glu
 50 55 60

Pro Ala Glu Lys His Leu Ser Gln Cys Met Arg Gln Cys Glu Arg Gln
 65 70 75 80

Glu Gly Gly Gln Gln Lys Gln Leu Cys Arg Phe Arg Cys Gln Glu Arg
 85 90 95

Tyr Lys Lys Glu Arg Gly Gln His Asn Tyr Lys Arg Glu Asp Asp Glu
 100 105 110

Asp Glu Asp Glu Asp Glu Ala Glu Glu Glu Asp Glu Asn Pro Tyr Val
 115 120 125

Phe Glu Asp Glu Asp Phe Thr Thr Lys Val Lys Thr Glu Gln Gly Lys
 130 135 140

Val Val Leu Leu Pro Lys Phe Thr Gln Lys Ser Lys Leu Leu His Ala
 145 150 155 160

Leu Glu Lys Tyr Arg Leu Ala Val Leu Val Ala Asn Pro Gln Ala Phe
 165 170 175

Val Val Pro Ser His Met Asp Ala Asp Ser Ile Phe Phe Val Ser Trp
 180 185 190

Gly Arg Gly Thr Ile Thr Lys Ile Leu Glu Asn Lys Arg Glu Ser Ile
 195 200 205

Asn Val Arg Gln Gly Asp Ile Val Ser Ile Ser Ser Gly Thr Pro Phe
 210 215 220

Tyr Ile Ala Asn Asn Asp Glu Asn Glu Lys Leu Tyr Leu Val Gln Phe

161102_Sequence listing Nachanmeldung Macadamia_ST25

225 230 235 240
 Leu Arg Pro Val Asn Leu Pro Gly His Phe Glu Val Phe His Gly Pro
 245 250 255
 Gly Gly Glu Asn Pro Glu Ser Phe Tyr Arg Ala Phe Ser Trp Glu Ile
 260 265 270
 Leu Glu Ala Ala Leu Lys Thr Ser Lys Asp Thr Leu Glu Lys Leu Phe
 275 280 285
 Glu Lys Gln Asp Gln Gly Thr Ile Met Lys Ala Ser Lys Glu Gln Ile
 290 295 300
 Arg Ala Met Ser Arg Arg Gly Glu Gly Pro Lys Ile Trp Pro Phe Thr
 305 310 315
 Glu Glu Ser Thr Gly Ser Phe Lys Leu Phe Lys Lys Asp Pro Ser Gln
 325 330 335
 Ser Asn Lys Tyr Gly Gln Leu Phe Glu Ala Glu Arg Ile Asp Tyr Pro
 340 345 350
 Pro Leu Glu Lys Leu Asp Met Val Val Ser Tyr Ala Asn Ile Thr Lys
 355 360 365
 Gly Gly Met Ser Val Pro Phe Tyr Asn Ser Arg Ala Thr Lys Ile Ala
 370 375 380
 Ile Val Val Ser Gly Glu Gly Cys Val Glu Ile Ala Cys Pro His Leu
 385 390 395
 Ser Ser Ser Lys Ser Ser His Pro Ser Tyr Lys Lys Leu Arg Ala Arg
 405 410 415
 Ile Arg Lys Asp Thr Val Phe Ile Val Pro Ala Gly His Pro Phe Ala
 420 425 430
 Thr Val Ala Ser Gly Asn Glu Asn Leu Glu Ile Val Cys Phe Glu Val
 435 440 445
 Asn Ala Glu Gly Asn Ile Arg Tyr Thr Leu Ala Gly Lys Lys Asn Ile
 450 455 460
 Ile Lys Val Met Glu Lys Glu Ala Lys Glu Leu Ala Phe Lys Met Glu
 465 470 475
 Gly Glu Glu Val Asp Lys Val Phe Gly Lys Gln Asp Glu Glu Phe Phe
 485 490 495
 Phe Gln Gly Pro Glu Trp Arg Lys Glu Lys Glu Gly Arg Ala Asp Glu

<210> 8
<211> 766
<212> PRT
<213> pecan

<400> 8

Leu Glu Thr Glu Asp Leu Ser Asn Glu Leu Asn Pro His His Asp Pro
1 5 10 15

Glu Ser His Arg Trp Glu Phe Gln Gln Cys Gln Glu Arg Cys Gln His
20 25 30

Glu Glu Arg Gly Gln Arg Gln Ala Gln Gln Cys Gln Arg Arg Cys Glu
35 40 45

Glu Gln Leu Arg Glu Arg Glu Arg Glu Arg Glu Arg Glu Glu Ile Val
50 55 60

Asp Pro Arg Glu Pro Arg Lys Gln Tyr Glu Gln Cys Arg Glu Thr Cys
65 70 75 80

Glu Lys Gln Asp Pro Arg Gln Gln Pro Gln Cys Glu Arg Arg Cys Glu
85 90 95

Arg Gln Phe Gln Glu Gln Gln Glu Arg Glu Arg Arg Glu Arg Arg Arg
100 105 110

Gly Arg Asp Asp Asp Asp Lys Glu Asn Pro Arg Asp Pro Arg Glu Gln
115 120 125

Tyr Arg Gln Cys Glu Glu His Cys Arg Arg Gln Gly Gln Gly Gln Arg
130 135 140

Gln Gln Gln Gln Cys Gln Ser Arg Cys Glu Glu Arg Phe Glu Glu Glu
145 150 155 160

Gln Arg Arg Gln Glu Glu Arg Glu Arg Arg Arg Gly Arg Asp Asn Asp
165 170 175

Asp Glu Glu Asn Pro Arg Asp Pro Arg Glu Gln Tyr Arg Gln Cys Gln
180 185 190

Glu His Cys Arg Arg Gln Gly Gln Gly Gln Arg Gln Gln Gln Cys
195 200 205

Gln Ser Arg Cys Glu Glu Arg Leu Glu Glu Glu Gln Arg Lys Gln Glu
210 215 220

Glu Arg Glu Arg Arg Arg Gly Arg Asp Glu Asp Asp Gln Asn Pro Arg
225 230 235 240

161102_Sequence listing Nachanmeldung Macadamia_ST25

Asp Pro Glu Gln Arg Tyr Glu Gln Cys Gln Gln Gln Cys Glu Arg Gln
 245 250 255
 Arg Arg Gly Gln Glu Gln Gln Leu Cys Arg Arg Arg Cys Glu Gln Gln
 260 265 270
 Arg Gln Gln Glu Glu Arg Glu Arg Gln Arg Gly Arg Asp Arg Gln Asp
 275 280 285
 Pro Gln Gln Gln Tyr His Arg Cys Gln Arg Arg Cys Gln Thr Gln Glu
 290 295 300
 Gln Ser Pro Glu Arg Gln Arg Gln Cys Gln Gln Arg Cys Glu Arg Gln
 305 310 315 320
 Tyr Lys Glu Gln Gln Gly Arg Glu Trp Gly Pro Asp Gln Ala Ser Pro
 325 330 335
 Arg Arg Glu Ser Arg Gly Arg Glu Glu Glu Gln Gln Arg His Asn Pro
 340 345 350
 Tyr Tyr Phe His Ser Gln Gly Leu Arg Ser Arg His Glu Ser Gly Glu
 355 360 365
 Gly Glu Val Lys Tyr Leu Glu Arg Phe Thr Glu Arg Thr Glu Leu Leu
 370 375 380
 Arg Gly Ile Glu Asn Tyr Arg Val Val Ile Leu Glu Ala Asn Pro Asn
 385 390 395 400
 Thr Phe Val Leu Pro Tyr His Lys Asp Ala Glu Ser Val Ile Val Val
 405 410 415
 Thr Arg Gly Arg Ala Thr Leu Thr Phe Val Ser Gln Glu Arg Arg Glu
 420 425 430
 Ser Phe Asn Leu Glu Tyr Gly Asp Val Ile Arg Val Pro Ala Gly Ala
 435 440 445
 Thr Glu Tyr Val Ile Asn Gln Asp Ser Asn Glu Arg Leu Glu Met Val
 450 455 460
 Lys Leu Leu Gln Pro Val Asn Asn Pro Gly Gln Phe Arg Glu Tyr Tyr
 465 470 475 480
 Ala Ala Gly Ala Gln Ser Thr Glu Ser Tyr Leu Arg Val Phe Ser Asn
 485 490 495
 Asp Ile Leu Val Ala Ala Leu Asn Thr Pro Arg Asp Arg Leu Glu Arg
 500 505 510

161102_Sequence listing Nachanmeldung Macadamia_ST25

Phe Phe Asp Gln Gln Glu Gln Arg Glu Gly Val Ile Ile Arg Ala Ser
 515 520 525
 Gln Glu Lys Leu Arg Ala Leu Ser Gln His Ala Met Ser Ala Gly Gln
 530 535 540
 Arg Pro Trp Gly Arg Arg Ser Ser Gly Gly Pro Ile Ser Leu Lys Ser
 545 550 555
 Gln Arg Ser Ser Tyr Ser Asn Gln Phe Gly Gln Phe Phe Glu Ala Cys
 565 570 575
 Pro Glu Glu His Arg Gln Leu Gln Glu Met Asp Val Leu Val Asn Tyr
 580 585 590
 Ala Glu Ile Lys Arg Gly Ala Met Met Val Pro His Tyr Asn Ser Lys
 595 600 605
 Ala Thr Val Val Val Tyr Val Val Glu Gly Thr Gly Arg Phe Glu Met
 610 615 620
 Ala Cys Pro His Asp Val Ser Ser Gln Ser Tyr Glu Tyr Lys Gly Arg
 625 630 635 640
 Arg Glu Gln Glu Glu Glu Glu Ser Ser Thr Gly Gln Phe Gln Lys Val
 645 650 655
 Thr Ala Arg Leu Ala Arg Gly Asp Ile Phe Val Ile Pro Ala Gly His
 660 665 670
 Pro Ile Ala Ile Thr Ala Ser Gln Asn Glu Asn Leu Arg Leu Val Gly
 675 680 685
 Phe Gly Ile Asn Gly Lys Asn Asn Gln Arg Asn Phe Leu Ala Gly Gln
 690 695 700
 Asn Asn Ile Ile Asn Gln Leu Glu Arg Glu Ala Lys Glu Leu Ser Phe
 705 710 715 720
 Asn Met Pro Arg Glu Glu Ile Glu Glu Ile Phe Glu Arg Gln Val Glu
 725 730 735
 Ser Tyr Phe Val Pro Met Glu Arg Gln Ser Arg Arg Gly Gln Gly Arg
 740 745 750
 Asp His Pro Leu Ala Ser Ile Leu Asp Phe Ala Gly Phe Phe
 755 760 765

<210> 9
 <211> 593

161102_Sequence listing Nachanmeldung Macadamia_ST25

<212> PRT
<213> walnut

<400> 9

Arg Gly Arg Asp Asp Asp Asp Glu Glu Asn Pro Arg Asp Pro Arg Glu
1 5 10 15

Gln Tyr Arg Gln Cys Gln Glu Tyr Cys Arg Arg Gln Gly Gln Gly Gln
20 25 30

Arg Gln Gln Gln Gln Cys Gln Ile Arg Cys Glu Glu Arg Leu Glu Glu
35 40 45

Asp Gln Arg Ser Gln Glu Glu Arg Glu Arg Arg Arg Gly Arg Asp Val
50 55 60

Asp Asp Gln Asn Pro Arg Asp Pro Glu Gln Arg Tyr Glu Gln Cys Gln
65 70 75 80

Gln Gln Cys Glu Arg Gln Arg Arg Gly Gln Glu Gln Thr Leu Cys Arg
85 90 95

Arg Arg Cys Glu Gln Arg Arg Gln Gln Glu Glu Arg Glu Arg Gln Arg
100 105 110

Gly Arg Asp Arg Gln Asp Pro Gln Gln Gln Tyr His Arg Cys Gln Arg
115 120 125

Arg Cys Gln Ile Gln Glu Gln Ser Pro Glu Arg Gln Arg Gln Cys Gln
130 135 140

Gln Arg Cys Glu Arg Gln Tyr Lys Glu Gln Gln Gly Arg Glu Arg Gly
145 150 155 160

Pro Glu Ala Ser Pro Arg Arg Glu Ser Arg Gly Arg Glu Glu Glu Gln
165 170 175

Gln Arg His Asn Pro Tyr Tyr Phe His Ser Gln Ser Ile Arg Ser Arg
180 185 190

His Glu Ser Glu Glu Gly Glu Val Lys Tyr Leu Glu Arg Phe Thr Glu
195 200 205

Arg Thr Glu Leu Leu Arg Gly Ile Glu Asn Tyr Arg Val Val Ile Leu
210 215 220

Asp Ala Asn Pro Asn Thr Ser Met Leu Pro His His Lys Asp Ala Glu
225 230 235 240

Ser Val Ala Val Val Thr Arg Gly Arg Ala Thr Leu Thr Leu Val Ser
245 250 255

161102_Sequence listing Nachanmeldung Macadamia_ST25

Gln Glu Thr Arg Glu Ser Phe Asn Leu Glu Cys Gly Asp Val Ile Arg
260 265 270

Val Pro Ala Gly Ala Thr Val Tyr Val Ile Asn Gln Asp Ser Asn Glu
275 280 285

Arg Leu Glu Met Val Lys Leu Leu Gln Pro Val Asn Asn Pro Gly Gln
290 295 300

Phe Arg Glu Tyr Tyr Ala Ala Gly Ala Lys Ser Pro Asp Gln Ser Tyr
305 310 315 320

Leu Arg Val Phe Ser Asn Asp Ile Leu Val Ala Ala Leu Asn Thr Pro
325 330 335

Arg Asp Arg Leu Glu Arg Phe Phe Asp Gln Gln Glu Gln Arg Glu Gly
340 345 350

Val Ile Ile Arg Ala Ser Gln Glu Lys Leu Arg Ala Leu Ser Gln His
355 360 365

Ala Met Ser Ala Gly Gln Arg Pro Trp Gly Arg Arg Ser Ser Gly Gly
370 375 380

Pro Ile Ser Leu Lys Ser Glu Ser Pro Ser Tyr Ser Asn Gln Phe Gly
385 390 395 400

Gln Phe Phe Glu Ala Cys Pro Glu Glu His Arg Gln Leu Gln Glu Met
405 410 415

Asp Val Leu Val Asn Tyr Ala Glu Ile Lys Arg Gly Ala Met Met Val
420 425 430

Pro His Tyr Asn Ser Lys Ala Thr Val Val Val Tyr Val Val Glu Gly
435 440 445

Thr Gly Arg Tyr Glu Met Ala Cys Pro His Val Ser Ser Gln Ser Tyr
450 455 460

Glu Gly Gln Gly Arg Arg Glu Gln Glu Glu Glu Ser Thr Gly Arg
465 470 475 480

Phe Gln Lys Val Thr Ala Arg Leu Ala Arg Gly Asp Ile Phe Val Ile
485 490 495

Pro Ala Gly His Pro Ile Ala Ile Thr Ala Ser Gln Asn Glu Asn Leu
500 505 510

Arg Leu Leu Gly Phe Asp Ile Asn Gly Glu Asn Asn Gln Arg Asp Phe
515 520 525

161102_Sequence listing Nachanmeldung Macadamia_ST25

Leu Ala Gly Gln Asn Asn Ile Ile Asn Gln Leu Glu Arg Glu Ala Lys
530 535 540

Glu Leu Ser Phe Asn Met Pro Arg Glu Glu Ile Glu Glu Ile Phe Glu
545 550 555 560

Ser Gln Met Glu Ser Tyr Phe Val Pro Thr Glu Arg Gln Ser Arg Arg
565 570 575

Gly Gln Gly Arg Asp His Pro Leu Ala Ser Ile Leu Asp Phe Ala Phe
580 585 590

Phe

<210> 10
<211> 625
<212> PRT
<213> Macadamia

<400> 10

Gln Cys Met Gln Leu Glu Thr Ser Gly Gln Met Arg Arg Cys Val Ser
1 5 10 15

Gln Cys Asp Lys Arg Phe Glu Glu Asp Ile Asp Trp Ser Lys Tyr Asp
20 25 30

Asn Gln Glu Asp Pro Gln Thr Glu Cys Gln Gln Cys Gln Arg Arg Cys
35 40 45

Arg Gln Gln Glu Ser Asp Pro Arg Gln Gln Gln Tyr Cys Gln Arg Arg
50 55 60

Cys Lys Glu Ile Cys Glu Glu Glu Glu Glu Tyr Asn Arg Gln Arg Asp
65 70 75 80

Pro Gln Gln Gln Tyr Glu Gln Cys Gln Lys Arg Cys Gln Arg Arg Glu
85 90 95

Thr Glu Pro Arg His Met Gln Ile Cys Gln Gln Arg Cys Glu Arg Arg
100 105 110

Tyr Glu Lys Glu Lys Arg Lys Gln Gln Lys Arg Tyr Glu Glu Gln Gln
115 120 125

Arg Glu Asp Glu Glu Lys Tyr Glu Glu Arg Met Lys Glu Gly Asp Asn
130 135 140

Lys Arg Asp Pro Gln Gln Arg Glu Tyr Glu Asp Cys Arg Arg His Cys
145 150 155 160

161102_Sequence listing Nachanmeldung Macadamia_ST25

Glu Gln Gln Glu Pro Arg Leu Gln Tyr Gln Cys Gln Arg Arg Cys Gln
 165 170 175
 Glu Gln Gln Arg Gln His Gly Arg Gly Gly Asp Leu Met Asn Pro Gln
 180 185 190
 Arg Gly Gly Ser Gly Arg Tyr Glu Glu Gly Glu Glu Lys Gln Ser Asp
 195 200 205
 Asn Pro Tyr Tyr Phe Asp Glu Arg Ser Leu Ser Thr Arg Phe Arg Thr
 210 215 220
 Glu Glu Gly His Ile Ser Val Leu Glu Asn Phe Tyr Gly Arg Ser Lys
 225 230 235 240
 Leu Leu Arg Ala Leu Lys Asn Tyr Arg Leu Val Leu Leu Glu Ala Asn
 245 250 255
 Pro Asn Ala Phe Val Leu Pro Thr His Leu Asp Ala Asp Ala Ile Leu
 260 265 270
 Leu Val Ile Gly Gly Arg Gly Ala Leu Lys Met Ile His Arg Asp Asn
 275 280 285
 Arg Glu Ser Tyr Asn Leu Glu Cys Gly Asp Val Ile Arg Ile Pro Ala
 290 295 300
 Gly Thr Thr Phe Tyr Leu Ile Asn Arg Asp Asn Asn Glu Arg Leu His
 305 310 315 320
 Ile Ala Lys Phe Leu Gln Thr Ile Ser Thr Pro Gly Gln Tyr Lys Glu
 325 330 335
 Phe Phe Pro Ala Gly Gly Gln Asn Pro Glu Pro Tyr Leu Ser Thr Phe
 340 345 350
 Ser Lys Glu Ile Leu Glu Ala Ala Leu Asn Thr Gln Thr Glu Arg Leu
 355 360 365
 Arg Gly Val Leu Gly Gln Gln Arg Glu Gly Val Ile Ile Arg Ala Ser
 370 375 380
 Gln Glu Gln Ile Arg Glu Leu Thr Arg Asp Asp Ser Glu Ser Arg Arg
 385 390 395 400
 Trp His Ile Arg Arg Gly Gly Glu Ser Ser Arg Gly Pro Tyr Asn Leu
 405 410 415
 Phe Asn Lys Arg Pro Leu Tyr Ser Asn Lys Tyr Gly Gln Ala Tyr Glu
 420 425 430

161102_Sequence listing Nachanmeldung Macadamia_ST25

Val Lys Pro Glu Asp Tyr Arg Gln Leu Gln Asp Met Asp Val Ser Val
435 440 445

Phe Ile Ala Asn Ile Thr Gln Gly Ser Met Met Gly Pro Phe Phe Asn
450 455 460

Thr Arg Ser Thr Lys Val Val Val Val Ala Ser Gly Glu Ala Asp Val
465 470 475 480

Glu Met Ala Cys Pro His Leu Ser Gly Arg His Gly Gly Arg Gly Gly
485 490 495

Gly Lys Arg His Glu Glu Glu Glu Glu Val His Tyr Glu Gln Val Arg
500 505 510

Ala Arg Leu Ser Lys Arg Glu Ala Ile Val Val Leu Ala Gly His Pro
515 520 525

Val Val Phe Val Ser Ser Gly Asn Glu Asn Leu Leu Leu Phe Ala Phe
530 535 540

Gly Ile Asn Ala Gln Asn Asn His Glu Asn Phe Leu Ala Gly Arg Glu
545 550 555 560

Arg Asn Val Leu Gln Gln Ile Glu Pro Gln Ala Met Glu Leu Ala Phe
565 570 575

Ala Ala Ser Arg Lys Glu Val Glu Glu Leu Phe Asn Ser Gln Asp Glu
580 585 590

Ser Ile Phe Phe Pro Gly Pro Arg Gln His Gln Gln Gln Ser Pro Arg
595 600 605

Ser Thr Lys Gln Gln Gln Pro Leu Val Ser Ile Leu Asp Phe Val Gly
610 615 620

Phe
625