

Tomato allergy: impact of genotype and environmental factors on the biological response

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Abstract

BACKGROUND: Food allergies are increasing in the European population. At present the onset of symptoms can be avoided only by elimination of a particular fruit or vegetable from the diet. A new approach is to develop hypoallergenic food products. This study characterises the allergenic potential of tomatoes, considering cultivation conditions, developmental stages and genotypes, in order to identify hypoallergenic fruits.

RESULTS: Patients with a history of tomato allergy were recruited for skin allergy tests. Tomatoes carrying distinct genotypes were grown under various cultivation conditions and harvested at different maturation stages. Cultivation conditions (nitrogen fertilisation, light exposure and plant nutrition) did not affect the skin reactivity in tomato-allergic patients. However, skin reactivity was significantly lower when using green-unripe compared with red-ripe tomatoes and when using landrace cultivars compared with cultivars bred for use in organic horticulture.

CONCLUSION: Depending on their genetic background and maturity level, some tomato cultivars elicit positive reactions in tomato-allergic patients in the skin allergy test. This novel finding should pave the way for the development of tomatoes with reduced allergenicity to relieve sufferers of tomato allergy.

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Keywords: food allergy; tomato; cultivars; environmental cultivation conditions

INTRODUCTION

Food allergies are increasing and affect 2–4% of the general adult population.¹ Among adults, fruits and vegetables are the main elicitors of food allergies. A geographical difference in sensitisation pattern between patient populations exists, mainly due to the specific geographical distribution of pollen and to local dietary habits. Thus peanut is the most frequent elicitor of food allergy in North America and Great Britain,^{2,3} whereas celery and carrot allergies are more frequent in central Europe.^{4,5}

Tomatoes (*Lycopersicon esculentum*) are a major component of diets around the world and thus are intensively consumed. The intake of raw tomatoes differs substantially throughout Europe: 5.9 g day⁻¹ are consumed in the Netherlands, 47.5 g day⁻¹ in Germany and 112.5 g day⁻¹ in Greece.⁶ Further, global tomato consumption has increased over the past two decades.⁷ In Germany, fresh tomatoes are consumed more frequently than cooked or processed products (raw, 47.5 g day⁻¹; cooked or processed, 14.9 g day⁻¹) and are more relevant in eliciting allergic reactions.⁶ The prevalence of tomato allergy ranges from 1.5% in northern Europe⁸ up to 16% in Italy⁹ among the food-allergic population. Clinical reactions to tomatoes are mostly local symptoms in the oral mucosa, but tomatoes can also elicit systemic reactions and can even cause serious illnesses. Affected individuals avoid tomatoes, which leads to decreased quality of life. As tomato allergy results partly from pollen cross-reactivity,

the majority of patients develop symptoms after eating fresh tomato fruits and can mostly tolerate processed tomato products. So far, limited data are available on the reactivity of immunoglobulin E (IgE) against such products.^{7,10} Allergens that are still present in processed tomato products are lipid transfer protein (Lyc e 3, LTP)⁷ and the putative allergens vicilin and legumin.¹⁰

Instead of avoidance, the development of plants with reduced allergenicity may offer a new therapeutic approach. Genetic engineering can be used to produce plant foods with reduced allergenicity. With gene-silencing technologies, any plant allergen can be targeted and hence a food with reduced allergenicity can be produced.^{11,12} Such approaches are highly effective in eliminating target proteins, but several aspects limit their

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Table 1. Settings of greenhouse experiments. The settings (S) were carried out in cabins of 60 m². Shown are duration of cultivation (D), cultivars and environmental conditions, i.e. daily mean relative humidity (RH), temperature (T), photosynthetic active radiation (PAR) and electrical conductivity (EC) of nutrient solution used

S	D (days)	Cultivar	RH (%)	T (°C)	PAR (mol m ⁻² day ⁻¹)	EC (dS·m ⁻¹)	Treatments
1	75	Counter ^a	61	22.5	20.1		Ammonium, nitrate or organic nitrogen at low or high rates
2	90	Counter	74	22.1	21.6 10.8	2 or 9 2 or 9	Salinity and radiation
3	71	Six different cultivars	78	21.8	20.2	2.8	LYC 457 ^b + LYC 1045 ^b Idyll ^c + Matina ^d Supersweet ^e + Classy ^e

Tomato breeders: ^a De Ruiter (now Monsanto), Bergschenhoek, The Netherlands; ^b Genbank Gaterleben, Gaterleben, Germany; ^c Gartenland Produktion GmbH, Aschersleben, Germany; ^d Hild Samen GmbH (now Nunhems), Marbach, Germany; ^e Syngenta Seeds, Basel, Switzerland.

application. These include the fact that tomato-allergic patients are sensitised towards several proteins in tomatoes. Therefore transgenic lines in which several genes corresponding to allergens are silenced would be needed. Even more important is the fact that major allergens from tomatoes are essential for plant function and cannot be completely silenced.¹² Finally, food products from genetically modified plants may be less acceptable to consumers. An alternative strategy for tomato-allergic patients may be the identification of low-allergenic tomato varieties or conditions for cultivation that reduce the allergenic potential. In this study we examined tomato cultivars with distinct genetic origins and cultivated them in different environmental conditions. To assess their allergenic potential, tomatoes were tested in tomato-allergic patients using the skin allergy test.

MATERIALS AND METHODS

Tomato cultivation

Tomatoes were cultivated in greenhouses of the Leibniz-Institute of Vegetables and Ornamental Crops (IGZ), Großbeeren, Germany using three different settings. An overview of these settings is given in Table 1.

In setting 1, different nitrogen forms were supplied in deficient or excess amounts defined by the demand of a regular plant. Tomatoes were grown in buckets, each filled with 10 L of a commercial horticultural substrate (Terreau Professionel GePAC Einheitserde Typ Null, Einheitserde Werkverband, Sinnatal-Jossa, Germany) and supplied with (in g) Ca(NO₃)₂·4H₂O (10/34), (NH₄)₂SO₄ (5.8/19), Horngrües (14/44), KH₂PO₄ (7), K₂SO₄ (30), K₂O/MgO (25), CaSO₄ (30, with nitrate 0/15) and (in mg) FeEDTA (1.2), MnSO₄ (43), H₃BO₄ (150), MoO₃ (6) CuSO₄ (9.4), ZnSO₄ (200). Tomatoes were irrigated manually once a day until drainage started.

In setting 2, tomato plants were grown in gullies (8 m × 0.2 m × 0.1 m) provided continuously with nutrient solution at low or high electrical conductivity (EC).¹³ At low EC, tomatoes were cultivated without any growth reduction, while at high EC the concentration of all macronutrients was increased in equal proportions to reach saline conditions, resulting in growth reduction. Half of the cabins were shaded with a net to diminish radiation at 50% (Table 1).

In setting 3, six different cultivars were grown in one chamber under the same conditions. The same design and nutrient solution recipe were used as in setting 2. The fruits of all cultivars were

harvested green-unripe and red-ripe. Three cultivars had a mean fruit size of about 80 g (medium) and three of about 20 g (small). In each fruit size group the cultivars were either a landrace, a cultivar bred for use in organic horticulture or a modern hybrid.

Participants

The inclusion criterion for the selection of patients was a positive history of adverse reactions to tomato. All patients (adults, ≥18 years) were recruited from the Allergy-Centre-Charité, Charité – Universitätsmedizin Berlin, Berlin, Germany. The study was approved by the local ethics committee (Charité – Universitätsmedizin Berlin, EC-No. 1832/Si.258) and all patients gave written informed consent before the investigations. The patients' characteristics are summarised in Table 2. Eight aeroallergen-sensitised subjects served as controls and gave written informed consent (Table 3).

Skin allergy test and tomato extract preparation

Skin allergy tests were performed by the prick-to-prick method with native material according to the recommendations of GA²LEN.¹⁴ The different tomato materials were applied in duplicate on the surface of the interior forearms. Histamine dihydrochloride (10 mg mL⁻¹, ALK-Abelló, Wedel, Germany) and saline solution (pH 7.4, ALK-Abelló) served as positive and negative controls respectively. The skin reaction was considered positive when the wheal diameter was ≥3 mm after 15 min in the absence of a reaction in the negative control.

Except for the green-unripe fruits, tomatoes were always harvested when red-ripe, i.e. stage 8–9 of the colour screening scale for tomato.¹⁵ The tomato materials were prepared freshly using a defined amount of fruits shortly before use. They were homogenised twice with a commercial knife mill (Grindomix GM 200, Retsch GmbH, Haan, Germany) for 10 s without creating a rise in temperature.

Specific IgE

IgE specific to tomato was measured with Phadia ImmunoCAP System, (Uppsala, Sweden) according to the manufacturer's instructions.

Statistical analysis

Skin reactivity data were subjected to variance analyses and nonparametric tests using SPSS Version 18.0 (SPSS, Inc., Chicago,

Table 2. Characteristics of patients recruited

Patient	Age (years)	Sex	slgE _{tomato} (kU L ⁻¹)	Symptoms to tomato case history
1	40	M	2.02	GIT
2	41	M	<0.35	OAS
3	36	M	0.86	OAS
4	43	M	<0.35	OAS
5	38	M	<0.35	R
6	18	F	0.83	OAS
7	18	F	ND	OAS
8	36	F	0.96	GIT
9	36	F	1.17	OAS
10	18	F	0.65	OAS
11	49	M	0.51	OAS, R, D
12	31	F	<0.35	OAS
13	45	M	0.89	OAS, U
14	41	F	<0.35	OAS, GIT, E
15	55	F	0.68	OAS, GIT
16	42	F	0.51	OAS, D
17	37	F	1.52	E, D
18	39	M	<0.35	OAS
19	48	F	2.2	OAS, Er
20	59	F	<0.35	OAS, Er, D
21	50	F	0.63	OAS, GIT
22	63	F	0.46	E
23	69	F	<0.35	OAS, GIT
24	27	F	2.83	OAS, E
25	35	F	<0.35	OAS, GIT
26	26	M	<0.35	OAS, R
27	36	F	18.3	OAS, D
28	41	F	3.56	OAS, D
29	56	F	6.87	R, D
30	32	M	0.58	OAS
31	35	F	<0.35	OAS
32	22	F	0.76	OAS, Er, D
33	46	F	<0.35	OAS, GIT
34	20	M	0.83	OAS
35	39	F	0.8	OAS, D
36	43	F	<0.35	OAS, D
37	51	F	<0.35	OAS, D
Total	Median (min–max)	F/M	Median (min–max)	
37	39 (18–69)	26/11	0.85 (0.46–18.30)	

M, male; F, female; ND, not done; GIT, symptoms of gastrointestinal tract; OAS, oral allergy syndrome; R, rhinitis; D, dyspnoea; U, urticaria; E, eczema; Er, erythema.

Table 3. Characteristics of control subjects

Subject	Age (years)	Sex	Symptom/Allergens
C1	42	M	Rhinitis/Grass
C2	33	F	Birch pollen sensitivity
C3	26	M	Rhinitis/Grass
C4	31	F	Perennial rhinitis/Grass, birch, house dust mite
C5	43	M	Rhinitis/Grass
C6	38	F	Rhinitis/Grass, birch
C7	67	F	Rhinitis/Grass, birch
C8	23	F	Rhinitis/Birch
Total	Median (min–max)	F/M	
8	36 (23–43)	5/3	

M, male; F, female.

Table 4A. Skin allergy test results. Numbers of patients with negative (wheal size <3 mm) and positive (wheal size ≥3 mm) reactions. The impact of environmental factors was investigated with cv. Counter. Patients 1–10 (*n* = 10) were tested with cv. Counter fertilised with deficient or excess supply of ammonium (NH₄⁺), nitrate (NO₃⁻) or organic nitrogen. Patients 11–37 (*n* = 27) were tested with cv. Counter cultivated under different light intensities and two salinities (EC2 and EC9, dS m⁻¹)

		Skin allergy test		Total	
		Negative	Positive		
<i>Nitrogen nutrition</i>					
<i>N form</i>	<i>N amount</i>				
NH ₄ ⁺	Deficient	2	8	10	
	Excess	2	8	10	
NO ₃ ⁻	Deficient	1	9	10	
	Excess	1	9	10	
Organic	Deficient	2	8	10	
	Excess	3	7	10	
Sum of N form	NH ₄ ⁺	4	16	20	NS
	NO ₃ ⁻	2	18	20	
	Organic	5	15	20	
Sum of N amount	Deficient	5	25	30	NS
	Excess	6	24	30	
<i>Light intensity</i>					
	Shaded	10	17	27	NS
	Unshaded	10	17	27	NS
<i>Salinity</i>					
	EC2	9	18	27	NS
	EC9	10	17	27	NS

NS, not significant.

IL, USA). Medians were separated by the Mann–Whitney *U* test procedure at *P* = 0.05. The wheal diameters of the skin reactions (oedema) were used when expressing the data in box plots. The patients reacting positively and negatively were compared. These results are summarised in Table 4.

RESULTS AND DISCUSSION

Influence of environmental cultivation conditions

Environmental cultivation conditions during tomato plant growth have a significant influence on several quality characteristics of

the fruits, such as their content of sugars, organic acids and health-related carotenoids.¹⁶ In this study we aimed to investigate the impact of environmental cultivation conditions on the allergenic potential of tomatoes.

The median skin reactivity against tomato plants (setting 1) treated with either deficient or excess supply of nitrogen, ammonium and nitrate differed slightly, but the differences were not statistically significant (Fig. 1). Furthermore, no differences were seen in the overall number of positive and negative skin reactions (Table 4A). The data indicate that none of the applied fertilisation treatments had a clear impact on the allergenic potential of

Table 4B. Skin allergy test results. Numbers of patients with negative (wheal size <3 mm) and positive (wheal size ≥3 mm) reactions. Impact of developmental stage and genotype. Patients 11–37 ($n = 27$) were tested with green-unripe and red-ripe tomatoes from six different genotypes, which were categorised in three tomato origins each comprising one small and one medium fruit size cultivar

			Skin allergy test		Total	
			Negative	Positive		
Genotype (fruit size)		Developmental stage				
Landrace	LYC 457 (small fruits)	Green	14	13	27	
		Red	10	17	27	
	LYC 1045 (medium fruits)	Green	12	15	27	
		Red	11	16	27	
Organic cultivation	Idyll (small fruits)	Green	10	17	27	
		Red	5	22	27	
	Matina (medium fruits)	Green	11	16	27	
		Red	4	23	27	
Modern hybrid	Supersweet (small fruits)	Green	12	15	27	
		Red	5	22	27	
	Classy (medium fruits)	Green	10	17	27	
		Red	5	22	27	
Sum of developmental stage		Green-unripe fruits	69	93	162	$P = 0.001$
		Red-ripe fruits	40	122	162	
Sum of fruit size (red fruits)		Small fruits	20	61	81	NS
		Medium fruits	20	61	81	
Sum of origin (red fruits)		Landrace	21	33	54	$P = 0.016$
		Organic cultivation	9	45	54	
		Modern hybrid	10	44	54	NS

P values are given for differences between tomatoes (green vs red; small vs medium; landrace vs organic cultivation vs modern hybrid). NS, not significant.

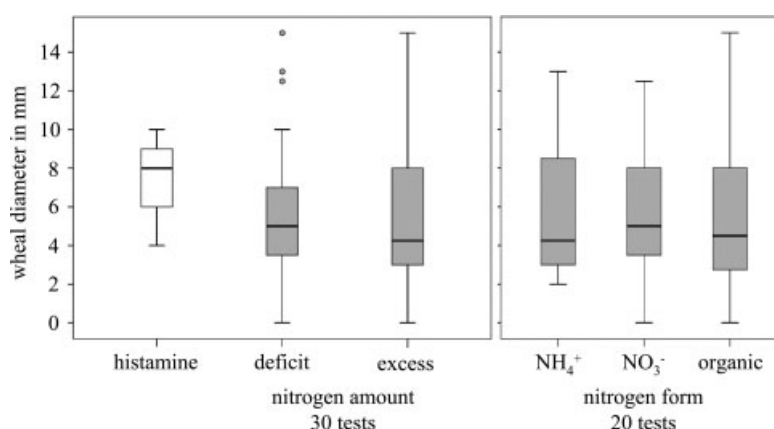


Figure 1. Skin test reactions to tomatoes from plants treated with different forms of nitrogen. Patients ($n = 10$) were tested with fruits from tomato plants (cv. Counter) fertilised with deficient or excess supply of ammonium (NH_4^+), nitrate (NO_3^-) or organic nitrogen. Histamine dihydrochloride (10 mg mL^{-1}) was used as positive control. The median is depicted as a black line and outliers are shown as small circles. No significant differences were detected. Control subjects ($n = 8$) were all negative when differently fertilised tomato materials were used.

tomato fruits. It has to be kept in mind, however, that the number of replications was less compared with the other tests (Table 4A vs 4B). Therefore effects of different nitrogen treatments may have been missed in the present study. Since nitrogen is involved in the biosynthesis of proteins known to be putative allergens, effects upon more severe conditions such as absolute nitrogen deficiency with a higher number of test persons cannot be excluded.

Tomato plants (setting 2) were also exposed to various light intensities and to nutrient solutions with different salinities. Again the degree of skin reactivity in tomato-allergic patients was not significantly affected by the specific tomato cultivation treatments. Neither the number of negative and positive skin reactions (Ta-

ble 4A) nor the wheal diameters (Figs 2A and 2B) differed between light intensity conditions or nutrient solution concentrations.

It has been shown in previous experiments that increased salinity (NaCl), increased nutrient solution concentration or a switch from nitrate to ammonium fertilisation significantly influences taste-related parameters of tomatoes.^{17,18} This effect has been attributed to mild abiotic stress generated by raising the EC and could be measured by the increase in proline concentration.¹⁹ Abiotic stress can induce the expression of particular genes encoding e.g. peroxidases,²⁰ chitinases²¹ or β -1,3-glucanase.²² As mentioned above, these proteins are described as putative allergens in tomato.^{23,24}

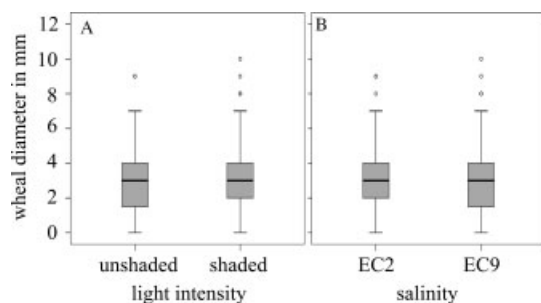


Figure 2. Skin test reactions to tomatoes from plants grown under different conditions. Patients ($n = 27$) were tested with fruits from tomato plants (cv. Counter) cultivated (A) with different light intensities and (B) at two salt concentrations measured as electrical conductivity (EC) of the nutrient solution. The result of the positive control (histamine dihydrochloride) is shown in Fig. 1. The median is depicted as a black line and outliers are shown as small circles. No significant differences were detected. Control subjects ($n = 8$) were all negative when differently treated tomato materials were used.

The conditions applied in the present settings seem not to be sufficient to induce the expression of particular genes in tomato fruits above a certain threshold. However, passing this threshold expression seems to be necessary to provoke a significant response in the skin allergy test. Another possibility might be that the group of patients investigated in this study was not sensitised to these stress-induced genes of tomato fruits. As the environmental cultivation conditions were performed with only one cultivar, it could also be that using other cultivars might lead to significant differences in allergenic potential if grown under different conditions. However, this seems not very likely, because the variation in adaptability to environmental conditions is small among different tomato cultivars as a consequence of inbreeding during domestication.^{25,26}

Impact of developmental stage of tomatoes

To study the impact of the developmental stage of tomatoes on skin reactivity in tomato-allergic patients, green-unripe and red-ripe fruits were harvested from six different genotypes (setting 3). The number of positive skin reactions (wheal size ≥ 3 mm) was increased by 30% with red-ripe fruits compared with green-unripe

fruits (Table 4B). Corresponding to this observation, the wheal diameters caused by red-ripe fruits were significantly larger than those caused by green-unripe fruits (Fig. 3A).

These results might indicate that the amount of allergens increases during fruit ripening. β -Fructofuranosidase (Lyc e 2)²⁷ and polygalacturonase (Lyc e PG)^{28,29} are important tomato allergens and their expression increases with the developmental stage of tomatoes. In contrast, other tomato allergens such as profilin (Lyc e 1)³⁰ and Lyc e 3 (LTP)³¹ are expressed more at earlier stages. A couple of tomato allergens, namely a chitinase (Lyc e Chi)²³ and a glucanase (Lyc e Glu)³² or a peroxidase (Lyc e Pox),³³ are only expressed at a very low level during normal fruit development. Thus the expression of allergens depends on the developmental stage of tomatoes. Based on the expression profiles, it can be speculated that the allergenicity of red-ripe fruits depends on the tomato allergens Lyc e 2 (β -fructofuranosidase) and Lyc e PG (polygalacturonase), while Lyc e 1 (profilin) and Lyc e 3 (LTP) are responsible for allergic reactions to fruits at the early developmental stage. Moreover, proteins expressed during seed development may be also present in red-ripe tomatoes. However, the expression profiles of recently identified allergens such as legumin and vicilin¹⁰ during the ripening process are not known so far.

Influence of genotype

Besides ripening and environmental factors during tomato plant growth, we analysed the influence of genetic background on the allergenic potential of the fruits (setting 3). For this purpose, three groups (landraces, cultivars bred for use in organic horticulture and modern hybrids) with two cultivars each were selected. The two cultivars of each group were distinguished by fruit size to examine the impact of assimilate distribution in the tomato fruits. The skin reactivity of the patients revealed no differences between smaller and larger fruits (Table 4B and Fig. 3B). On the one hand, with a larger fruit size, more assimilate products are distributed among the fruits.³⁴ On the other hand, larger fruits contain more water, a lower dry matter proportion and thus fewer assimilates per gram of fruit compared with small fruits. This might be associated with a lower number of proteins that are active in carbohydrate metabolism. Such proteins are the known allergens Lyc e 1 (β -fructofuranosidase) and Lyc e PG (polygalacturonase) mentioned above. However, the fruit sizes chosen for the present investigation were not important in respect of skin reactivity.

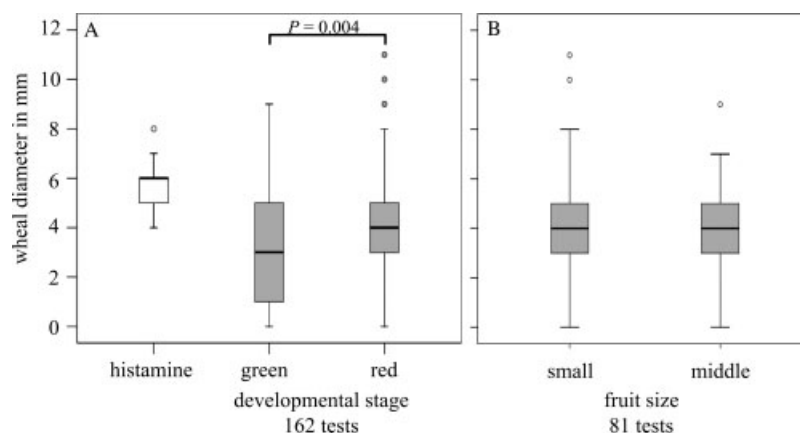


Figure 3. Skin test reactions to tomatoes from different developmental stages and with different fruit size. (A) Green-unripe and red-ripe tomato fruits from six tomato genotypes and (B) cultivars with small and medium size fruits were compared ($n = 27$). Histamine dihydrochloride (10 mg mL^{-1}) was used as positive control. The median is depicted as a black line and outliers are shown as small circles. Differences were significant ($P = 0.004$) between green-unripe and red-ripe tomato fruits. Control subjects ($n = 8$) were all negative when green-unripe and red-ripe tomatoes were tested.

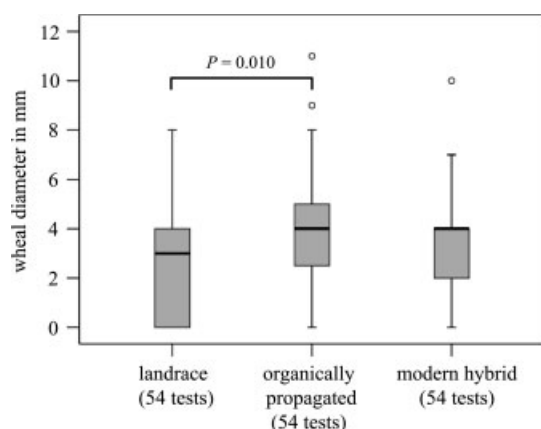


Figure 4. Skin test reactions to tomatoes from cultivars varying in their origin. Patients ($n = 27$) were tested with fruits from landraces (LYC 457 and LYC 1045), from cultivars bred for use in organic horticulture (Idyll and Matina) and from modern hybrid cultivars (Supersweet and Classy). The result of the positive control (histamine dihydrochloride) is shown in Fig. 1. The median is depicted as a black line and outliers are shown as small circles. Differences were significant ($P = 0.010$) between landraces and cultivars bred for use in organic horticulture. Control subjects ($n = 8$) were negative to all tested cultivars.

The overall numbers of negative and positive skin reactions of the six different genotypes among tomato-allergic patients revealed that more patients reacted (wheal diameter ≥ 3 mm) against cultivars bred for use in organic horticulture and modern hybrids in comparison with landraces (Table 4B). Indeed, skin reaction to cultivars bred for use in organic horticulture was significantly stronger than that to landraces (Fig. 4). The molecular basis for the observed differences in biological activity between the tomato genotypes is presently unknown.

It has been recognised previously that cultivars from certain food-producing plants may exert different allergenicities.³⁵ However, reliable scientific experiments to confirm this observation are limited. Bolhaar *et al.*³⁵ investigated a number of apple cultivars that were classified according to their allergenicity by the skin allergy test and confirmed the results for two cultivars by oral provocation. Our results have not been confirmed by oral provocation tests yet, but such studies are considered for future investigations. Nevertheless, the skin allergy test is an elegant tool to screen biological differences in various tomato materials, because it is fast and results are linked to the sensitisation profile of the patients. A limitation of using native extracts is the lack of standardisation. We tried to consider this limitation by preparing composite samples of four fruits for each investigated tomato treatment. The protein proportions of the landrace (cv. Reisetomate) and the cultivar bred for use in organic horticulture (cv. Matina) were quite similar (cv. Reisetomate $418 \pm 96 \mu\text{g mL}^{-1}$, cv. Matina $491 \pm 71 \mu\text{g mL}^{-1}$). With the inclusion of a control group, unspecific skin reactions could be excluded. To avoid protein degradation, the homogenisation procedure was kept short under stable temperature conditions.

CONCLUSION

Our results indicate that various environmental conditions as they typically occur in horticultural practice might not affect the expression of allergens in tomatoes to a large, biologically relevant extent. However, we cannot exclude that the impact of

cultivation conditions may be different on patients' reaction, since our settings exhibited several limitations: (i) the number of tomato-allergic patients was relatively low; (ii) individual skin reactivity varied strongly in the nitrogen fertilisation setting; (iii) only one cultivar was tested. Other conditions such as biotic stress situations caused by infection with pathogens may be relevant and should be considered in future studies. Intriguingly, we demonstrated that the origin of tomato cultivars led to significant differences in skin reactivity. Material from such contrasting genotypes can be used for analysing expression patterns and allelic sequence differences to get a deeper insight into the molecular basis for variations in allergenic reaction. Those genes identified may be used as functional markers in programmes for breeding low-allergenic tomatoes, which would offer a new strategy to retain the quality of life of tomato-allergic subjects.

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