



This is the peer reviewed version of the following article: Jin, Jing, Ling Gao, Lan Zhao, Zhong-shan Gao, Xiong-wei Li, Han-bing Xie, and Jun-bei Ni et al. 2019. "Selection Of Pru P 3 Hypoallergenic Peach And Nectarine Varieties". *Allergy*. Wiley. doi:10.1111/all.14102, which has been published in final form at <https://doi:10.111/all.14102>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions <http://www.wileyauthors.com/self-archiving>.

Document downloaded from:



PROF. ZHONGSHAN GAO (Orcid ID : 0000-0001-6944-9955)

Article type : Letter to the Editor

Selection of Pru p 3 hypoallergenic peach and nectarine varieties

To the Editor,

Peach is an important fruit consumed worldwide. However, it is also one of the most frequently reported allergenic fruits¹. Component diagnosis of peach allergy indicates Pru p 1, Pru p 2, Pru p 3 Pru p 4, Pru p 7 and Pru p 9 are involved^{2,3}. Pru p 3 is the dominant allergen responsible for severe allergic reaction⁴ and it is considered to be the primary sensitizer to other LTPs in Mediterranean and Central Europe⁵.

The levels of Pru p 3 differ between varieties⁶. To date, measurement of Pru p 3 in a limited number of peach and nectarines from Spain, US and Italy has been reported⁷. Significant variation of allergen concentration in processed foods containing peach has also been observed⁸. The content of Pru p 3 of peach/nectarine determines the potential risk for peach allergic patients.

China is the origin of peach with representative genetic diversity to be explored for hypoallergenic varieties⁹. A core collection of 103 varieties cultivated in Jiaxing, Zhejiang Province were selected to represent this diversity, including 23 nectarines and 80 peach varieties (with fruit hair, round or flat, 77 cultivated, three wild) (Supplementary Table 1). The soluble solid content (SSC), ripening date and peach aroma intensity were recorded. Specific methods are detailed in the Supporting Information. Pru p 3 was quantified by ELISA based on our previous research⁶.

Significant differences in Pru p 3 content were identified in individual varieties ($P<0.0001$) (Figure 1A). Most nectarine varieties had low Pru p 3 content with pedigree of ‘Armking’ and

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/ALL.14102](https://doi.org/10.1111/ALL.14102)

This article is protected by copyright. All rights reserved

'Mayfire' (Supplementary Table 1), while a large variation was observed in peach: the lowest ($3.5\mu\text{g/g}$) in a wild peach, and the highest ($64.4\mu\text{g/g}$) in flavorsome yellow flesh peaches. In cultivated peach and nectarine, the level was higher than in wild varieties, and usually higher in peach than nectarine. In addition, fruit harvest month greatly influenced the Pru p 3 content ($P<0.001$). Peach varieties ripening late generally had higher levels than earlier ones: $40.19\mu\text{g/g}$ on average for varieties ripening in August/September, about three times the level of those in May (Figure 1B). Fruit flesh color also reflected the Pru p 3 content ($P=0.0072$, $n=100$). The results showed that hypoallergenic varieties were mainly yellow flesh nectarines and red flesh peaches (Figure 1C, D). Correlation analysis between Pru p 3 and soluble solid content (SSC) and the influence of aroma showed that higher Pru p 3 content related to higher SSC ($P=0.0006$, $r=0.3394$, $n=98$) and stronger aroma ($P=0.0002$) (Figure 1E, F), indicating that good quality peaches had high allergenic potential. The Pru p 3 content of flat peaches, becoming more popular in Mediterranean countries and China, is expected to be high, as demonstrated in Supplementary Table 2. There was 4% to 30% variation between years (2016-2018) for the same variety. The distribution of Pru p 3 in different parts of peach fruit differs greatly: the content in peel was 13 to 60 times higher than in pulp (Supplementary Table 2).

Immunocytochemical observations of Pru p 3 in four varieties with significantly different Pru p 3 content showed striking differences. In low Pru p 3 content 'Hu You 278' (nectarine, $4.02\mu\text{g/g}$) (Figure 1 G1) and peach variety 'Xue Bu Dai' (red flesh, $4.00\mu\text{g/g}$, Figure 1G2), small amounts of Pru p 3 was located in the pericarp layer, and less in the mesocarp. In high Pru p 3 content yellow flesh peach variety 'Mei Jin' ($37.42\mu\text{g/g}$, Figure 1G3) and 'Jin Shuo' ($57.89\mu\text{g/g}$, Figure 1G4), the fluorescent signals were clearly stronger than 'Xue Bu Dai' and 'Hu You 278' in both the pericarp and mesocarp cells. All three peach varieties (with hair) (Figure G2, 3, 4) contained high Pru p 3 in the hair. These results were consistent and data are shown in Supplementary Table 2. The Pru p 3 content in the pulp of a yellow flesh peach cultivar such as 'Jin Shuo', was higher than that in the whole fruit of some nectarines or red flesh peaches. This indicates that, although peeling the fruit is theoretically an effective way to reduce Pru p 3, it is not always practical and does not always alleviate the risk of peach allergic reaction. The correct choice of variety is better.

Skin prick testing (SPT) was performed on nine patients from Shanxi and Zhejiang provinces, recruited on the basis of their clinical history and a positive peach ImmunoCAP. All of them had provided written informed consent and approved by the local ethics committee (authorization No. 2011-R-1, Second Affiliated Hospital, College of Medicine, Zhejiang University), in collaboration with the Third People's Hospital of Datong, Shanxi (authorization No. 2015-001). The identified low Pru p 3 'Hu You 278' (nectarine, 4.02 μ g/g) and high Pru p 3 variety 'Mei Jin' (peach cultivar, 37.42 μ g/g), according to our quantification and immunocytochemical localization, were tested to assess the sensitization of varieties with different Pru p 3 content in allergic individuals. All nine allergic subjects had a positive SPT to fresh peach cv 'Mei Jin', higher than those with nectarine cv Huyou 278, and one patient was negative to nectarine (Table 1). ImmunoCAP results showed that 9/9 were positive to peach, 7/9 positive to Pru p 3 and /or Art v 3, 2/9 positive to Pru p 4 and none of them positive to Pru p 1 (Table 1).

In summary, Pru p 3 content differed considerably depending on the variety, related to fruit type, flesh color and ripening date. Low risk varieties were nectarines and red flesh peach, maturing in May to July and with low or mild fruit quality. This research provides a directive for evaluating potential Pru p 3 levels for patients and clinical doctors. We identified several hypoallergenic nectarines (May Fire, Hu You 278) and three red-flesh peach varieties 'Xue Bu Dai', 'Zi Xue Tao' and 'Wu Yue Xian'. Because of the narrow genetic background of nectarines worldwide from limited founder cultivars such as 'Armking' and 'Mayfire', hypoallergenic nectarines are recommended for further clinical trials.

Legend

Figure 1. Comparison of Pru p 3 content in different variety groups and immunocytochemical localization of Pru p 3 in low and high content varieties.

A: Pru p 3 content in different fruit types. **B:** different harvest month. **C:** flesh color of nectarines. **D:** flesh color of peaches. **E:** Influence of soluble solid content (SSC) and **F:** aroma intensity. **G:** Immunocytochemical localization of Pru p 3 in (1) ‘Hu You 278’, (2)- ‘Xue Bu Dai’, (3) ‘Mei Jin’ and (4) ‘Jing Shuo’. Scale bar = 200 μm , 100x magnification. Difference between groups was assessed by Kruskal-Wallis nonparametric test followed by Dunn’s multiple comparison test (**A, B, D, F**) and Kolmogorov-Smirnov test (**C**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns, not significant. Data expressed as mean \pm SE.

Reference

1. Gao ZS, Yang ZW, Wu SD, et al. Peach allergy in China: a dominant role for mugwort pollen lipid transfer protein as a primary sensitizer. *J Allergy Clin Immunol*, 2013, 131(1), 222-224.
2. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, et al. EAACI Molecular Allergology User's Guide. *Pediatr Allergy Immunol*, 2016, 27 Suppl 23:1-250.
3. Somoza ML, Garrido-Arandia M, Victorio-Puche L, et al. Peach tree pollen and Pru p 9 may induce rhinoconjunctivitis and asthma in children. *Pediatr Allergy Immunol*, 2019, 00:1-4.
4. Ma S, Yin J, and Jiang N. Component-resolved diagnosis of peach allergy and its relationship with prevalent allergenic pollens in China. *J Allergy Clin Immunol*, 2013, 132:764-767.
5. Mothes-Luksch N, Raith M, Stingl G, et al. Pru p 3, a marker allergen for lipid transfer protein sensitization also in Central Europe. *Allergy*, 2017, 72:1415-1418.
6. Gao ZS, Ma YT, Zhou X, et al. Quantification of peach fruit allergen lipid transfer protein by a double monoclonal antibody-based sandwich ELISA. *Food Anal Methods*, 2016, 9(4), 823-830.
7. Ahrazem O, Jimeno L, Lopez-Torrejon G, et al. Assessing allergen levels in peach and nectarine cultivars. *Ann Allergy Asthma Immunol*, 2007, 99(1), 42-47.
8. Duffort OA, Polo F, Lombardero M, et al. Immunoassay To quantify the major peach allergen Pru p 3 in foodstuffs. differential allergen release and stability under physiological conditions. *J Agri Food Chem*, 2002, 50(26), 7738-7741.
9. Li XW, Meng X, Jia HM, et al. Peach genetic resources: diversity, population structure and linkage disequilibrium. *BMC Genetics*, 2013.14(1), 84.

Author's name

Jing Jin^{1*}, Ling Gao^{1*}, Lan Zhao¹, Zhong-shan Gao^{1,2,3}, Xiong-wei Li⁴, Han-bing Xie¹, Jun-bei Ni¹, Ke-xin Gan¹, Shan-dong Wu², Zheng-wen Ye⁴, Jun Luo⁴, Ke Cao⁵, Rui-juan Ma⁶, Miao-jin Chen⁷, Pere Arús⁸, Serge A. Versteeg³, Hui-ying Wang⁹, Meiling Liu¹⁰, Hui-juan Jia¹, Ronald van Ree³

¹ College of Agriculture and Biotechnology, Zhejiang University, 310058, Hangzhou, China

² Allergy Research Center, Zhejiang University, 310058, Hangzhou, China

³ Departments of Experimental Immunology and of Otorhinolaryngology, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands

⁴ Forest & Fruit Tree Institute, Shanghai Academy of Agricultural Sciences, Shanghai, 201403, China

⁵ Zhengzhou Fruit Research Institute, China Academy of Agricultural Sciences, Zhengzhou, China

⁶ Horticultural Institute, Jiangsu Academy of Agricultural Sciences, Zhong-Lin Street 50, Nanjing, 210014, China

⁷ Fenghua Honey Peach Institute, Xikou, Fenghua, Zhejiang Province, 315521, China

⁸ IRTA, Centre de Recerca en Agrigenòmica CSIC-IRTA-UAB-UB, Campus UAB – Edifici CRAG, Cerdanyola del Vallès (Bellaterra), 08193 Barcelona, Spain

⁹ Department of Allergy, the Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, 310013, China.

¹⁰ Department of Allergy, The Third People's Hospital of Datong, Datong, Shanxi, 037008, China

*Jing and Gao contributed equally to this research.

Correspondence: Prof. Zhongshan Gao, E-mail address: gaozhongshan@zju.edu.cn; and Dr. Xiongwei Li, lixiongweisea@163.com

Acknowledgements

This study was funded by the National Natural Science Foundation of China (grant 31272131 and 31372040), Shanghai Science and Technology Committee Rising-Star Program (19QB1404600)

and The Key Project for New Agricultural Cultivar Breeding in Zhejiang Province, China (2016C02052-5). Dr. WANG Lirong, Dr. YU Mingliang, Dr. SHEN Zhijun, Dr. NIU Liang, Dr. WANG Zhiqiang, Mr. MA Zhisheng, Mr. LIU Hangkong, Dr. TIAN Jianbao and Mr. WU Dajun provided some peach accessions. Ms WANG Xuefeng and Ms ZHAO Xiuzhen performed the skin prick test. Dr. ZHANG Xianqi and Ms WANG Tingting provided assistance in the sera collection.

Conflict of Interest

Gao ZS has received grants from National Natural Science Foundation of China (31272131) and the Key Project for New Agricultural Cultivar Breeding in Zhejiang Province (2016C02052-5), Dr. Li XW has received grant from Shanghai Science and Technology Committee Rising-Star Program; Dr. Jia HJ has received grants from National Natural Science Foundation of China (31372040) and the Key Project for New Agricultural Cultivar Breeding in Zhejiang Province (2016C02052-5). The remaining authors declare that they have no relevant conflict of interests.

Authors' contributions

ZSG, RvR and JHJ conceived and planned the study. JJ, GL, LZ, XWL, HBX and KXG collected peach samples and extracted total proteins. JJ, LG, LZ, JBN, SDW and SAV established ELISA method and data analyses. XWL, KC, ZWY, JL, RJM, KC, MJC, PA and HJJ selected the core peach collection. MLL and HYW performed the diagnosis and skin prick tests. JJ, GL, ZSG, XWL and RvR wrote the manuscript. All authors read and approved the final manuscript.

Keywords

Allergen; peach; Pru p 3; hypoallergenic varieties; protein quantification

Table 1. Diagnostic profiles of nine peach allergic patients and skin prick test with low and high Pru p 3 content varieties.

Subject	Code	Age	Gender	Tolerance	Symptoms to peach	SPT wheel diameter (mm)				ImmunoCAP(KUA/L)				
						Mei Jin	Hu You 278	Positive control	Negative control	Peach	Pru p 1	Pru p 3	Pru p 4	Art v 3
1	HZ-7	48	M	Some nectarine	OAS,GI, U	7x8	4x3.5	4x3	0x0	1.32	0.00	1.68	0.00	0.35
2	HZ-22	26	M	None	GI	7x6	5x4	6x4	0x0	29.0	0.00	24.0	0.02	4.23
3	HZ-23	25	F	Nectarine	OAS	5x4	0x0	5x4	0x0	0.37	0.00	0.51	0.00	0.00
4	S-17	28	M	Some nectarine	U, C	5x5	2x2	5x5	0x0	1.49	0.00	0.91	0.00	2.67
5	DT-177-2	9	M	None	AS,U	15x10	4x5	4x5	0x0	16.2	0.00	19.6	4.03	20.0
6	DT-166	34	M	None	U	18x12	8x7	4x5	0x0	4.12	0.00	0.24	0.01	20.5
7	DT-241	17	F	None	U, As	13x12	9x8	5x5	0x0	48.7	0.00	80.4	23.8	73.7
8	DT-397	14	M	Some nectarine	OAS, GI	9x7	6x5	5x4	0x0	0.35	0.00	0.22	0.0	0.00
9	DT-398	36	M	None	U, GI	10x9	7x7	4x5	0x0	5.39	0.00	1.78	0.0	92.4

Symptoms in peach allergy were recorded by patients in a written consent. Symptoms: OAS-oral allergy syndrome; GI: gastro-intestinal tract symptoms including vomiting and abdominal pain; U- Urticaria; AS-Asthma; C-Conjunctivitis; ‘Mei Jin’ is peach, Pru p 3 content is 37.42 μ g/g; ‘Hu You 278’ is nectarine, Pru p 3 content is 4.02 μ g/g. Results of skin testing performed with fresh peach, the size of wheal was expressed as two perpendicular diameters.

This article is protected by copyright. All rights reserved

