biology and life sciences forum

MDPI

Proceeding Paper

Variability of genomic profile of ypr-10 gene in *Citrus sinensis* L. Osbeck

Dagmar Moravčíková [1]* and Jana Žiarovská [2]

[1] Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Plant and Environmental Sciences, Tr. A. Hlinku 2, 949 76, Nitra, Slovak Republic; xmoravcikova@uniag.sk
[2] Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Plant and Environmental Sciences, Tr. A. Hlinku 2, 949 76, Nitra, Slovak Republic; jana.ziarovska@uniag.sk
* Correspondence: jana.ziarovska@uniag.sk

Abstract: Citrus fruits enjoy widespread consumption globally, being among the most popular fruits. They are highly regarded for their nutritional composition, offering a range of beneficial nutrients. However, it's important to acknowledge that they can also elicit allergic reactions in sensitized individuals, which presents a contrasting aspect. Bet v 1 cross reacting allergen is major birch pollen allergen and it the most commonly sensitizing allergen in central Europe. Bet v 1 belongs to the group of PR-10 proteins in the plant kingdom that cause a various allergic reaction. Bet v 1 allergen has a number of isoforms and homologues. These homologues genes are inherited from a common ancestor and subsequent amino acid similarity. It can cause the phenomen cross-reactivity in food allergies. The aim of the study was analyzing the length polymorphism variability of the Bet v 1 homologs in orange varieties by using degenerated and nondegenerated primers. A total of 8 varieties of *Citrus sinensis* L. Osbeck were used in the analysis. BBAP technique (Bet v 1 based amplified polymorphism) was used to detect the length variability of fingerprints of allergen encoding genes of Bet v 1 homologs. Degenerated primer combination and only a one from nondegenerated variant of primers provided fingerprints, that were unique for every individual variety of analyzed oranges. In all other primer variants, from 2 up to the 4 varieties generated the same BBAP profile, what indicate the higher degree of Bet v 1 homologs seguentional conservativity when compared to other fruit species.

Keywords: *Citrus sinensis* L. Osbeck, Bet v 1, BBAP, polymorphism

1. Introduction

Oranges are one of the most grown species and account for more than half of the world's citrus production [1]. *Citrus sinensis* L. Osbeck contains various flavonoids, flavonols, polymethoxy flavonoids, flavanones and coumarins [2]. In addition to its excellent nutritional value, *Citrus sinensis* L. Osbeck is also known for its use in medicine because possesses antiproliferative activity, antibacterial activity, antifungal activity antiparasitic activity, insecticidal activity and many others health benefits [3]. Oranges are divided into sweet oranges, which are divided into: navel oranges, white oranges, and blood oranges. And then we have a group of sour oranges [4].

Plant species have a large number of proteins in common, including allergenic proteins. An increasing number of proteins potentially expressed in all plant species have been identified from decades of molecular biology studies and genome sequencing. Many genes encoding homologous proteins have been found in the genomes. Bet v 1 (major allergen of birch pollen) and its homologues belong to the PR-10 (pathogenesis-related class 10) protein family [5]. Research about Bet v 1 allergen dates back to 1989 [6]. Many plants contain food allergens that are Bet v 1 homologues, suggesting that people allergic to birch pollen often suffer from the PFS (pollen food syndrome) syndrome. when such a

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Published: date

phenomenon occurs we talk about cross-reactivity [7]. Cross-reactivity can be described as the similarity between two allergens, and the more similar they are, the more likely it is that cross-reactivity will occur [8]. Franzese and co-authors reported that the cross-reaction is a consequence of a similar epitope structure of the allergen to which the same antibodies bind [9]. Reactions to plant foods associated with birch pollen (Bet v 1) are considered to be the most common form of food allergy in adults in Central and Northern Europe [10]. Studies show that cross-reactions occur between oranges and other foods such as peanuts [11]. Database AllerBase contains up to 27 isoallergens Bet v 1 [12]. When we refer to isoallergens, we are talking about allergens that are homologous and exhibit shared biochemical characteristics. These shared properties include a similar molecular size, comparable or identical known biological functions, and an amino acid sequence identity of at least 67%. It's important to note that each isoallergen can has multiple highly similar forms (> 90% identity), which are commonly referred to as variants or isoforms [13]. Structurally homologous Bet v1 isoforms may have different properties in terms of allergic sensitization and Th2 polarization. This is probably due to differential susceptibility to proteolytic cleavage [14]. Database also contains cross-reactive allergens which include the allergen Mal d 1, which is the main allergen of *Malus domestica* [15], Api g 1, major allergen of *Apium graveolens* [16] and many others. The knowledge of the Bet v 1 homologues is increasing, but the knowledge of the *ypr10* gene and its potential applicability in different genomic techniques is still limited. However, a certain percentage of homology has been found [17].

Four primer pairs were designed for the development of the BBAP technique for the comparison of triplets for different amino acids (histidine/asparagine/glutamine/lysine). In developing the primers, the authors focused specifically on two amino acid segments. These segments were subjected to BLAST (Basic Local Alignment Search Tool) analysis with fruit species with established genomic sequences. Forward primer was designed for a region of high homology in *Malus domestica* [18]. Degenerate primer has degeneracy situated at positions 12 (S) and 14 (K), meaning that position 12 can be occupied by either guanine or cytosine, and position 14 can be filled with either thymine or guanine [19, 20].

2. Methods

Plant material and DNA isolation

In the study we used 8 varieties of *Citrus sinesis* L. Osbeck (Salustiana, Navelina, Navel Late, Mid Knights, Odmiana, Lane Late, Valencia). Total genomic DNA was isolated by using Thermo scientific GeneJET purification mini kit, according to the manufacturer's protocol. The samples were subjected to a PCR analysis to confirm their functionality. In this analysis, the presence of ITS (Internal Transcribed Spacer) sequences, which are universally present in organisms belonging to the Eukarya domain, were examined. The presence of ITS was verified on 1.5% agarose gel.

BBAP analysis

In our analysis, the BBAP technique was used to detect the homologues of Bet v 1 allergen in *Citrus sinensis* L. Osbeck. Five different reverse primers were used in the PCR analysis according to the methodology of Žiarovská and Urbanová [18]: R1 (5′- aaccacaccatcaccgac - 3′), R2 (5′ – aaccacaccatcaacgac - 3′), R3 (5′ – aaccacaccatgaccgac - 3′), R4 (5′ – aaccacaccatgaacgac - 3′) and one degenerated primer (5′ - ttggtgtggtastkgctg - 3′). One forward primer was used in analysis (5′ – cctggaaccatcaagaag - 3′). The premix itself consisted of 5 ul of Mastermix (2x Elizyme HS Robust Mix), forward primer and reverse primer in 400 nM concentrations, H_2O and 4 ul of DNA. All components were pipetted to a final volume of 10 ul. The following temperature and time regimes were used for thermal cycling on the PCR cyclers: initial denaturation 95°C for 5 minutes, denaturation

95°C for 45 seconds, annealing 54°C for 45 seconds, elongation 72°C for 35 seconds and the last step was final elonfation 72° for 10 minutes. Results from electroforeogram were processed by using GelAnalyzer. Binary matrix was created from fragments distribution in gel, and followed dedrogram by using DendroUPGMA [21]. Jaccard coefficient has been used to compare between sets of variables.

3. Results and discussion

Due to a phenomenon called cross-reaction, allergy to oranges is often associated with pollinosis and sensitization to other plants [22]. Using reverse primer R1, a total of 56 fragments were amplified in all *Citrus sinensis* varieties. These fragments were visualized and evaluated on agarose gels, indicating the presence of approximately 200 bp (base pair) fragments in each variety. When the reverse primer R2 was used, slightly more fragments (58) were produced. Conversely, when the reverse primer R3 was used the smallest number of fragments, only 38 being detected. The reverse primer R4 resulted in the production of 57 fragments. Additionally, the use of the degenerate primer led to the amplification of 48 fragments. By using primers R2, R3, R4 we detected Bet v 1 homologues with size around 388 bp. The main allergen of birch Bet v 1 gained its notoriety thanks to the phenomenon of cross-reaction. It is likely that homologues of Bet v 1 found in several plants cause the so-called cross-reaction phenomenon in humans [10]. The variability of homologues of Bet v 1 with the BBAP technique used was monitored in *Malus domestica* [20], and in cereals, specifically in *Avena Sativa* [23]. In a study conducted by Žiarovská and co - authors did an analysis where allergen Bet v 1 homologues were identified in a range of 30 plant species. These species included *Ficus carica*, *Carica papaya*, *Pyrus communis*, *Punica granatum*, *Vaccinium myrtillus*, *Ananas comosus*, *Citrus reticulata*, *Annona cherimola*, *Castanea sativa*, and *Citrus × limon* [17]. Notably, the study also investigated *Citrus sinensis*, the focus of our own investigation. The presence of Bet v 1 homologues in these diverse plant species suggests a potential role in allergenicity and highlights the relevance of understanding these homologues across various plant taxa. Urbanová 2021 applied the BBAP technique to different vegetable species to see what profiles and how much variability there is between species. The vegetables included *Allium cepa*, *Beta vulgaris*, *Spinacia oleracea*, *Daucus carota* and others. *Apium graveolens* was also analyzed in the same study [24]. Bohle in their study already reported that in these vegetable species the Bet v 1 homologues are present [25]. Several techniques have already been used to detect Bet v 1 homologues in plants such as *Cannabis sativa* [26]. Comparing and searching for conserved stretches of PR genes has also been addressed by Juskyté and her colleagues. In their study comparing the sequences of a putative PR gene among different crops, including *Citrus sinensis* [27].

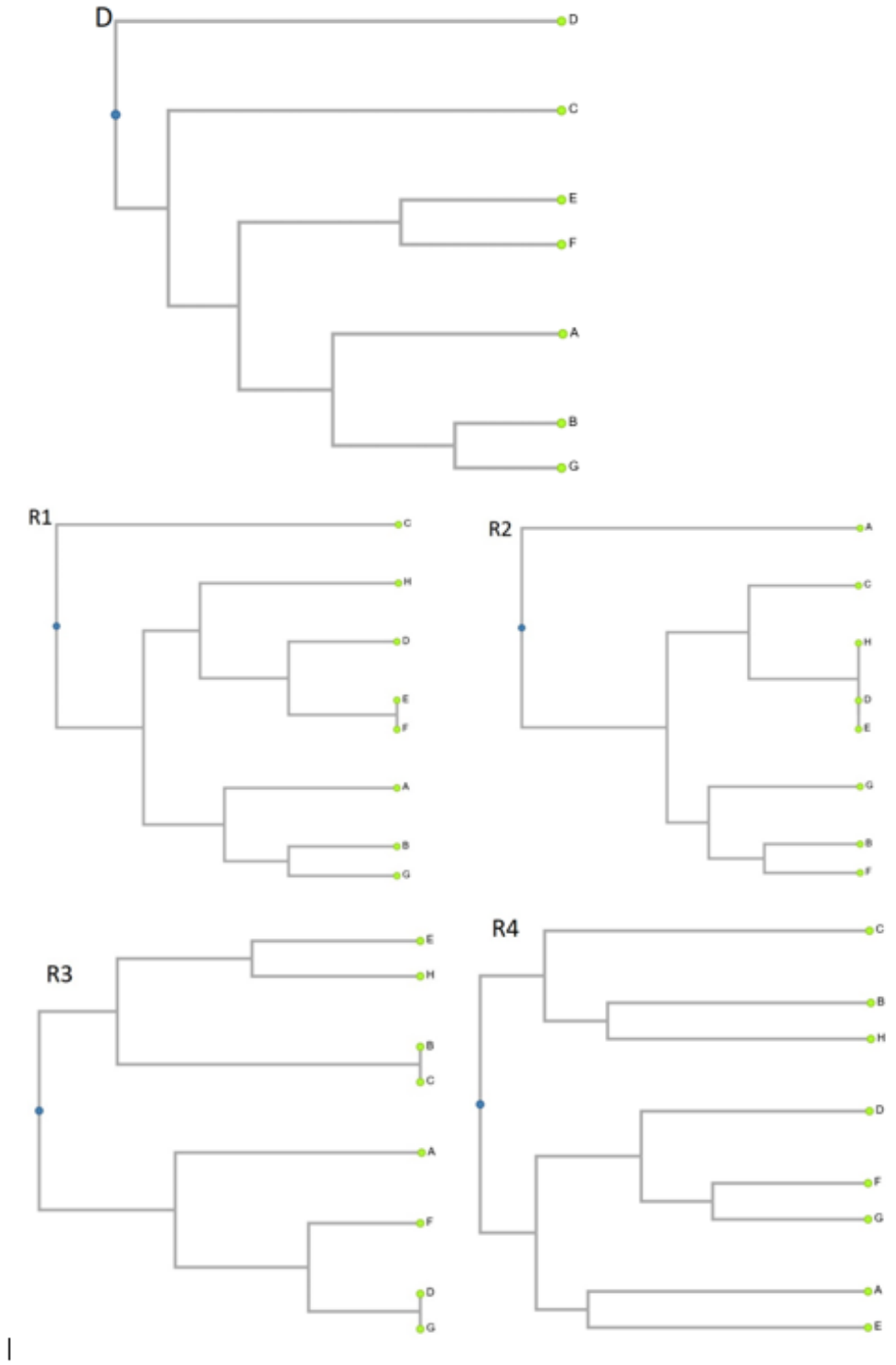

Figure 1. Dissimilarity dendrograms for length polymorphism of Bet v 1 homologues in analysis of different varieties in *Citrus sinenes* L. Osbeck. The letters represent the following varieties of *Citrus sinensis*: A = Salustiana, B = Navelina, C = Navel Late, D = Mid Knights, E = Odmiana, F = Lane Late, G = Navel and H = Valencia.

Cophenetic correlation coefficient when using primer R1 is 0.83, for R2 it is 0.91, for R3 it is 0.94, for R4 it is 0.79 and for D it is 0.86. Genetic distance was from 0.000 (Odmiana and Lane Late) to 0.545 (Odmiana and Navel Late, Lane Late and Navel Late) by using primer R1, from 0.000 (Odmiana and Mid Knights, Valencia and Mid Knights, Valencia

and Odmiana) to 0.556 (Navel and Salustiana) by using primer R2, from 0.000 (Navel and Mid Knights, Navelina and Navel Late) to 0.667 (Salustiana and Valencia, Mid Knights and Valencia) by using primer R3, from 0.222 (Navel and Lane Late) to 0.636 (Navel and Valencia) by using primer R4, and from 0.167 (Navel and Navelina) to 0.889 (Mid Knights and Navel Late) by using primer D. From results of distance matrix and dendrograms we can conclude that when we use primer R1 and R2, the same profile was degenerated between some varieties, but on the contrary, by using primers R3, R4 and D, there were different profiles between each variety.

4. Conclusion

It is likely that in Central Europe, where birch is abundant, homologues of the Bet v1 allergen in plants such as *Citrus sinensis* L. Osbeck may play a role in allergy to *Citrus sinensis*. This analysis provides valuable information about the variability between each variety of *Citrus sinensis* L. Osbeck. The successful application of this BBAP technique to *Citrus sinensis* L. Osbeck varieties suggests that they have a wide range of practical uses and as we have found out from another studies this technique can be applied to different types of vegetables and fruits with consistent results. This universal applicability indicates that the BBAP technique can be utilized across multiple vegetable and fruit species, allowing for efficient and reliable analysis of genetic variability.

Author Contributions: DM and JŽ contributed to the proceeding paper equally

Funding: This publication was supported by the Operational program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAO. 2021. *Citrus*. © **2021**
2. Luo, J.; Yuan, H.; Mao, L.; Wu, J.; Jiang, S.; Yang, Y.; Fu, Y.; Liu, L.; Chen, S.; Wang, W. The Young Fruit of Citrus Aurantium L. or Citrus Sinensis Osbeck as a Natural Health Food: A Deep Insight into the Scientific Evidence of Its Health Benefits. *Arabian Journal of Chemistry* **2023**, 16, 104681, doi:10.1016/j.arabjc.2023.104681.
3. Dongre, P.; Doifode, C.; Choudhary, S.; Sharma, N. Botanical Description, Chemical Composition, Traditional Uses and Pharmacology of Citrus Sinensis: An Updated Review. *Pharmacological Research - Modern Chinese Medicine* **2023**, 8, 100272, doi:10.1016/j.prmcm.2023.100272.
4. Calabrese, F. Origin and history. In *Citrus*, Dugo, G.; Giacomo, A. D. Publisher: The Taylor & Francis **2002**, 642, ISBN 0-203-21661-X.
5. Alessandri, C.; Ferrara, R.; Bernardi, M.L.; Zennaro, D.; Tuppo, L.; Giangrieco, I.; Tamburrini, M.; Mari, A.; Ciardiello, M.A. Diagnosing Allergic Sensitizations in the Third Millennium: Why Clinicians Should Know Allergen Molecule Structures. *Clinical Translation Allergy* **2017**, 7, 21, doi:10.1186/s13601-017-0158-7.
6. Breiteneder, H.; Kraft, D.. The History and Science of the Major Birch Pollen Allergen Bet v 1. *Biomolecules* **2023**, 13.7: 1151. https://doi.org/10.3390/biom13071151
7. Biedermann, T.; Winther, L.; Till, S.J.; Panzner, P.; Knulst, A.; Valovirta, E. Birch Pollen Allergy in Europe. *Allergy* **2019**, 74, 1237–1248, doi:10.1111/all.13758
8. Aalberse, R. C. Assessment of allergen cross-reactivity. *Clinical and Molecular Allergy* **2007**, 5, 1-6. https://doi.org/10.1186/1476-7961-5-2
9. Franzese, Ch. B; Damask, C. C.; Wise, S. K.; Ryan, M. W. *Handbook of Otolaryngic Allergy.* Publisher: Thieme Medical Publisher, Incorporated **2019** 306. ISBN 9781626239074.
10. Kleine-Tebbe, J.; Ballmer-Weber, B. K.; Breitneder, H.; Vieths, S. Bet v 1 and its Homologs: Triggers of Tree-Pollen Allergy and Birch Pollen-Associated Cross-Reactions. In *Molecular Allergy Diagnostics,* Klene-Tebbe, J., Jacob, T. Publisher: Cham : Springer, **2017**, 21-42, ISBN 978-3-319-42499-6.

11. Glaspole, I.N.; de Leon, M.P.; Rolland, J.M.; O'Hehir, R.E. Anaphylaxis to Lemon Soap: Citrus Seed and Peanut Allergen Cross-Reactivity. *Annals of Allergy, Asthma & Immunology* **2007**, 98, 286–289, doi:10.1016/S1081-1206(10)60720-X.
12. Kadam, K.; Karbhal, R.; Jayaraman, V.K.; Sawant, S.; Kulkarni-Kale, U. *AllerBase*: A Comprehensive Allergen Knowledgebase. Database **2017**, bax066, doi:10.1093/database/bax066.
13. Pomés, A.; Davies, J.M.; Gadermaier, G.; Hilger, C.; Holzhauser, T.; Lidholm, J.; Lopata, A.L.; Mueller, G.A.; Nandy, A.; Radauer, C.; et al. WHO/IUIS Allergen Nomenclature: Providing a Common Language. *Molecular Immunology* **2018**, 100, 3–13, doi:10.1016/j.molimm.2018.03.003.
14. Grutsch, S.; Fuchs, J. E.; Ahammer, L.; Kamenik, A. S.; Liedl, K. R.; Tollinger, M. Conformational Flexibility Differentiates Naturally Occurring Bet v 1 Isoforms. In *International Journal of Molecular* **2017**, 18, ISSN 1422-0067.
15. Fahlbusch, B.; Rudeschko, O.; Müller, W.D.; Schlenvoigt, G.; Vettermann, S.; Jäger, L. Purification and Characterization of the Major Allergen from Apple and Its Allergenic Cross-Reactivity with Bet v 1. *International Archives of Allergy and Immunology* **1995**, 108, 119–126, doi:10.1159/000237128.
16. Gepp, B.; Lengger, N.; Bublin, M.; Hemmer, W.; Breiteneder, H.; Radauer, C. Chimeras of Bet v 1 and Api g 1 Reveal Heterogeneous IgE Responses in Patients with Birch Pollen Allergy. *Journal Allergy Clinnical Immunology* **2014**, 134, 188–194, doi:10.1016/j.jaci.2013.12.1073.
17. Žiarovská, J.; Zeleňáková, L. Application of genomic data for PCR Screening of BET v 1 conserved sequence in clinically relevant plant species. *Systems Biology, IntechOpen*, **2018**
18. Žiarovská, J.; Urbanová, L. Utilization of Bet v 1 homologs based amplified profile (BBAP) variability in allergenic plants fingerprinting. *Biologia*, **2022**, 77.2: 517-523.
19. Fu, L.; Cherayil. B.; Shi, H.; Wang, Y.; Zhu, Y. Species and structure of food allergens: epitopes and cross-reactivity. In: *Food Allergy: From Molecular Mechanisms to Control Strategies*, Fu, L.; Cherayil. B.; Shi, H.; Wang, Y.; Zhu. Publisher: Springer, Singapore, **2019**, 13–39, ISBN 978-981-13-6927-8
20. Speváková, I.; Urbanová, L.; Kyseľ, M.; Bilcikova, J.; Farkasová, S.; Ziarovska, J. BBAP Amplification Profiles of Apple Varieties. *Science, Technology and Innovation* **2021**, 13, 1–6, doi:10.5604/01.3001.0015.5233.
21. Garcia-Vallvé, S.; Palau, J.; Romeu, A. Horizontal Gene Transfer in Glycosyl Hydrolases Inferred from Codon Usage in Escherichia Coli and Bacillus Subtilis. *Molecular Biology and Evolution* **1999**, 16, 1125–1134, doi:10.1093/oxfordjournals.molbev.a026203. http://genomes.urv.cat/UPGMA/
22. Iorio, R.A.; Del Duca, S.; Calamelli, E.; Pula, C.; Lodolini, M.; Scamardella, F.; Pession, A.; Ricci, G. Citrus Allergy from Pollen to Clinical Symptoms. *PLoS One* **2013**, 8, e53680, doi:10.1371/journal.pone.0053680.
23. Farkasová, S.; Droppa, M.; Žiarovská, J. variability of amplified profiles generated by BBAP in avena sativa l. *Journal of microbiology, biotechnology and food sciences 2023*, 12, 5, e9545, https://doi.org/10.55251/jmbfs.9545
24. Urbanová, L., Žiarovská, J. Variability of DNA based amplicon profiles generated by Bet v 1 homologous among different vegetable species. 1, *Acta fytotechnica et zootechnica* **2021**. https://doi.org/10.15414/afz.2021.24.mi-apa.1-6
25. Bohle, B.; Radakovics, A.; Jahn-Schmid, B.; Hoffmann-Sommergruber, K.; Fischer, G.F.; Ebner, C. Bet v 1, the Major Birch Pollen Allergen, Initiates Sensitization to Api g 1, the Major Allergen in Celery: Evidence at the T Cell Level. *European Journal of Immunology* **2003**, 33, 3303–3310, doi:10.1002/eji.200324321.
26. Ebo, D.G.; Decuyper, I.I.; Rihs, H. P.; Mertens, C.; Van Gasse, A.L.; Van der Poorten, M. L.M.; De Puysseleyr, L.; Faber, M.A.; Hagendorens, M. M.; Bridts, C. H.; et al. IgE-Binding and Mast Cell–Activating Capacity of the Homologue of the Major Birch Pollen Allergen and Profilin from Cannabis Sativa. *The Journal of Allergy and Clinical Immunology* **2021**, 9, 2509-2512.e3, doi:10.1016/j.jaip.2021.02.012.
27. Juškytė, A.D.; Mažeikienė, I.; Stanys, V. Putative Genes of Pathogenesis-Related Proteins and Coronatine-Insensitive Protein 1 in Ribes Spp. *Plants* **2022**, 11, doi:10.3390/plants11030355.