Chapter 1

Pollen Allergenicity is Highly Dependent on the Plant Genetic Background: The "Variety"/"Cultivar" Issues

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1. Introduction

Type I hypersensibility to pollen is an important cause of allergy worldwide. In other types of allergy like the food allergic symptoms or very frequently the oral allergy syndrome (OAS), clear differences between varieties/cultivars of the same or highly-related plant species have been described as regard to the expression of allergens and their allergenic importance.

Pioneer studies were carried out in date palm tree over the later years of the last century (Kwaasi et al 1999, 2000). Such studies indicated that allergenicity to date fruit was a cultivar-specific phenomenon, and laboratory data showed that individual cultivars varied in their number of IgE immunoblot bands. Sera from fruit-allergic as well as pollen-allergic patients recognized common fruit-specific epitopes. Also, there was heterogeneity in patient responses to the different extracts. Nevertheless, a number of common allergens were responsible for cross-reactivity between the cultivars.

Up to date, similar studies have been carried out in an important number of plants, mainly those producing edible fruits like apple (Asero et al 2006; Rur 2007; Matthes and Schmitz-Eiberger 2009; Vlieg-Boerstra et al 2011), peach (Brenna et al 2004; Ahrazem et al 2007; Chen et al 2008), cherry (Verschuren, http://www.appliedscience.nl/doc/Onderzoek_111117_Martie_Verschuren.pdf), nectarine (Ahrazem et al 2007), tomato (Dölle et al 2011), strawberry (Muñoz et al 2010), and lichy (Hoppe et al 2006) among others, and in seeds like cereals (Nakamura et al 2005), buckwheat (Maruyama-Funatsuki et al 2004) and peanuts (Kang et al 2007; Kottapallia et al 2008).

Numerous analysis have raised the question that pollen grains, similarly to fruits may notably differ among different varieties/cultivars in terms of pollen micromorphology, as well as in their physiological characteristics (e.g. viability, vigour, ability to germinate, compatibility…) (Castro et al 2010; Ribeiro et al 2012), and eventually in their allergenic content. However, literature devoted to the comparison of the pollen allergenic characteristics intra- and inter- varieties is still relatively scarce. This article reviews most of these investigations.

2. Taxonomy of allergenic plants

Excellent reviews have been made as regard to the taxonomical classification of the allergenic plants (Yman1982; Takhtajan 1997; D'Amato et al 1998; Mothes et al 2004; Mohapatra et al 2004; Esch 2004; Radauer et al 2006). Moreover, several broad databases have compiled profuse and well-documented information linking the most relevant plant allergenic sources, the identified allergens and their taxonomical classification. They include Pharmacia (Pharmacia Diagnostics, 2001) and later Phadia/Thermo Fisher Scientific (http://www.phadia.com/en/Allergen-information/ImmunoCAP-Allergens/Allergen-components-list/), the Allergome database of allergenic molecules (Mari et al 2009; http://www.allergome.org/index.php) and the official site for the systematic allergen nomenclature approved by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee (http://www.allergen.org/index.php). Independently of the widespread presence of cross-reactivity, most allergens are described in these works and databases as characterized in a single species (e.g. rBet v 2 Profilin, Birch= *Betula verrucosa*). Only a minority are referenced to taxonomical entities different to species, either to a combination of related species, cultigens or hybrids (e.g. *Musa acuminata* / *sapientum* / *paradisiaca*) or to a heterogeneous group of more than one (often numerous) species (e.g. *Eucalyptus* spp. Note that these abbreviations are not italicized or underlined, and can easily be confused with the abbreviations "ssp." or "subsp." referring to subspecies.). In several cases, allergens are referred to taxonomic ranks of higher entity than species (e.g. *Theaceae*). Only a few allergens are univocally attributed to infraspecific plant categories like varieties (e.g. *Brassica oleraceae var. italica*, *var. gemmifera*, *var. capitata*, *var. botrytis*).

As regards to pollen allergen analysis, two alternatives, apparently opposite, although somehow complementary strategies are defined:

Mothes et al (2004) analyzed cross-reactivities to pollens of trees of the Fagales order, fruits and vegetables, between pollens of the Scrophulariales and pollens of the Coniferales. They proposed a classification of tree pollen and related allergies based on major allergen molecules instead of botanical relationships among the allergenic sources, suggesting Bet v 1 as a marker for Fagales pollen and related plant food allergies, Ole e 1 as a possible marker for Scrophulariales pollen allergy and Cry j 1 and Cry j 2 as potential markers for allergy to Coniferales pollens. Another work analyzed pollen allergen sequences with respect to protein family membership, taxonomic distribution of protein families, and interspecies variability

(Radauer and Breteneder 2006). These authors managed to classify all pollen allergens known to date into a limited number of protein families, and divide them into ubiquitous (e.g. profilins), present in certain families (e.g. pectate lyases), or limited to a single taxon (e.g. thaumatin-like proteins). This approach provides invaluable help in issues like the prediction of cross-reactivity, the design of diagnostic methods and the assessment of the allergenic potential of novel molecules. A similar approach is described by Moreno-Aguilar (2008).

On the other hand, different authors are contributing to define the specific allergenic composition of pollens, going deeper into the taxonomical classification usually observed (this is, characterizing the allergenic composition of pollens at infraspecific level), and abounding into the analysis of pollen allergenic polymorphism. Advantages of such strategy have been outlined before (Alché et al 2007). Diverse examples of this strategy are depicted next.

3. Infraspecific botanical names

In botany, an infraspecific name is that corresponding to any taxon below the rank of species. Such names are constructed based in the use of trinomial nomenclature, regulated by the International Code of Botanical Nomenclature (ICBN) (McNeill et al 2006), which includes: genus name, specific epithet, connecting term indicating the rank (not part of the name, but required), and finally the infraspecific epithet. It is habitual to italicize all three parts of the name, but not the connecting term. Five different taxonomical ranks below the species are explicitly allowed in the ICBN:

a. subspecies - recommended abbreviation: subsp. ("ssp." also widely used)
b. varietas (variety) - recommended abbreviation: var.
c. subvarietas (subvariety) - recommended abbreviation: subvar.
d. forma (form) - recommended abbreviation: f.
e. subforma (subform) - recommended abbreviation: subf.

A **subspecies** is a taxonomical rank formed by individuals of the same species which are capable of interbreeding and producing fertile offspring. However, they often do not interbreed in nature due to geographic isolation or other factors (http://en.wikipedia.org/wiki/Subspecies). The differences between subspecies are usually less distinct than the differences between species, but more distinct than the differences between varieties.

A **botanical variety** is a taxonomic rank below that of species, characterized by differential appearance from other varieties. However, varieties retain the ability to hybridize freely among themselves, providing they become in contact. Usually, varieties are geographically separated. Varieties are named by using the binomial Latin name followed by the term "variety" (usually abbreviated as "var.") and the name of the variety in italics.

Subvarieties, forms and subforms constitute taxonomic ranks of "secondary" importance and are more rarely used. For example, a form usually designates a group with a noticeable but minor deviation. Some botanists believe that there is no need to name forms, since there are theoretically countless numbers of forms based on minor genetic differences (http://en.wikipedia.org/wiki/Form_(botany)).

The term **cultivar** is defined as a plant or group of plants selected for desirable characteristics that can be maintained by propagation (http://en.wikipedia.org/wiki/Cultivar). Most cultivars have been obtained after using agronomical methods, or in some cases, selected from wild populations. Crops and even trees used in forestry are usually cultivars that have been selected for desirable characteristics including improved production, resistance to pests, flavor, timber production etc. Naming of cultivars is recommended by the International Code of Nomenclature for Cultivated Plants (ICNCP) (Brickell et al, 2009), and is formed of the scientific botanical name (Latin) followed by the term "cultivar" (usually abbreviated as "cv.") and a cultivar epithet bounded by single quotation marks, for example: *Olea europaea* cv. 'Picual'.

The terms "cultivar" and "variety" are not equivalent. Although different, both terms are often used as synonyms: thus, "grape varieties" are habitually used in viticulture nomenclature to indicate what should be in reality cultivars, according to the International Code of Nomenclature for Cultivated Plants, since grapes are mostly propagated by cuttings. The same applies to "olive varieties", which should be properly named "olive cultivars". In both, and in many other cases, cuttings are the most frequently selected propagation method, as agronomical, physiological and anatomical properties are not maintained in a stable-manner under sexual reproduction. However, usage of the term variety is well fixed in both viticulture and oliviculture, therefore, a change to the correct term (cultivar) is unlikely to occur.

Finally, the term **cultigen** represents to a plant that has been deliberately altered or selected by humans. It is therefore the result of artificial (anthropogenic) selection. Their naming and origin can be very varied, as it is subjected to different rules and criteria (http://en.wikipedia.org/wiki/Cultigen).

4. Pollens with described differential allergenicity within infraspecific taxonomical ranks

Up to date, the presence of differential allergenicity within infraspecific taxonomical ranks has been demonstrated in the pollen of a significant number of plant species at the allergenic context. Next, we describe pollen allergens in these plants, as well as the most representative literature describing such differences.

4.1. Date palm (*Phoenix dactilifera* L.)

The most relevant allergenic questions regarding this plant are compiled in the following web pages: http://www.phadia.com/en/Allergen-information/ImmunoCAP-Allergens/Food-of-Plant-Origin/Fruits/Date/ (Phadia), and http://www.allergome.org/script/dettaglio.php?id_molecule=1925 (Allergome).

Briefly, *Ph. dactilifera* pollen contains allergens of 14.3 kDa, 27-33 kDa, 54-58 kDa and 90 kDa (Postigo et al 2009). The presence of cross-reactivity among the different individual species of tree pollen of members of the genus could be expected (Yman 1982), and RAST inhibition

studies have demonstrated significant cross-reactivity between *P. canariensis* and *P. dactylifera* pollen (Blanco et al 1995).

Kwaasi et al (1994) compared pollen crude extracts from ten cultivars of this tree for their antigenic and allergenic potentials. The results of the tests performed on 6 confirmed atopic patients, including skin prick tests, ELISA, IgG and IgE immunoblotting analyses, peripheral blood lymphocyte proliferation and concomitant interleukin-4 (IL-4) production indicated sharp inter-cultivar heterogeneity. One of the cultivars even failed to elicit any skin test reactivity or bind IgE in atopic sera as determined by the indicated assays. The authors therefore suggest that the antigenicity and allergenicity of date palm pollen is more of a cultivar-specific phenomenon than a species-specific phenomenon, which is governed by the number, quantities or both of the major allergen epitopes possessed by that variety or cultivar. Nevertheless, a number of common allergens are responsible for cross-reactivity between the cultivars.

It has been later demonstrated that antigens and allergens of date fruits cross-react with date pollen allergens and date fruit-sensitive as well as date pollen-allergic patients' sera recognize the same group of date fruit IgE-binding components (Kwaasi et al 1999). Therefore, the cultivar issue is also tremendously important in selecting date cultivars for allergen standardization (Kwaasi et al 2000).

4.2. Arizona cypress (*Cupressus arizonica* L.)

The most relevant allergenic questions regarding this plant are brought together in the following web pages: http://intapp3.phadia.com/en/Allergen-information/ImmunoCAP-Allergens/Tree-Pollens/Allergens/Arizona-cypress-/ (Phadia), and http://www.allergome.org/ script/dettaglio.php?id_molecule=1793 (Allergome).

In brief: *Cupressaceae* pollen is characterized by a low protein concentration and high carbohydrate content. Allergens from the Arizona cypress tree have been isolated, characterized, and their diagnostic significance established (Penon 2000). They include Cup a 1, a 43-kDa protein, characterized as a pectate lyase (Di Felice et al1994; 2001; Afferni et al 1999; Aceituno et al 2000; Alisi et al 2001; Mistrello et al 2002; Iacovacci et al 2002; Arilla et al 2004), rCup a 1 (Aceituno et al 2000; Iacovacci et al 2002), Cup a 2, a polygalacturonase (Di Felice et al 2001; de Coana et al 2006), Cup a 3, a thaumatin-like protein (Cortegano et al 2004; Togawa et al 2006; Suarez-Cervera et al 2008) and Cup a 4, a calcium-binding protein (de Coana 2010). *C. arizonica* and *C. sempervirens* extracts are highly cross-reactive at the IgE level and have a number of common epitopes. Two major IgE-reactive components of approximately 43 kDa and 36 kDa have been shown to be present in both (Barletta et al 1996). *C. sempervirens* shows a wider diversity of allergens, whereas *C. arizonica* shows a higher content of the major 43 kDa allergen (Leduc et al 2000). Extensive cross-reactivity also occurs with other family members, which include *Juniperus oxycedrus*, *Chamaecyparis obtusa* and *Thuja plicata*.

In general, species of the *Cupressaceae* family are a very important cause of allergies in various geographical areas, especially North America, Japan, and Mediterranean countries.

Incidence is growing spectacularly as a consequence of these species been widely used for reforestation, for wind and noise barriers, and ornamentally in gardens and parks, as well as for reforestation (Bousquet et al 1993; Caiaffa et al 1993).

Shahali et al (2007) performed a comparative study of the pollen protein contents in two major varieties of *Cupressus arizonica* (*C. arizonica* var. *arizonica* and *C. arizonica* var. *glabra*) planted in Tehran. Their investigations revealed noticeable differences in protein content of each variety, with a new major protein of c.a. 35 kDa present in the extracts, with high reactivity to the sera from allergic patients. Such band showed even more relevance than the major allergen Cup a 1 (45 kDa), reported as the most representative protein in pollen extracts of Mediterranean countries. Due to the fact that many different Arizona cypress tree varieties exist (recognized on the basis of distribution and of foliage, cone and bark characteristics and furthermore by using RAPDs markers) (Bartel et al 2003), the presence of huge differences in reactivity is expected.

4.3. Birch (*Betula verrucosa*, Synonym*: B. pendula*)

Relevant allergenic information concerning this plant (one of the best characterized allergenic sources up to date) is listed in the next web pages: http://intapp3.phadia.com/en/Allergen-information/ImmunoCAP-Allergens/Tree-Pollens/Allergens/Common-silver-birch-/http://intapp3.phadia.com/en/Allergen-informtion /ImmunoCAP-Allergens/Tree-Pollens/Allergens/Arizona-cypress-/ (Phadia), and http://ww w. allergome.org/script/dettaglio.php?id_molecule=1741 (Allergome).

In short: Birch pollen contains at least 29 antigens (Wiebicke et al 1987). Allergens of molecular weights of 29.5, 17, 12.5, and 13 kDa have been isolated (Florvaag et al 1988; Hirschl, 1989). The following allergens have been characterized: Bet v 1, a 17 kDa protein displaying ribonuclease activity and characterized as a PR-10 protein (Breiteneder et al 1989; Elsayed et al 1990; Grote et al 1993; Scheiner, 1993; Swoboda et al 1994; Taneichi et al 1994; Bufe et al 1996; Holm et al 2001; Mogensen et al 2002; Vieths et al 2002) , Bet v 2, a 15 kDa profilin (Elsayed and Vik, 1990; Valenta et al 1991a,b,c; Grote et al 1993; Scheiner, 1993; Seiberler et al 1994; Wiedemann et al 1996; Engel et al 1997; Domke et al 1997; Fedorov et al 1997; Vieths et al 2002), Bet v 3, a 24 kDa calcium-binding protein (Seiberler et a. 1994; Tinghino et al 2002). Bet v 4, a 9 kDa Ca-binding protein (Engel et al 1997; Twardosz et al 1997; Ferreira et al 1999; Grote et al 2002), Bet v 5, a 35 kDa isoflavone reductase-related protein (Vieths et al 1998; Karamloo et al 1999; Stewart and McWilliam, 2001), Bet v 6, a 30-35 kDa protein, PCBER (Phenylcoumaran benzylic ether reductase) (Karamloo et al 2001), Bet v 7, a 18 kDa protein, characterized as a cyclophilin (Cadot et al 2000) and Bet v 11 (Moverare et al 2002).

A large number of these allergens have been expressed as recombinant proteins, including rBet v 1 (Ferreira et al 2003), rBet v 2 (Valenta et al 1991a-c; Niederberger et al 1998; Susani et al 1995; Valenta et al 1993), rBet v 3 (Valenta et al 1991a-c; Seiberler et al 1994), rBet v 4 (Engel et al 1997; Twardosz et al 1997; Ferreira et al 1999), rBet v 5 (Karamloo et al 1999) and rBet v 6 (Vieths et al 2002).

As significant allergenic behaviors, Bet v 1 displays a considerable degree of heterogeneity and consists of at least 20 isoforms which differ in their IgE-binding capacity (Bet v 1a to Bet v 1n), (Breiteneder et al 1989; Elsayed and Vik, 1990; Karamloo et al 1999; Friedl-Hajek et al 1999). Birch pollen-allergic individuals may not be sensitized to any of the major birch pollen allergens.

Evidence of cross-reactivity of birch allergens among different sources is very high: Cross-reactivity exists between pollens from species within the *Betulaceae* family or belonging to closely related families (Valenta et al 1991a-c, 1993; Yman 1982, 2001; Eriksson et al 1987; Jung et al 1987; Ipsen et al 1985; Breiteneder et al 1993; Kos et al 1993; Wahl et al 1996). Moreover, the presence of numerous so-called cross reactivity syndrome have been described, including the "Birch-Mugwort-Celery syndrome" (Ballmer-Weber et al 2000) and the "Celery-Carrot-Birch-Mugwort-spice syndrome" when Carrot and Spices are included (Pauli et al 1985; Dietschi et al 1987; Helbling 1997; Wüthrich and Dietschi 1985; Stäger et al 1991). The major birch pollen allergen, Bet v 1, and the apple allergen Mald 1 share allergic epitopes leading to IgE cross-reactivities (Ebner et al 1991; Vieths et al 1994; Matthes and Schmitz-Eiberger 2009). Especially during the birch pollen season, an increase in clinical reactions to apples occurs (Skamstrup-Hansen et al 2001). The most common manifestation of allergy to food in Birch pollen-allergic individuals is oral allergy syndrome (OAS).

Selection and breeding of hypoallergenic trees or the application of genetic modification to develop these may potentially reduce the allergenic load caused by birch. This and other objectives have led to the development of studies to characterize genes encoding Bet v 1 isoforms (Schenk et al 2006, 2009). Such studies included the screening of different *Betula* species and different *Betula pendula* cultivars. In total, fourteen different Bet v I-type isoforms were identified in three cultivars, of which nine isoforms were entirely new (Schenk et al 2006). A major conclusion of this study is that a single birch tree may produce a mixture of isoforms with varying IgE reactivity, and that this fact should be taken into account in investigations towards sensitization and immunotherapy. Variability of Bet v I and closely related PR-10 genes in the genome was established by Schenk et al (2009) in eight birch species including *B. pendula* and a particular *B. pendula* cultivar named 'Youngii'. Expression studies of these genes were also carried out by using Q-TOF LC-MS^E methods.

A recent publication by Schenk et al (2011) analyzes antigenic and allergenic profiles of pollen extracts from several genotypes of birch species, including several hybrids, and four cultivars of *Betula pendula* by SDS-PAGE and Western blot using pooled sera of birch-allergic individuals. Tryptic digests of the Bet v 1 were subjected to LC-MS^E analysis. Considerable differences in Bet v 1 isoform composition exist between birch genotypes.

Schenk et al (2008) reviewed the controversial taxonomy of *Betula*, and the various classifications historically proposed. The basic chromosome number of *Betula* is $n = 14$, and the species form a series of polyploids with chromosome numbers of $2n = 28$, 56, 70, 84, 112, and 140. Moreover, several of the recognized *Betula* species have a hybrid origin. The

simultaneous occurrence of polyploidization, extensive hybridization, and introgression complicates even more taxonomical studies in the genus. These authors also reviewed the different methods and alternative markers (including DNA markers) used to reconstruct species relationships within the genus *Betula*. The authors examined the use of AFLPs for this purpose in 107 *Betula* accessions from 23 species and 11 hybrids. At least 9 well determined subspecies, varieties, or cultivars of *Betula pendula* were included in this study along another 24 infraspecies-undetermined accessions of this species. This gives an idea of the wide germplasm involved, and the difficulty of characterizing the different allergenic variants present in the corresponding pollen.

4.4. Japanese cedar (*Cryptomeria japonica*, Synonym: *Cupressus japonica*)

Relevant allergenic information concerning this plant is assembled in the web pages http://www.phadia.com/en/Allergen-information/ImmunoCAP-Allergens/Tree-Pollens/Allergens/Japanese-cedar-/ (Phadia), and http://www.allergome.org/script/dettaglio.php?id_molecule=1784 (Allergome).

The following allergens have been characterized in this source: Cry j 1, a 45-50 kDa protein, a pectate lyase, is considered a major allergen (Yasueda et al 1983; Taniai et al 1988; Griffith et al 1993; Sone et al 1994; Taniguchi et al 1995; Hashimoto et al 1995; Okano et al 2001; Goto et al 2004; Okano et al 2004; Maeda et al 2005;Takahashi et al 2006; Midoro-Horiuti et al 2006; Kimura et al 2008), Cry j 2, a polygalacturonase, also considered a major allergen (Sakaguchi et al 1990; Namba et al 1994; Komiyama et al 1994; Taniguchi et al 1995; Ohtsuki et al 1995; Futamura et al 2006; Goto-Fukuda et al 2007), Cry j 3, a 27 kDa protein characterized as a thaumatin, and a PR-5 protein (Fujimura et al 2007; Futamura et al 2002, 2006), Cry j 4, a Ca-binding protein (Futamura et al 2006), Cry j IFR, an isoflavone reductase (Kawamoto et al 2002), Cry j, a chitinase (Fujimura et al 2005), Cry j AP, a Aspartic Protease (Ibrahim et al 2010a), Cry j CPA9, a serin protease (Ibrahim et al 2010b), and Cry j LTP, a Lipid Transfer Protein (Ibrahim et al 2010c). Moreover, a number of other antigenic proteins have been isolated but not characterized, including proteins of 7, 15 and 20 kDa (Matsumura et al 2006).

Cross-reactivity among conifer pollens has been documented (Aceituno et al 2000; Midoro-Horiuti et al 1999; Ito et al 1995). This could be explained by the high similarity between the Japanese cedar allergen Cry j 1 and the major allergens of Mountain cedar (Jun a 1), Japanese cypress (Cha o 1) and *Cupressus arizonica* (Cup a 1). Other cross-reactivities include tomato fruit (Kondo et al 2002), latex (Fujimura 2005) and *Cupressus sempervirens* (Panzani et al 1986).

Cry j 1 and Cry j 2 are major allergens. However, concentrations of these allergens vary greatly in pollen from different individual Japanese cedar trees (Goto-Fukuda et al 2007). Most basically, there are 2 varieties of Japanese cedar trees: the popular diploid and the less popular triploid. These trees are not very different morphologically. In a comparison of the major allergens Cry j 1 and Cry j 2, the triploid tree pollen extract was shown to have lower

concentrations of both. The pollen from this variety may thus be less allergenic (Kondo et al 1997). Conspicuous differences were detected in the presence of the Cry j 1 allergen in two kinds of cultivar: 'Mio' and 'Masuyama' (Saito and Teranishi, 2002).

4.5. Olive tree (*Olea europaea* L.)

Relevant allergenic information concerning this plant is compiled in the web pages http://www.phadia.com/en/Allergen-information/ImmunoCAP-Allergens/Tree-Pollens/Allergens/Olive-/(Phadia), and http://www.allergome.org/script/dettaglio.php?id_molecule=1888 (Allergome). Furthermore, a very recent article by Esteve et al (2012) reviews the information available about the characterized olive allergens at present, the procedures used for such physicochemical and immunological characterization, as well as for extraction and production of olive allergens. Up to date, twelve allergens have been identified in olive pollen while just one allergen has been identified in olive fruit. Additional reviews on olive pollen allergens include the chapters by Jimenez-Lopez et al, Morales et al, and Zienkiewicz et al included in this book.

Olive pollen is by far the most studied allergenic pollen at infraspecific taxonomical level. An important point to explain this is the fact that the olive germplasm (extremely rich although still unexplored in its totality), is the subject of numerous analysis carried out in order to characterize cultivar identity. These works include the use of morphological traits (Barranco and Rallo, 1984; Cimato et al, 1993; Barranco et al, 2005; Caballero et al, 2006) as well as molecular methods, which started with the use of isoenzyme markers (Ouazzani et al, 1993; Trujillo et al, 1995) and at a later stage have been carried out utilizing DNA markers as RFLPs (Besnard et al 2001), RAPDs (Belaj et al, 2001; Fabbri et al, 1995), AFLPs (Angiolillo et al, 1999) and microsatellite markers (SSRs). SSRs are one of the most reliable methods used in olive cultivar characterization (Baldoni et al, 2009; La Mantia et al, 2005). SSRs markers have been successfully used in germplasm bank classification and contributed to a better management of several olive collections around the world (Khadari et al, 2003; Muzzalupo et al, 2006; Fendri et al 2010). In order to provide a better world-wide applicable tool for olive DNA typing, a list of 11 SSRs markers has been selected among microsatellites available for olive cultivar characterization (Baldoni et al, 2009). These works have led to the publication of different olive cultivar catalogues (Barranco and Rallo 1984; Cimato et al 1993; Barranco et al 2000; Caballero et al 2006).

Earlier evidence of the relationships between olive allergen polymorphism and the cultivar origin of olive pollen was reviewed by Alché et al (2007), with particular reference to the publications available at that time, including those by Barber et al 1990, Geller-Bernstein et al (1996), Waisel and Geller-Bernstein (1996), Castro (2001, 2003), Carnes Sanchez et al (2002), Conde Hernandez et al (2002), Hamman-Khalifa et al (2003, 2005), Alché et al (2003), Napoli et al (2006) and Fernandez-Caldas et al (2007). Further confirmation at the molecular level has risen since, based in the use of powerful cloning, proteomics (peptide mapping and N-glycopeptide analysis) and bioinformatics methods. These include the analysis of

numerous cDNA and peptide/glucan sequences from Ole e 1 (Napoli et al, 2008; Hamman Khalifa et al, 2010; Castro et al, 2012; Jiménez-López et al, 2011; Soleimani et al, 2012a,b), Ole e 5 (Zafra, 2007), Ole e 2 (Jiménez López, 2008; Morales et al, 2008; Jiménez-López et al, 2012b), and Ole e 11 (Jiménez-López et al, 2012a). Moreover, the reactivity of a broad panel of olive pollen cultivar extracts to diverse patient' sera has been also analyzed in Jordan (Jaradat et al, 2011). Recently, a novel multiplex method for the simultaneous detection and relative quantification of pollen allergens has been set up (Morales 2012; Morales et al, 2012). This method will help to investigate pollen allergen polymorphism within cultivars in combination with patient's reactivity, by notably improving the specificity and capacity of the biochemical and immunological assays. The present book also includes remarkable analyses of olive varietal polymorphism in those chapters by Jimenez-Lopez et al, Morales et al, and Zienkiewicz et al

5. Conclusions and future perspectives

The past and recent developments in the analysis of the differential allergenicity of pollens from heterogeneous infraspecific taxonomic ranks described above, confirm the need of rethinking current strategies for basic research on pollen allergen characterization, and the design of diagnosis and specific immunotherapy approaches. These issues, raised and discussed initially by us (Alché et al, 2007) for olive pollen allergens, seem to be valid for a broader number of species, as stated here. Extensive pollen allergen polymorphism is known to represent a general feature over the plant kingdom. The limitation of the study of this polymorphism just to the level of species represents a restriction which may limit both basic knowledge and more importantly the efficacy and the future development of strategies to detect and contest human pollen allergy. Although the use of marker allergens for order, genera or even plant families may represent an invaluable tool (Mothes et al, 2004), relevant differences in patient's reactivity occur even among close-related taxonomical ranks (e.g. van Ree 2002; Asero et al, 2005; Fenaille et al, 2009; Wallner et al, 2009a,b; Jaradat et al, 2011) therefore determining that even close allergenic compositions are not always "fully equivalent". The analysis of allergenic variability in infraspecific taxonomical ranks should be considered a "must" that can be easily incorporated into most developing and evolving trends in allergy analysis and clinics, namely the design of highly specific and personalized natural extracts, hypoallergens, the design and production of recombinant allergens, hybrid molecules, high-throughput diagnosis, new forms of allergen administration and release, the analysis of allergen cross-reactivity etc. (Schenk et al, 2006, 2011; Gao et al, 2008; Wallner et al, 2009a,b).

Agricultural and environmental strategies to reduce the impact of pollen allergy involving the use of differential infraspecific taxonomic ranks are not to be discarded either. They may include the primary screening of relatively less allergenic varieties as proposed for wheat, buckwheat and other food sources (Nair and Adachi, 2002; Nakamura et al, 2005; Spangenberg et al, 2006), and the future design of varieties/hybrids with reduced pollen production, limited period of flowering, or even androsteril characteristics in a similar way

of that proposed for the Gilissen et al (2006a,b) for the production of hypoallergenic plant foods by selection, breeding and genetic modifications.

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