

REVIEW

Grass pollen allergens globally: the contribution of subtropical grasses to burden of allergic respiratory diseases

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Summary

Grass pollens of the temperate (Pooideae) subfamily and subtropical subfamilies of grasses are major aeroallergen sources worldwide. The subtropical Chloridoideae (e.g. *Cynodon dactylon*; Bermuda grass) and Panicoideae (e.g. *Paspalum notatum*; Bahia grass) species are abundant in parts of Africa, India, Asia, Australia and the Americas, where a large and increasing proportion of the world's population abide. These grasses are phylogenetically and ecologically distinct from temperate grasses. With the advent of global warming, it is conceivable that the geographic distribution of subtropical grasses and the contribution of their pollen to the burden of allergic rhinitis and asthma will increase. This review aims to provide a comprehensive synthesis of the current global knowledge of (i) regional variation in allergic sensitivity to subtropical grass pollens, (ii) molecular allergenic components of subtropical grass pollens and (iii) allergic responses to subtropical grass pollen allergens in relevant populations. Patients from subtropical regions of the world show higher allergic sensitivity to grass pollens of Chloridoideae and Panicoideae grasses, than to temperate grass pollens. The group 1 allergens are amongst the allergen components of subtropical grass pollens, but the group 5 allergens, by which temperate grass pollen extracts are standardized for allergen content, appear to be absent from both subfamilies of subtropical grasses. Whilst there are shared allergenic components and antigenic determinants, there are additional clinically relevant subfamily-specific differences, at T- and B-cell levels, between pollen allergens of subtropical and temperate grasses. Differential immune recognition of subtropical grass pollens is likely to impact upon the efficacy of allergen immunotherapy of patients who are primarily sensitized to subtropical grass pollens. The literature reviewed herein highlights the clinical need to standardize allergen preparations for both types of subtropical grass pollens to achieve optimal diagnosis and treatment of patients with allergic respiratory disease in subtropical regions of the world.

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Introduction

Grass pollens (GP) are amongst the most clinically important allergen sources worldwide and are responsible for triggering allergic rhinitis and exacerbating asthma [1, 2]. Allergenic pollens of the Poaceae grasses arise from three separate subfamilies including the temperate Pooideae and subtropical Chloridoideae (e.g. *Cynodon dactylon*; Bermuda grass) and Panicoideae (e.g. *Paspalum notatum*; Bahia grass) subfamilies. Research has mainly addressed allergic sensitivity and allergen recognition of temperate GP (e.g. *Phleum pratense*; Timothy, or *Lolium perenne*; Ryegrass) in populations living in temperate climates from Europe and North America. The subtropical grasses are abundant in war-

mer regions that are home to a vast and increasing proportion of the global population, including parts of Africa, India, Asia, Australia, United States of America (e.g. Florida and California) as well as Central and South America [3, 4]. Although all GP show a degree of allergenic relatedness, there are clinically important differences in immune recognition of pollen allergens from different subfamilies of grasses.

Subtropical grasses are phylogenetically and ecologically distinct from temperate grasses, but their distribution is likely to increase with the advent of global warming. Temperature and carbon dioxide increases associated with global warming are likely to lead to increased biomass of subtropical grasses [5] and increase in pollen production [6, 7]. In such a scenario,

it is conceivable that the contribution of subtropical GP to the burden of allergic respiratory disease (hay fever and asthma) will increase. Whilst the distribution of temperate and subtropical grasses fits reasonably well with the latitudinal climate gradients, they can co-exist in temperate climates. Secondary, late summer peaks in airborne GP were observed to coincide with flowering of subtropical grasses including *Sorghum halepense* (Johnson) in Washington, USA [8], and *C. dactylon* in Italy [9].

Future improvements in diagnosis and treatment of patients with allergic respiratory diseases in subtropical regions, who are primarily sensitized to subtropical GP allergens, would be gained by increased knowledge of (i) allergic sensitivity to subtropical GP allergens in relevant populations, (ii) a better appreciation of the allergenic components of subtropical GP allergens and (iii) the immunological cross-reactivity between subtropical and temperate GP allergens. It is apparent that patterns of sensitization detected by skin prick test (SPT) or serum IgE measurement vary with geographic region and depend on the primary sensitizing GP. This differential immune recognition of subtropical GP has significance for the efficacy of allergen immunotherapy treatment of patients primarily sensitized to subtropical GP.

Allergic sensitivity to subtropical grass pollens

Patients from temperate regions

Patients with GP allergy who are born and sensitized in a temperate region show primary sensitivity to temperate GP but they also show allergic sensitivity and serum IgE reactivity with subtropical GP, albeit to a lower level. European studies of 143 subjects revealed that specific serum IgE to subtropical GP extracts of *C. dactylon*, *P. notatum* and *S. halepense* were not as tightly correlated with IgE reactivity to *P. pratense* GP as other temperate GP [10]. A larger panel of over 5000 subjects from Northern European and North American clinics confirmed this markedly lower correlation of IgE reactivity between *P. pratense* and *C. dactylon* GP than amongst the temperate GP [11].

Rossi et al. [12] report IgE reactivity with allergen components of *P. pratense* GP in *C. dactylon* GP-allergic patients from north-west Italy. Phenological studies confirmed that *C. dactylon* grass contributes to a small secondary peak of airborne pollen from June to July [9]. However, the patients in the Rossi study showed six times higher specific IgE to *P. pratense* GP (median 48.9 kUA/L) than *C. dactylon* (median 8.0 kUA/L), suggesting primary sensitivity to temperate GP [12], consistent with their typical pattern of IgE reactivity with components of *P. pratense* GP [13, 14]. Similarly,

in a temperate region of Australia, 85% of GP-allergic patients from Victoria had SPT responses to the subtropical *P. notatum* GP [15]. However, patients from Victoria showed significantly higher serum IgE reactivity with *L. perenne* GP than *P. notatum* or *C. dactylon* GP [16], consistent with their exposure to *L. perenne* GP as the dominant fodder grass in Victoria and primary sensitivity to temperate GP.

Patients from subtropical regions

Patients in regions with subtropical climates show evidence of allergic sensitivity to pollens from a wide variety of species derived from both subfamilies of subtropical grasses (Fig. 1, Table 1). Pollen of other local Chloridoideae; *Chloris gayana* (Rhodes grass) and *Eragrostis* sp have been identified as important allergen sources in Queensland, Australia [17–19], South Africa [20], and India [21]. Panicoideae species of the Andropogonae tribe; *Dichanthium sericeum* [17] and *Sorghum verticilliformum* [21] and Paniceae tribe, *Stenotaphrum secundatum* (Buffalo grass) [20], are reported allergen sources in Queensland, Australia, India, and South Africa, respectively. *P. dilatatum* (Dallas grass) has been identified as allergen source in south and south-west USA [22, 23]. Whilst its pollen was IgE cross-reactive with *P. notatum* pollen, *P. dilatatum* GP showed less potent SPT than *P. notatum* GP in Florida, USA [24]. *P. notatum* was identified as a clinically important GP allergen source in southern parts of USA and Australia [15, 24–27].

The subtropical GP appear to play a more important role in allergic disease in subtropical than tropical countries of Asia as apparent by higher frequency of allergic sensitivity to GP assessed by SPT (Table 1). Positive SPT to subtropical GP were observed in 38–55% of patients with respiratory allergies in South Africa and West Bengal, India [39, 42], compared with 10–22% in Taiwan, Thailand, and Malaysia [35–38]. Detection of allergic sensitivity was limited by the availability of particular GP for diagnostic testing, where other species of local subtropical grasses were likely to be contributing to pollen allergy [37], potentially leading to under representation of allergic sensitivity to GP. In Thailand and Taiwan, allergic sensitivity with pollen of temperate grasses (e.g. *P. pratense* and *Dactylis glomerata*; Orchard grass) was reported, but frequency of positive SPT to temperate GP was slightly lower than with *C. dactylon* or *S. halepense* [35, 36]. The capacity for temperate grasses to thrive in the tropical environments is unlikely. These positive SPT to temperate GP may reflect sensitization to pollens of local grasses.

In South Africa, Potter et al. observed that 38% of 209 children presenting with allergic disease were sensi-

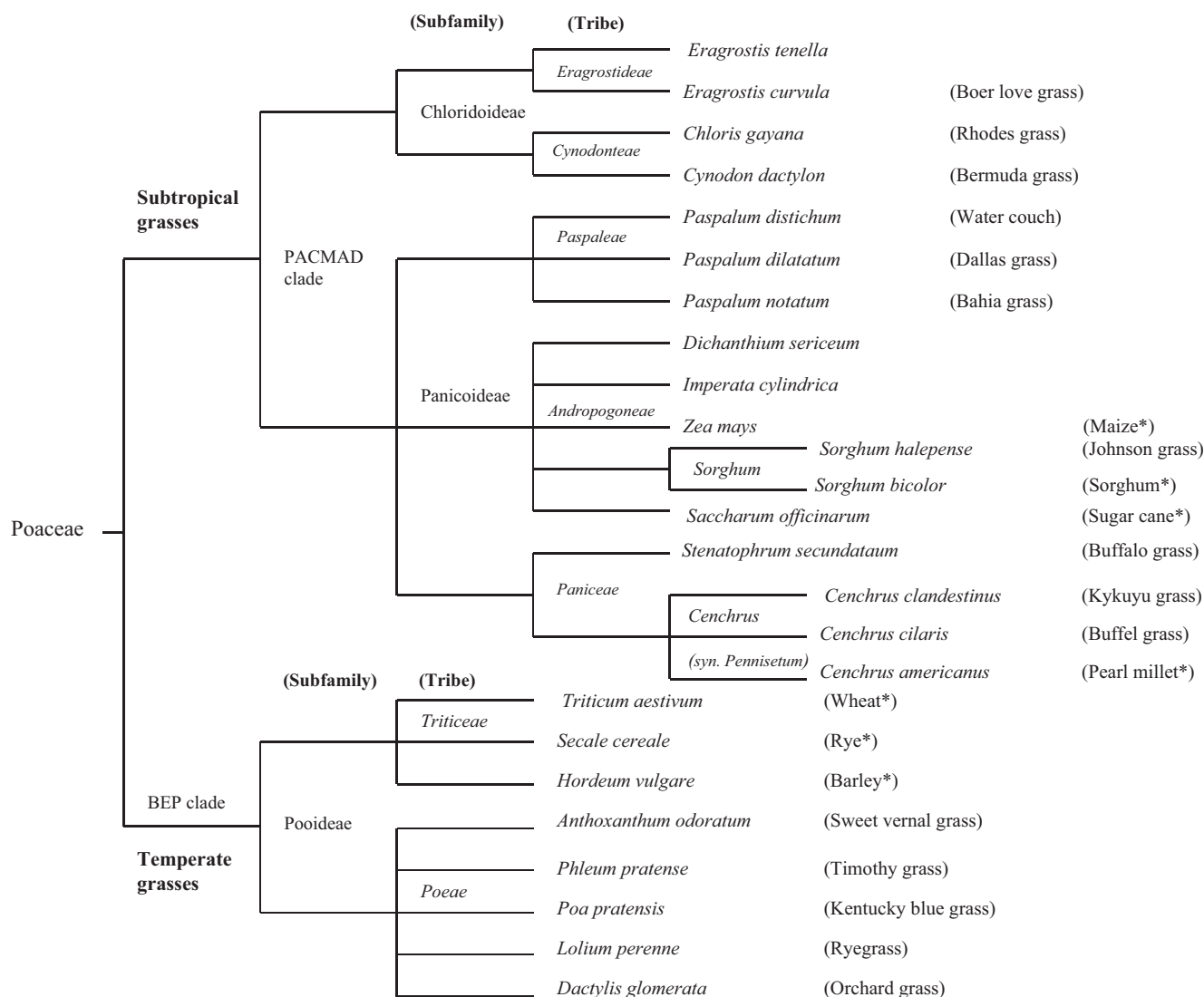


Fig. 1. The phylogenetic relationships within the Poaceae family of grasses. Temperate grasses of the Pooideae subfamily are biologically distinct from the subtropical grasses belonging to Chloridoideae and Panicoideae subfamilies. The separate tribes within the subfamilies are named, and taxa levels are shown in brackets. Species of each subfamily that produce allergenic pollen are shown (common name). *Agriculturally important subtropical crops with allergenic pollen. Phylogenetic representation was generated by PhyloDendron (<http://iubio.bio.indiana.edu/treeapp/>).

tive to a mixture of South African GP [42]. Of a subset of 25 of these children who had pollen allergy, 92% showed serum IgE reactivity with *P. pratense*, even though *P. pratense* grass is not reported to occur in the region. Although other temperate grass species were observed in the West Cape region, the Panicoideae subtropical grasses predominate in South Africa [42]. West-ritchnig et al. [32] report allergic reactivity of patients from Zimbabwe and found higher frequency reactivity with *C. dactylon* (38%) than *P. pratense* (26%). It was not clear whether the reactivity with *P. pratense* was due to cross-recognition of common allergenic components or a cosensitivity due exposure of the subjects to *P. pratense*. IgE reactivity with allergenic components of *P. pratense* was measured. Compared with European

populations, patients from Zimbabwe showed substantially lower frequencies of IgE reactivity with most *P. pratense* GP allergens, with the exception of Phl p 4. The absence of IgE reactivity with Phl p 2 and substantially reduced reactivity with Phl p 5 (100% vs. 27%) and Phl p 6 (52% vs. 15%), in the Zimbabwe patient group is consistent with the reported absence of these allergen families in subtropical GP (reviewed section 2.). The high frequency of IgE reactivity with Phl p 4 in the Zimbabwe patients might reflect the dominance of this allergen family in *C. dactylon* [43], shared conformational epitopes between Cyn d 4 and Phl p 4 [44], or the presence of cross-reactive carbohydrate determinants. The pattern of allergen component recognition indicates that the subtropical GP are the likely primary sensitizing

Table 1. Clinically important subtropical grass pollen allergen sources

Genus species	Country	Patient group	Skin test (number, test*)	Serum IgE (number)	References
<i>Cynodon dactylon</i>	India: Delhi,	GP-allergic	52% (133, IDST)	40% (66)	[21, 28]
	Lucknow	As and AR	12.5% (48)		[29]
	Australia: Qld,	GP-allergic, AR	84% (48)	81.5% (55)	[17, 18, 30]
	Vic.	GP-allergic, AR		91% (51)	[16]
	Saudi Arabia	Perennial AR	61% (54)		[31]
	Zimbabwe	Any allergy	38% (650)		[32]
		Any allergy	35.3% (1046)		[33]
		AR	50.4% (341)		
		As	48.1% (104)		
	USA	Population	18.15 (50 000)		[34]
	Taiwan	AR	2.1% (419)		[35]
	Thailand	AR	17% (100)		[36]
	Malaysia	As	20.5% (200)		[37]
<i>Imperata</i> sp.	India: Delhi,	GP-allergic	22% (133, IDST)		[28]
	Lucknow	As and AR	20.8% (48)		[29]
<i>Zea mays</i> (corn)	India: Lucknow	As and AR	6.3% (48)		[29]
	Zimbabwe	Any allergy	28% (650)		[32]
		Any allergy	29% (1046)		[33]
		AR	39% (341)		
		As	28.8% (104)		
<i>Sorghum halepense</i> (Johnson grass)	Australia: Qld	GP-allergic, AR	77% (48)	80% (55)	[17, 18, 30]
	Taiwan	AR	10% (419)		[35]
	Thailand	AR	21% (100)		[36]
		As (children)	14% (100)		[38]
	USA	Resp. allergy			[23]
<i>Sorghum bicolor</i> [†] (Sorghum)	India: Delhi,	GP-allergic	16% (133, IDST)		[28]
	Lucknow	As and AR	8.3% (48)		[29]
<i>Saccharum officinarum</i> (sugar cane)	India, West Bengal	Resp. allergy	55% (370)		[39]
			54.3% (350)		[40]
<i>Paspalum notatum</i> (Bahia grass)	USA: south Florida	Resp. allergy			[22]
		Resp. allergy	246 (429)		[24]
	Australia: Qld,	GP-allergic, AR	81% (48)	80% (55)	[30]
	Vic.	GP-allergic AR		92% (51)	[16]
	Thailand	AR	16% (100)		[36]
	Malaysia	As	6.5% (200)		[37]
					[37]
<i>Cenchrus ciliaris</i> (Buffel grass)	India: Delhi,	GP-allergic	22% (133, IDST)		[28]
	Lucknow	As and AR	20.8% (48)		[29]
	Australia, Qld	GP-allergic			[17, 18]
<i>C. americanus</i> [‡] (Pearl millet)	India: Delhi,	GP-allergic	32% (133, IDST)		[28]
	Lucknow	As and AR	6.3% (48)		[29]
<i>C. clandestinus</i> [§] (Kikuyu)	South Africa	GP-allergic	43 out of 104		[41]

IDST, intradermal skin test; AR, allergic rhinitis; As, asthma; RA, respiratory allergy; GP, grass pollen; Qld; Queensland (subtropical climate state), Vic.; Victoria (temperate climate state).

*Skin prick test unless stated; [†]*syn. S. vulgare*; [‡]*syn. Pennisetum typhoides*; [§]*syn. Pennisetum clandestinum*, blank spaces indicate no data were reported for this test.

GP allergen sources for patients from Zimbabwe. Similarly, a case review study of 1046 sequential patients with allergic disease from Zimbabwe, showed higher positive SPT, 35.3% frequency (median 10 mm diameter) with *C. dactylon* compared with a temperate GP mixture (25.5% frequency, 8 mm diameter) [33].

In Australia, allergic sensitization to *P. notatum* and *C. dactylon* in patients from subtropical northern New

South Wales has been associated with asthma in children and allergic rhinitis in adults [27]. In patients from the subtropical region of Queensland, *C. dactylon* and *P. notatum* GP, elicited greater allergic sensitivity (by SPT) than *L. perenne* GP, and levels of IgE reactivity were significantly higher with *P. notatum* than with *L. perenne* [30]. Within a subset of subjects who were born and sensitized in Queensland, the profile of IgE

cross-reactivity showed greater avidity of interaction of serum IgE with the subtropical (*P. notatum*, *S. halepense*, and *C. dactylon*) than *L. perenne* GP, consistent with primary sensitization to subtropical GP [30].

As reviewed above, the frequency and median levels of SPT diameters, or serum IgE reactivity with subtropical or temperate GP, differ between pollen sources and between patient groups. This difference in level of reactivity has been observed much earlier; the lower level of SPT responses to *C. dactylon* compared with temperate GP (*S. cereale*, *Ph. communis*, *L. perenne*, and *P. pratense*) depended on the European country of origin of patients [45]. Patients with GP allergy from the temperate region Victoria, reacted at similar frequency of IgE reactivity with *L. perenne*, *C. dactylon*, and *P. notatum*, which was paralleled by similar frequency of IgE reactivity with the major allergens of these three grasses; Lol p 1, Pas n 1, and Cyn d 1 [16]. However, the level of IgE reactivity with whole *L. perenne* GP or allergen component (Lol p 1) was significantly higher than IgE reactivity with *P. notatum* and *C. dactylon* GP, or their components Pas n1 and Cyn d 1, respectively. This difference in level of IgE reactivity was consistent with asymmetric patterns of IgE cross-reactivity (reviewed section 3) and the differential capacity of these GP extracts or purified allergens, to activate basophils of pollen allergic individuals. Up to 500-fold higher concentrations of *C. dactylon* or *P. notatum* GP, or Pas n 1 and Cyn d 1, compared with *L. perenne* GP or Lol p 1, were required to activate basophils from blood of patients primarily sensitized to *L. perenne* GP [16], indicating that observed differences in levels of IgE reactivity are likely to be clinically meaningful. The studies reviewed here exemplify the regional differences in allergic sensitivity to different types of subtropical GP.

Subtropical grass pollen allergen components

Recent research has boosted the number of allergen components described for subtropical GP (Table 2). After removal of the major allergen Cyn d 1, sixty different IgE binding proteins were observed on immunoblots of *C. dactylon* GP separated according to charge and size [46]. The role of carbohydrate moieties in IgE binding to these components of *C. dactylon* GP remains to be investigated. However, many of these IgE binding proteins were isoforms of novel GP allergens not previously described (e.g. Cyn d 22 and Cyn d 24). Sequences for Cyn d, 15, Cyn d 2, and Cyn d 23 are listed in the Allergome database (www.allergome.org), and names of Cyn d 5, Cyn d 6, Cyn d 11, and Cyn d 13 are on the same site, but no evidence of their protein expression in mature pollen, IgE reactivity with patient sera, or other supporting data on their allergenic

status has been published. Two new classes of allergens discovered within the lipid soluble fraction of the pollen coat; exoxylanase, and cysteine protease, of *C. dactylon* showed IgE reactivity in five and six of eight patients, respectively, who had high IgE levels to *C. dactylon* [47].

The major group 1 pollen allergen of *P. notatum*, a basic β -expansin protein designated Pas n 1, was cloned, expressed, and characterized [65]. Independently identified was a 33 kDa allergen of *P. notatum* with a neutral pH [26, 66]. The amino terminal peptide showed 55% identity with the cloned sequence of Pas n 1; this allergen is likely to be the neutral isoform of Pas n 1. Of 55 GP-allergic patients with allergic rhinitis or asthma from Victoria, 85% showed IgE reactivity with recombinant (r)Pas n 1 [65]. rPas n 1 activated basophils and inhibited serum IgE reactivity with the natural Pas n 1 allergen of *P. notatum* GP. cDNA sequences for two isoforms of Pas n 13 have been cloned. Purified Pas n 13 showed IgE reactivity in 49% of 71 GP-allergic subjects [68].

Sor h 1, of the allergenic subtropical weed species *S. halepense* (Johnson grass), had been cloned, and rSor h 1 was shown to react with monoclonal antibodies (mAb) to group 1 allergens [69]. IgE reactivity with Sor h 1 was demonstrated in 76% of a relevant population of 64 GP-allergic patients with allergic rhinitis from the subtropical region of Queensland [70]. The next most dominant allergen component of *S. halepense* GP, the group 13 allergen Sor h 13, was purified and characterized. Of the same 64 GP-allergic patients with allergic rhinitis, 29 (46% overall) showed IgE reactivity with Sor h 13 by ELISA [70]. Bashir et al. [47] observed an IgE-reactive homologue of the newly described cysteine protease by immunoblotting a lipid soluble coat protein fraction of *S. halepense* GP (Sor h CP).

The Panicoideae subfamily contains a number of agriculturally important subtropical crop species such as *S. bicolor* (synonym *S. vulgare*; Sorghum). Although *S. bicolor* and *S. officinarum* are major crops in subtropical regions and elicit allergic sensitivity in India, information on allergenic components is lacking [28, 39, 40], with the exception of Sor b 1 [78, 79]. Pollen of another Panicoideae crop maize pollen (*Zea mays*) is an important allergen source for some patients in regions where these species are propagated. Maize was reported as an allergen in India [29] (Table 1), and Europe [73–75]. Notably, most European patients who experienced allergic respiratory symptoms upon occupational exposure to maize pollen had strong histories of GP allergy suggesting primary allergic sensitivity to *P. pratense* [73, 75]. Serum IgE from maize-allergic patients reacted with Zea m 1 and Zea m 13 [73, 74]. Two patients also reacted with Zea m 3 but not the equivalent allergen of *P. pratense*, suggesting a maize-specific allergic

Table 2. (A) Allergens characterized from subtropical Chloridoideae grass pollens. (B) Allergens characterized from subtropical Panicoideae grass pollens

Allergen	Protein family	Evidence for allergenic activity	References
(A)			
Cyn d 1	Beta-expansin, 29–31 kDa, basic and neutral isoforms	- Major allergen with high frequency of IgE reactivity in allergic patients. 12 isoforms of Cyn d 1 by mass spectrometry, and multiple isoforms cloned and characterized, activates basophils, immunodominant unique T-cell peptide epitopes mapped (lacking X-R), B-cell epitopes for IgE and IgG4 mapped by synthetic peptides, expressed cDNA fragments.	[48–54] [16, 48, 55–59]
Cyn d 4* (BG60)	Metallo-flavoprotein, berberine bridge glycoprotein, 60 kDa, pI 9.3	- IgE reactivity in all 18 and SPT in two patients with AR or As, biochemical characterization. 5 of 10 patients with seasonal AR, 2D IgE immunoblotting, proteomics. - Crystal structure surface of Cyn d 4 has on 25% surface similarity with other group 4 allergens (e.g. Phl p 4), IgE epitopes conformation dependent.	[43, 44, 46]
Cyn d 7	2 EF-hand Ca binding domains, 12 kDa	- Serum IgE reactivity with lambda phage clone expressing Cyn d 7 blocked by Bet v 4, 69% identity with Bet v 4, IgE X-R depended on the Ca²⁺ binding domain 2. 3 of 30 (10%) GP-allergic patients with AR by IgE immunoblotting of Cyn d 7 expressing bacteriophage.	[60, 61]
Cyn d 12	Profilin, 14.1 kDa	- IgE reactivity in 20% of 30 subjects with GP sensitivity, Spain. 2 of 10 patients with seasonal AR, 2D IgE immunoblotting, proteomics.	[46, 62]
Cyn d 22	Enolase, 45 kDa	All of 10 patients with seasonal AR, multiple isoforms, pI 5.1–6.1, 2D IgE immunoblotting.	[46]
Cyn d 24	Pathogenesis related protein, 21 kDa	29% of 21 GP-allergic patients by IgE immunoblotting. 23 of 35 (65%) GP-allergic patients, purified by chromatography, mass spectrometry and carbohydrate characterization. - three of 10 patients with AR, pI 5.8–5.9, 2D IgE immunoblotting.	[46, 54, 63]
Cyn d BG46K*	Cytochrome C oxidase	IgE reactivity with serum pool of 12 GP-allergics, purified by affinity chromatography, 4 isoforms, pI 5.87–6.0.	[64]
(B)			
Pas n 1	Beta-expansin, 28–30 kDa, neutral and basic isoforms	- rPas n 1 IgE in 47 of 55 (85%) GP-allergic patients. - Deglycosylated 28, 31 and 33 kDa allergens of <i>P. notatum</i> retained IgE reactivity. - The N-terminal peptide of 33 kDa allergen matched the beta-expansin family; 11/20 (55%) identity with N-terminal of Pas n 1.0101. - nPas n 1 IgE in 91% of 51 GP-allergic patients, activated basophils.	[16, 26, 65–67]

(continued)

Table 2. (continued)

Allergen	Protein family	Evidence for allergenic activity	References
Pas n 13*	Polygalacturonase, 50–55 kDa	3 immunodominant T-cell epitopes; 2 specific to Pas n 1 without T cell X-R with equivalent Lol p 1 and Cyn d 1 peptides. 48% of 71 GP-allergic patients had IgE reactivity with nPas n 13, activated basophils, 4 isoforms (pI 6.3–7.8) by 2D immunoblotting, partial cDNAs for two isoforms cloned.	[68]
Sor h 1	Beta-expansin, 30 kDa	- Sequence cloned and rSor h 1 shown to react with mAb to group 1 allergen. 49 of 64 (76%) GP-allergic patients with AR, multiple isoforms detected in proteome.	[69] [70]
Sor h 13*	Polygalacturonase	29 of 64 (45.3%) GP-allergic patients with AR, six variants by 2D immunoblotting.	[70]
Zea m 1	Beta-expansin	- Biochemical characterization of four Zea m 1 isoforms. - Crystal structure solved for Zea m 1. - IgE reactivity immunoblotting in all patients tested, albeit with small numbers.	[71–75]
Zea m 3*	Homology with C terminal domain of expansins	3 isoforms by 2D IgE immunoblots with pooled sera from German patients with <i>P. pratense</i> and <i>Z. mays</i> pollen sensitivity. - Two of eight patients with RA elicited by maize pollen exposure. These sera did not show IgE reactivity with Phl p 3. No reaction with mAb to Phl p 3.	[73, 74]
Zea m 13*	Polygalacturonase	4 isoforms by 2D immunoblots with pooled sera from German patients with <i>P. pratense</i> and <i>Z. mays</i> pollen sensitivity. - IgE reactive with patient with RA elicited by maize pollen exposure.	[73, 74, 76, 77]

AR, allergic rhinitis; RA, respiratory allergy; GP, grass pollen; 2D, two-dimensional gel electrophoresis, X-R, cross-reactivity.

*Not verified by the International Union of Immunological Societies.

response in addition to *P. pratense* allergy. Whilst other maize allergens have been described (Zea m 2, Zea m 12, and Zea m 14), there is no strong evidence that these molecular components are allergens of pollen grains or show IgE reactivity with relevant patient sera. For instance, the allergen Zea m 14, a lipid transport protein, was described as a food allergen in maize seed rather than mature pollen [80].

Qualitative differences in pollen allergen components of subtropical and temperate grasses

With 63% amino acid identity, the sequence of Sor h 1 was not as similar to Pas n 1 as might have been expected [65]; however, *P. notatum* is from a tribe separate to that of *S. halepense* (Fig. 1). Pas n 1 encodes a basic isoform, whereas the sequence of Sor h 1 encodes a neutral isoform. It will be important in future to directly compare the frequency and affinity of IgE binding between these basic and neutral group 1 allergens of the Panicoideae GP. Notably, the Chlori-

doideae subfamily allergen Cyn d 1 is as different from Pas n 1 (67% amino acid identity) as Pas n 1 is different from the temperate GP allergens (64–66% amino acid identity). Thus, phylogenetically, subtropical GP allergens are distinct from temperate GP allergens and also show substantial sequence differences from each other.

Notably, the groups 2 and 5 GP allergens have not been found in any grass outside the Pooideae subfamily [81, 82]. These allergens are reportedly missing from pollen of *C. dactylon* grass [83], *P. notatum* [16, 65], as well as Buffalo (*Stenotaphrum secundatum*) and *Eragrostis* [20]. Phl p 5 and Lol p 5 appear to be potent allergens recognized at high frequency by patients with temperate grass pollen allergy [14, 81, 84, 85]. The consequence of the absence from subtropical GP of group 5 and the related group 6 allergen family, upon the pollen biology and the capacity for subtropical GP to elicit primary sensitization is not known.

Whilst group 2 allergen sequences have been reported for *C. dactylon* (Cyn d 2), Rice (*Oryza sativa*,

(Ory s 2 [86, 87]), and maize (*Zea m 2*; [88]), evidence of IgE reactivity or a status as pollen allergens have not been confirmed. Marth et al. [82] noted that *C. dactylon* lacked immunoreactive group 2 allergen, and group 2 allergens were not observed in a detailed proteomic study of *C. dactylon* GP allergens [46].

Cross-reactivity between temperate and subtropical grass pollen allergens

Despite the capacity for partial cross-reactivity between temperate and subtropical GP, this cross-reactivity is incomplete and depends on the patient population being tested. Pooled serum IgE from GP-allergic patients affinity enriched and eluted from the major GP allergens of *L. perenne*, Lol p 1 and Lol p 5, did not cross-react with *P. notatum* or *C. dactylon* but bound allergens of *L. perenne* and *Phalaris aquatica* (Canary grass) GP [15]. Moreover, IgE affinity purified on the major allergen group 1 allergen band of *P. notatum* reacted more strongly with *L. perenne* than with *P. notatum* GP extract by ELISA, suggesting stronger IgE avidity for allergens of *L. perenne* than *P. notatum* GP in these patients from the temperate region of Victoria [16]. There was asymmetric cross-inhibition between *P. notatum* and *L. perenne* GP in these patients; IgE reactivity with *L. perenne* could not be blocked by *P. notatum* but IgE reactivity with *P. notatum* grass was blocked by *L. perenne* GP in seven of eight patients tested [16]. The data from this cross-reactivity study are consistent with *L. perenne* being the predominant clinically important source of GP in the Victorian cohort. The opposite pattern of asymmetric cross-inhibition occurred between *P. notatum* and *L. perenne* GP in patients from Queensland; serum IgE reactivity with *P. notatum*, *C. dactylon*, or *S. halepense* GP grass could not be fully inhibited by *L. perenne* GP, whereas IgE reactivity with *L. perenne* GP in the five patients tested was inhibited by all other GP, with the occasional exception of *C. dactylon*. Moreover, there was only partial or no reciprocal cross-reactivity between subtropical grass pollens of different subfamilies; GP of *C. dactylon* did not fully inhibit IgE with *P. notatum* and *S. halepense* GP and vice versa [30].

These findings are consistent with studies of patients from Florida who showed no *in vivo* cross-reactivity between GP of *P. pratense* and *P. notatum* in nasal provocation tests in a handful of subjects who were sensitized to either *P. pratense* or *P. notatum* [25]. Using IgE inhibition assays with pooled sera from volunteers in the US army, Martin et al. [89] observed 'unique allergenicity' for *P. notatum* compared with a range of 'Northern' temperate GP (e.g. *P. pratense* and *L. perenne* GP), and 'Southern' subtropical GP (e.g. *P. notatum*, *C. dactylon*, and *S. halepense*). *C. dactylon*

and *P. notatum* showed limited capacity to inhibit pooled serum IgE reactivity with pollens of 'northern' grasses (*L. perenne* and *P. pratense*) from US army volunteers whose origin was unknown. Interestingly, in the converse experiments, these same northern grasses inhibited most but not all IgE reactivity with *P. notatum* grass leading the authors to infer unique allergenicity of *P. notatum* [89].

Allergenic differences between *C. dactylon* and temperate GP are well recognized [10, 11, 90]. For example, in RAST, inhibition assays with pooled sera from patients recruited from Spain, Italy, and Switzerland; 500-fold higher concentration of *C. dactylon* GP was required to inhibit IgE reactivity with *L. perenne* or *P. pratense* than temperate GP (*L. perenne*, *S. cereale*, and *P. pratense*) [45]. This is consistent with the known primary sensitization of European subjects to temperate GP.

For patients from South Africa, pollens of Chloroid grasses; *Eragrostis* and *Cynodon*, shared similar IgE cross-reactivity patterns. Likewise, the Panicoid GP, *Stenotaphrum* and *Cenchrus*, showed comparable IgE cross-reactivity with each other [20]. However, there was incomplete cross-inhibition between pollens of different grass subfamilies revealing subfamily-specific IgE recognition of *Cynodon*, *Eragrostis*, *Stenotaphrum*, and *Cenchrus* grasses, which are locally abundant sources of clinically important pollen allergens in South Africa [91]. Notably, there was genus-specific IgE reactivity with *Eragrostis* GP that was not inhibited by the related *Cynodon* GP.

Cross-inhibition assays performed with sera from patients with GP allergy in India involving pollen from five locally abundant subtropical grasses; Chloroid; *Cynodon*, and Panicoids; *Cenchrus*, *Imperata*, *Pennisetum*, and *Sorghum*, revealed asymmetric IgE cross-inhibition consistent with the subfamily of GP. Vastly different amounts of particular GP extracts were required to give 50% inhibition of pooled patient serum IgE reactivity; whereas less than 10 ng of *Imperata*, *Pennisetum*, *Cenchrus*, and *Cynodon* pollen extract was needed to block 50% of IgE reactivity with *Sorghum*, greater than 5 µg from *Imperata* or *Sorghum* was required to block 50% of IgE reactivity to *Cenchrus* [28]. These studies provide evidence of subfamily-specific IgE binding to antigenic determinants of particular subtropical GP allergens that are not represented in other GP. Moreover, GP derived from each species showed unique IgE cross-inhibition patterns.

Importantly, unique T-cell peptide epitope recognition of the major group 1 allergens Cyn d 1 of *C. dactylon* and Pas n 1 of *P. notatum* have been observed [57, 67]. Whilst these allergens contain an immunodominant T-cell epitope encompassing the conserved key enzy-

matic determinant of β -expansins (histidine, phenylalanine-aspartate; HFD motif [72]) shared with Lol p 1 of *L. perenne* and Phl p 1 of *P. pretense*, additional T-cell epitopes in the carboxy terminal domain are unique to each allergen. Such distinct T-cell recognition is likely to critically affect engagement of allergen-specific T regulatory cells during specific immunotherapy [92]. The inference from these studies are that allergens of the subtropical grasses of both Panicoideae and Chloridoideae subfamilies are immunologically distinct from those of temperate pollens and, from each other, in respect to both the T- and B-cell epitope recognition.

Problems requiring further research

It is important to assess the allergenic potency of local GP sources and their allergen components in subtropical regions of the world in particular India, Africa, Australia, and southern parts of USA where both Panicoideae and Chloridoideae GP appear to be relevant allergens for patients with allergic respiratory diseases. Evidence of the contribution of pollens from both subfamilies of subtropical grasses in large cohorts of well-defined patients who are born and sensitized in subtropical regions, is necessary to underpin development of more specific diagnosis and therapies to optimally address the needs of such patients.

Whilst temperate grass pollen extracts have been standardized for allergen content [93], standardization of subtropical grass pollen extracts has not been undertaken, with the exception of *C. dactylon* GP [49]. However, a prerequisite for accurate, sensitive, and specific diagnosis is standardization of whole pollen allergen extracts, particularly for the clinically important subtropical grasses such as *P. notatum*, *S. halepense*, *C. dactylon*, for which considerable evidence of allergenicity is now available, and potentially *Cenchrus* sp and *Eragrostis* sp. Further research is clearly needed to achieve standardization of allergen content for subtropical grass pollens because the group 5 allergens by which temperate grass pollens are currently standardized upon [93] appear to be absent from subtropical GP. If group 5 allergens are present in subtropical GP, then they are sufficiently different and have eluded detection thus far. The group 1 allergens are an obvious candidate for standardization of subtropical GP. The difference between the basic and neutral Panicoideae group 1 allergens needs to be investigated from a biochemical and immunological point of view, prior to completion of standardization.

The availability of defined allergen components would improve our understanding of the allergenic importance of particular molecular components of subtropical GP, particularly those restricted to sub-

tropical GP. Ideally, subtropical GP allergen components will be incorporated into future multi-allergen microarray assay platforms for component resolved diagnosis [94] to improve the clinical applicability of these tools for accurate diagnosis of patients in subtropical regions.

Summary and conclusions

This review has revealed three levels of evidence regarding the importance of subtropical GP for the global burden of allergic respiratory diseases. (i) Patients with allergic rhinitis and asthma from subtropical regions of the world including parts of Australia, USA, Africa, and India, show higher allergic sensitivity and subfamily-specific immunoreactivity with pollen of both subfamilies of subtropical grasses, compared with temperate GP. (ii) A wide variety of species of Chloridoideae and Panicoideae grasses produce allergenic pollens for which there is limited knowledge about the allergenic components. Pollens of subtropical GP contain the major group 1 allergens as well as novel allergen families amongst their molecular components that are not observed in temperate grasses. Notably, subtropical GP lack the key clinically important groups 2 and 5 allergens of temperate grasses. Importantly, (iii) differences in biological origin, environmental distribution, and allergen content are reflected in distinct immunological recognition of subtropical and temperate GP. Thus, whilst there are some shared allergenic components and antigenic determinants, there are clinically relevant subfamily-specific differences, at T- and B-cell levels, between pollen allergens of subtropical and temperate grasses. This synopsis highlights the clinical need to standardize reagents for diagnosis and allergen immunotherapy treatment of patients primarily sensitized to subtropical GP allergens.

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improving diagnosis and treatment of subtropical grass pollen allergy.

Conflict of interests

The authors declare no conflict of interest.

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