ACTINIDIA ARGUTA PLOIDY LEVEL VARIATION IN RELATION TO *PSEUDOMONAS SYRINGAE* PV. *ACTINIDIAE* SUSCEPTIBILITY

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Abstract

Ploidy level variation is a very common phenomena in *Actinidia sp.* and can change the genetic contribution of an individual to the next generation's gene pool. Over the years the importance of polyploidy is well documented, studies have provided interesting insights into genomic consequences of polyploidy. Emerging evidence of duplication through polyploidization in model plant has stimulated the research on the relationship between early polyploidization events, the success of the polyploidy and the long-term fate of new species. But we still know very little about the mechanisms responsible for establishing and evolutionary success of polyploid lines. One possibility is that polyploid lines are less susceptible to the pathogens than diploid progenitor species. Focusing on the potential consequences of polyploids *Actinidia arguta* and *Pseudomonas syringae* pv. *actinidiae* susceptibility, first we established the ploidy level for the *Actinidia arguta* collection, and then we artificially inoculated the bacterial pathogen in order to evaluate whether ploidy level influences host pathogen interaction obtaining useful results.

Key words: polyploidy; interspecific hybrid; flow cytometry; kiwifruit bacterial canker; host-pathogen interaction.

Counting chromosomes in *Actinidia* species and their hybrids is technically difficult due to the high numbers of chromosomes involved and their small size. Chromosome numbers have been reported for only a few species but the data support a polyploid sequence 2n = 2x, 2n = 4x, 2n = 6x, 2n = 8x with x= 29 (Watanabe *et al*., 1990; Yan *et al*., 1994).

Many variations in ploidy level have been generated by polyploidization within and across species (Otto and Whitton, 2000; Greilhuber, 2005).

Intraspecific and interspecific ploidy level variation is associated with a suite of connections between polyploidy and biological phenomena (Leitch, 2008; Bierzychudek, 1985), and has the potential to influence fitness-related traits (Weider, 1993; Kearney *et al*., 2005).

In plants there are many advantages mentioned. It is the observation of hybrid vigor, or heterosis, whereby the polyploidy offspring of two diploid progenitors is more vigorous and healthy than either of the two diploid parents (Woodhouse *et al*., 2009).

This observation can have several possible explanations, like: maintaining heterozygosity throughout generations; gene redundancy, new polyploid offspring created have twice as many copies of any particular gene and the ability to diversify gene function over time.

In other words, extra copies of genes that are not required for normal organism function might end up being used in new and entirely different ways, leading to new opportunities in evolutionary selection (Adams, Wendel, 2005). Although data are scarce (Stover, 1986; Nuismer and Thompson, 2001), theory suggests ploidy level can deeply influence dynamics and host-pathogen interaction (Nuismer and Otto, 2004; Oswald and Nuismer, 2007).

Despite the well-documented historical importance of polyploidy, the mechanisms responsible for the establishment and evolutionary success of novel polyploid lineages remain unresolved.

One possibility is that novel polyploid lineages are initially less susceptible to pathogens than the diploid progenitor species (Oswald, Nuismer, 2007).

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Here we discuss whether ploidy level of selected hardy kiwifruit (*Actinidia arguta*) could mediate the susceptibility to *Pseudomonas syringae* pv. *actinidiae* (Psa), the agent of kiwifruit bacterial canker (Renzi *et al.,* 2012).

MATERIAL AND METHOD

Plant material

Ploidy levels of nine *Actinidia* accessions representing five of *A. arguta (*baby kiwi), with green and red pulp, one interspecific hybrid, currently available in Italy, two varieties with green pulp *A. deliciosa* 'Hayward*'* and one *A. cinensis* 'Soreli*'* were determinate by flow cytometry. 'Soreli' is the last variety introduced in yellow fruit and is reaching high production and significant growth expectations (Testolin, Cipriani, 2010).

The plant material included a collection of plants like: *Actinidia arguta*, *Actinidia deliciosa*, *Actinidia chinensis* and one interspecific crosses of *Actinidia;* the total of 130 plants were kept in a growth chamber.

Ploidy level content determined by flow cytometry

For ploidy level we have evaluated six seletions of *Actinidia arguta*, two of *A. deliciosa* 'Hayward*'*, one of *A. chinensis* 'Soreli*'* and one interspecific hybrid, *A. arguta x A. deliciosa,* using flow cytometry (*tab. 1*).

Table 1

Kiwifruit genotypes list used in experiment (*Actinidia* spp.)

Species	Genotype	No. plants
Actinidia arguta	R8P23	10
Actinidia arguta	R10P10	10
Actinidia arguta	R9P18	10
Actinidia arguta	R8P1	10
Actinidia arguta	R9P16	10
Actinidia arguta x deliciosa	P1	10
Actinidia deliciosa'Hayward'	Hk	10
Actinidia deliciosa'Hayward'	H8	30
Actinidia chinensis'Soreli'	S	30
Total		**130**

Flow cytometric analysis was performed for each of the *Actinidia* accessions by collecting leaf samples (3-5 per each), young fully expanded leaves collected from the shoot tips; to maintain an adequate moister and to ensure a correct conservation of tissues, the leaves were placed in plastic bags in a refrigerator at 4ºC.

Using the method of Galbraith *et al*. (1983), 50 mg of *Actinidia* leaf tissue without midribs was placed in plastic Petri dishes chopped with a razor blade adding 0.5 ml of ice-cooled homogenization (Otto I buffer) to homogenize the tissues and release the nuclei, and was kept on ice for 5 minutes.

After filtration through 42-µm nylon mesh, the nuclear suspension were treated with 2.5 ml of a staining solution containing 10 ml Tris, 50 mM sodium citrate, 2 mM MgCl2, 1% (w/v) PVP 30, 0.1% (v/v) Triton X-100 and 1 mg/ml propidium iodide (PI), pH 7.5, letting the samples stand 5 min prior to flow cytometric analysis. Samples were run on a Coulter Epics XL-MCL flow cytometer EXPO 32 ADC; for each accession was gated between 5000 - 10000 events of a sample.

Relative fluorescence intensity was assessed using FL2, a green light source detector (excitation 488 nm). Data were interpreted using WinMDI 2.9 software. *Pisum sativum cv. Ctirad* was used as an internal standard. The measurements were triplicate by using three leaf samples collected from each plant.

Artificial inoculation of bacteria

A known strain of *Pseudomonas syringae pv. actinidiae*, CFBP7285, was used for the artificial inoculation of the *Actinidia* selections. The strain was cultured on nutrient agar, supplemented with 5% sucrose, and incubated at 25–27ºC. Inoculation of pathogen was made by spraying the plantlets with bacterial inoculum at a concentration of 10^8 cfu/ml and the parameters inside the growth chamber were monitored along all experiments.

The *Actinidia* plantlets were placed in a growth chamber with controlled parameters; temperatures were maintained at 25°C for 16 h (light) and at 15°C for 8 h (darkness); relative humidity (RH) was maintained at 60 to 70%.

The plants were approximately 20 cm high at the moment of their artificial bacterial infection. There were 44 plantlets of different varieties of *Actinidia arguta*: 40 plantlets with two different varieties of *Actinidia deliciosa* 'Hayward*'*; 28 plantlets of *Actinidia chinensis* 'Soreli*'* and 8 plantlets of interspecific hybrid*, A. arguta x A. deliciosa*.

From the total number of plantlets utilized, half of them were considered as positive control (sprayed without injury by bacterial suspension) and half of the plantlets were inoculated after procuring slight injury (to simulate natural rubbing) on leaves. In addition, 20 plantlets for each variety/hybrid were inoculated by the same methodologies but, as negative control only with a sterile bacteriological saline (0.85% NaCl).

During the experiments (4 h before and 4 h after each inoculation), the plants were maintained at 95% RH level. In order to evaluate whether polyploids should, in general, be less susceptible to *Pseudomonas syringae* pv. *actinidiae* than their diploid progenitors, after artificial inoculation, all symptoms have been daily monitored.

The identification of re-isolated bacteria analysis were made by appropriate polymerase chain reaction - PCR (Balestra *et al*., 2013).

Repetitive-sequence PCR

To identify Psa bacterial cells from symptoms recorded on kiwifruit plants of *Actinidia* spp. we have collected leaf samples from all plantlets, in order to run the polymerase chain reaction (PCR) analysis. From each strain there was extracted 30 ng genomic DNA.

Briefly, a loopful of pure bacterial culture was suspended into Eppendorf tubes containing sterile water and mixed with a vortex. Subsequently, the tubes were centrifuged for 3 min at 8 000 rot/min. and heated at 95ºC for 10 min, for denaturation. The pallet was prepared with qPCR Master Mix and after a centrifugation of 3 sec at 8 000 rot/min., the supernatant was used and thermal cycling was carried out in a Bio-Rad MJ Mini thermal cycler.

Sequence typing (ST) was performed like rep-PCR in order to reveal the relationships among Psa strains. DNA was extracted using the lysis method described above. Gene fragments were amplified and sequenced with specific primers (Koh and Nou, 2002, Rees-George *et al*., 2010).

All PCR reactions were performed in a Bio-Rad MJ Mini thermal cycler. PCR amplification was performed and products of PCR were separated by gel electrophoresis and capillary gel electrophoresis using the QIAexcel DNA fast analysis, system that uses capillary gel electrophoresis; using the AM 320 method.

RESULTS

Ploidy level content

The ploidy levels of *Actinidia* plants were distinguished by flow cytometry. Among *Actinidia spp.* tested, we found: *Actinidia arguta* (2n=2x) R9P18, R8P23 as diploid plants; R10P10, R8P1, R9P16 as tetraploid plants *Actinidia arguta* (2n=4x); for *Actinidia deliciosa* 'Hayward' (2n=6x) HK, H8 as hexaploid; for *Actinidia chinensis* 'Soreli' (2n=4x) tetraploid; for interspecific crosses *A. arguta x A. deliciosa* (2n=2x) P1 as diploid (*table 2*).

Table 2

Ploidy level estimation of kiwifruit genotypes (*Actinidia spp.*)

Species	Genotype	Ploidy level	No. chromosomes
Actinidia arguta	R8P23	2x	58
Actinidia arguta	R10P10	4x	116
Actinidia arguta	R9P18	2x	58
Actinidia arguta	R8P1	4x	116
Actinidia arguta	R9P16	4x	116
Actinidia arguta x deliciosa	P1	2x	58
Actinidia deliciosa'Hayward'	Hk	6x	174
Actinidia deliciosa'Hayward'	H8	6x	174
Actinidia chinensis'Soreli'	S	4x	116

The results of an analysis are described as a mono-parametric (fluorescence intensity) histograms peaks, each of them representing the fluorescence intensity of a population of nuclei. Their intensity (position of the histogram) is proportional to the amount of nuclear DNA (*fig.1*).

The comparison between the position of the sample's peak and the internal standard's peak gives a ratio of relatives intensities.

The ploidy levels are calculated by comparing the experimental values with the value obtained from *Pisum sativum* sample having a known ploidy level.

As a result, ploidy analysis in which the DNA contents of *Arguta spp.* collection, nuclei were compared to those from a specie having known ploidy (*Pisum sativum 2n=2x=14*).

This comparison can be made either between the two analyses performed under identical conditions or, in our case, through mixing the two samples for simultaneous measurement. (A, *A. arguta* R8P23 ; B, *A. chinensis 'Soreli'*; C, *A. arguta* R10P10, D, *A. deliciosa var. deliciosa cv.* Hayward K;

Isolation of bacteria *Pseudomonas syringae* pv. *actinidiae* (Psa)

Psa re-isolations from diseased leaves of *A. arguta, A. chinensis* and *A. deliciosa* were carried out and experiments of artificial inoculation of *Pseudomonas syringae pv. actinidiae* have shown different levels of susceptibility to Psa. Psa symptoms (leaf spots) appeared within 7 days for *A. arguta* and the interspecific hibrid after artificial inoculation of the bacteria (*fig. 1*). While for *Actinidia deliciosa* 'Hayward' *and* for *Actinidia chinensis* 'Soreli' Psa symptoms (leaf spots) appeared within 21 days (*fig. 2*).

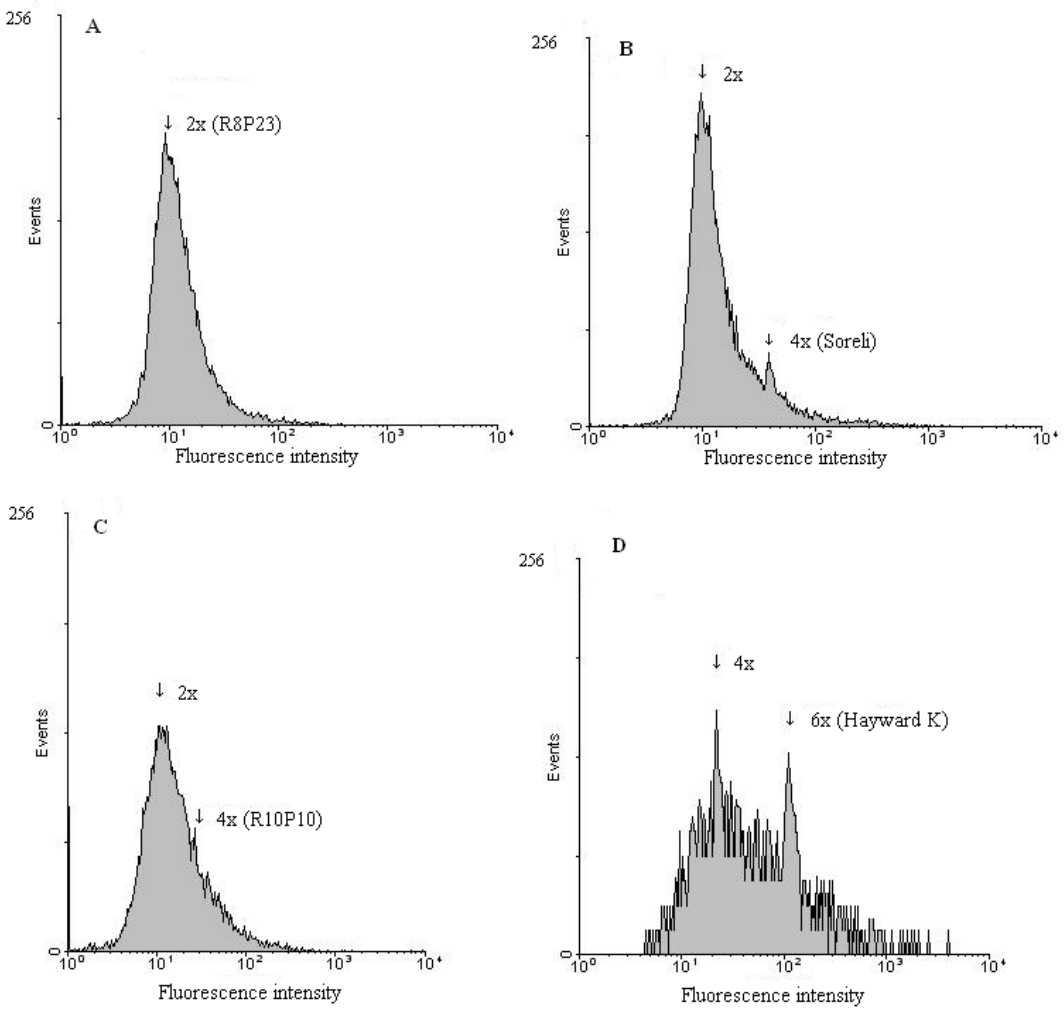

Figure 1. **Frequency distribution histograms of fluorescence intensity stained with propidium iodide (PI)**

On *A. arguta* plants Psa caused the wilting of some leaves within 21 days after the artificial inoculation. When it was inoculated by injury onto *A. chinensis* plants, it caused typical bacterial leaf spots. By daily observations the symptoms on leaves of different *Actinidia* species, were registered at different time. For the diploid individuals of *Actinidia arguta* we observed a higher susceptibility to *Pseudomonas syringae pv. actinidiae*, as well as for the diploid interspecific hybrid. Instead, tetraploid individuals of *Actinidia arguta* showed less susceptibility to Psa, similar to that one of the hexaploids *Actinidia deliciosa* 'Hayward'. After one week, there were recorded the first Psa symptoms on *A. arguta* (R8P23, R9P18), and the hybrid (P1) which are diploids, with an higher number of spots. On the other *A. arguta* selections the symptoms appear clearly after three weeks, like in the case of *A. chinensis* (tetraploid), and of *A. deliciosa* (Hk, H8) hexaploid, and the number of spots were clearly lower (*table 3, 4*).

The number of spots (symptoms) resulted higher on *A. arguta* (R8P23, R9P18), and the hibrid (P1) which are diploids, showing their higher level of susceptibility to Psa. A different level of disease incidence was recorded between the plants leaves inoculated with or without injury. It shows to have deep more damaging effect in case of plants with injury (*figs. 4-5*).

Repetitive-sequence PCR

The identity of bacterial strains re-isolated from symptoms caused by artificial inoculation was obtained by multiplex PCR applied (*fig. 6*). The Psa isolates obtained from *A. arguta, A. chinensis and A. deliciosa* isolated showed the same pattern profile upon PCR performed by the original Psa strain CFBP 7285 artificially inoculated. The bacteria re-isolates resulted identical to those originally isolated from *Actinidia chinensis* host plants affected by kiwifruit bacterial canker symptoms (*table 5*).

DISCUSSIONS

To determine ploidy, the number of basic chromosome sets in cell nuclei, using chromosome counting in dividing cells is an unambiguous way and is time consuming, that is way a high-throughput solution is to use flow cytometry, making possible a rapid and reliable ploidy estimation. Ploidy is predicted to influence adaptation directly, yet whether disease resistance or susceptibility to pathogens behave the same in different ploidy backgrounds has not been well studied. It has often been assumed theoretically that

aside from dominance, selective parameters do not differ between cells of varying ploidy. The dynamics of evolution are expected to vary between populations that differ in ploidy (Gerstein, 2012). Being a polyploid organism, it offers several advantages. One advantage of having more than one set of chromosomes is that there is a "backup". If a gene on one chromosome is damaged or missing, there are another copies of it in other chromosomes. Moreover, it adds to the genetic diversity of an organism's offspring. More chromosomes mean more genes, which means a bigger gene pool. Having a bigger gene pool means that offspring will be more diverse and that the overall chances that an organism's offspring will survive/will increase. Recent studies show that polyploidy could directly influence immune response to a pathogens' attack. The addition of a new genome may increase allelic diversity.

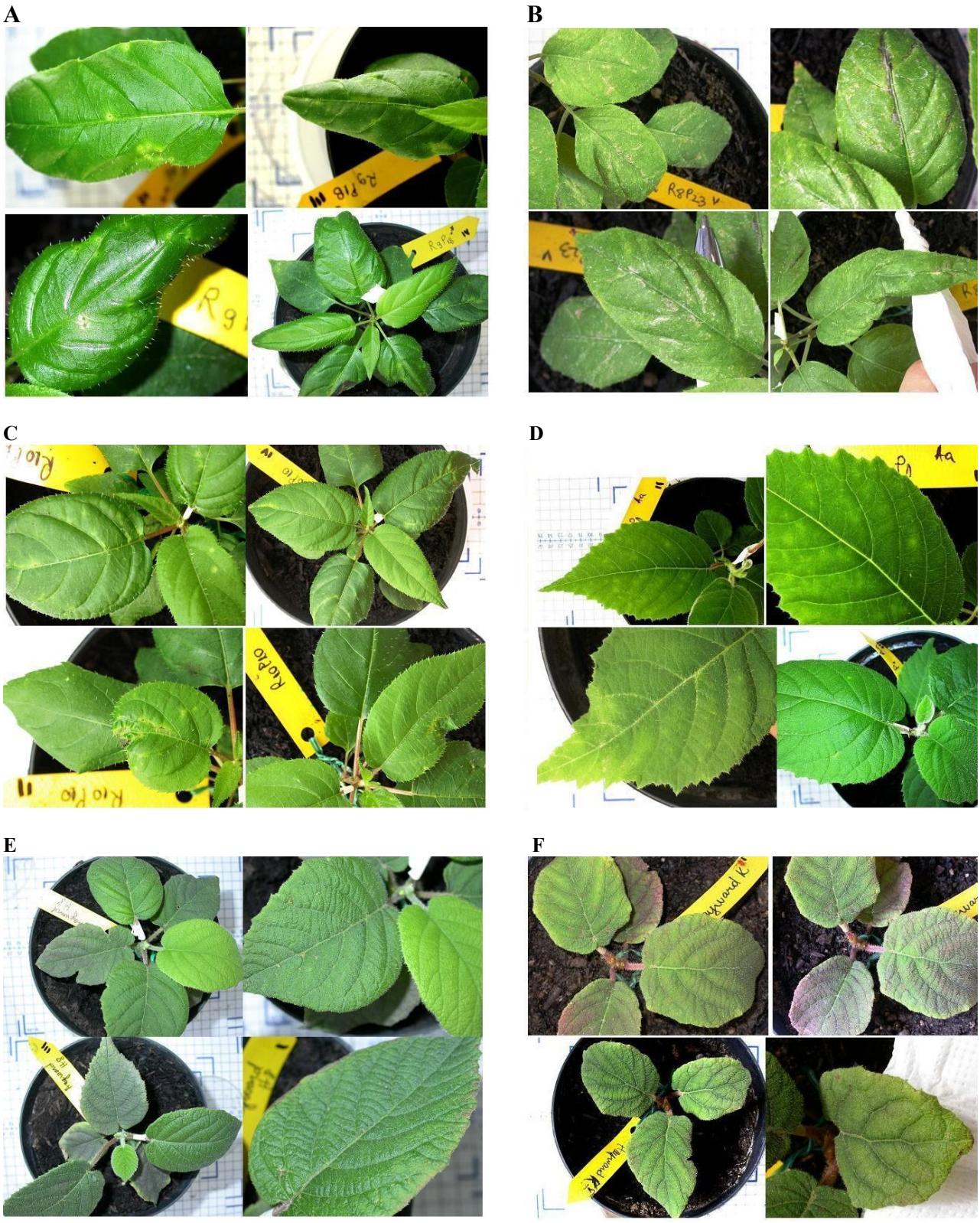

Figure 2. ***Actinidia spp.*** **leaves, as they appear at 7 days after artificial inoculation of bacteria** ***Pseudomonas syringae pv. actinidiae*** **(A:R9P18, B:R8P23, C:R10P10, D:P1, E:Hayward K, F:Hayward 8)**

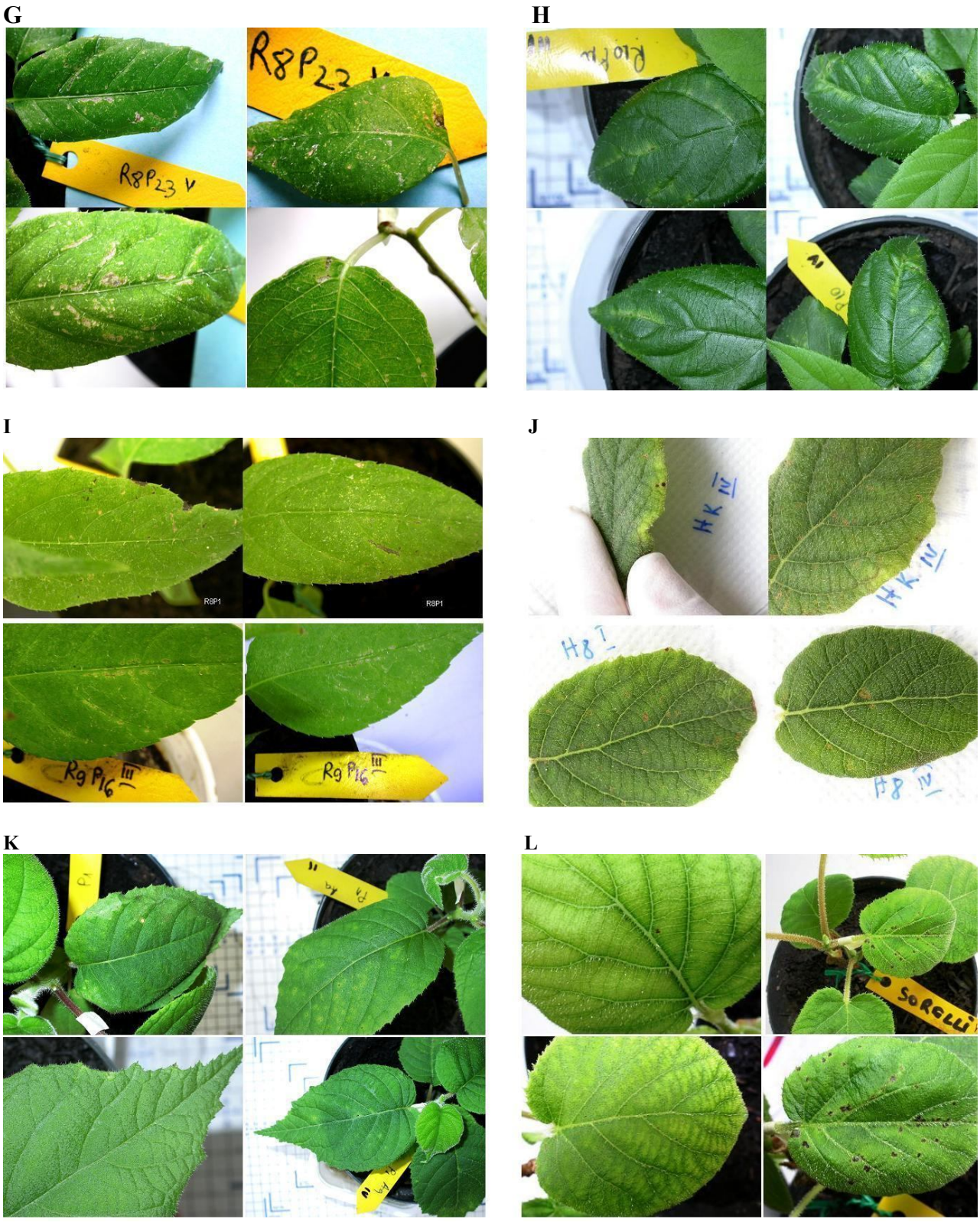

Figure 3. ***Actinidia spp.*** **Leaves at 21 days after artificial inoculation of bacteria *Pseudomonas syringae pv. actinidiae* (G: R8P23, H:R10P10, I: R8P1& R9P16, J: Hayward k& Hayward 8, K:P1, L:Soreli)**

Table 3

Number of spots caused by *Pseudomonas syringae pv. actinidiae* Strain CFBP 7285 on *Actinidia* collection for plants without injury

Lines without injury (spraying)	Average foliar surface	Total no. of spots per leaf (7 dd. after inoculation)	Total no. of spots per leaf (14 dd. after inoculation)	Total no. of spots per leaf (21 dd. after inoculation)	No. of spots/cm²	No. of spots/cm²	No. of spots/cm²
R10P10	15.04	1.00	2.11	2.11	0.07	0.14	0.14
R9P18	15.85	1.27	5.14	5.14	0.08	0.32	0.32
R8P1	14.21	0.14	0.22	0.44	0.01	0.02	0.03
R9P16	13.97	0.00	0.41	0.41	0.00	0.03	0.03
R8P23	13.46	0.45	0.58	1.05	0.03	0.04	0.08
P1	19.54	0.29	11.13	11.13	0.01	0.57	0.57
Hayward K	15.8	0.00	0.50	0.88	0.00	0.03	0.06
Hayward 8	16.9	0.00	0.00	0.34	0.00	0.00	0.02
Soreli	21.5	0.84	2.24	2.53	0.04	0.10	0.12

Table 4

Number of spots caused by *Pseudomonas syringae pv. actinidiae* Strain CFBP 7285 on *Actinidia* collection for plants with injury

Lines with injury	Average foliar surface	Total no. of spots per leaf (7 dd. after inoculation)	Total no. of spots per leaf (14 dd. after inoculation)	Total no. of spots per leaf (21 dd. after inoculation)	No. of spots/cm²	No. of spots/cm²	No. of spots/cm²
R10P10	15.04	0.00	6.86	6.86	0.00	0.46	0.46
R9P18	15.85	0.00	13.33	13.33	0.00	0.84	0.84
R8P1	14.21	0.71	0.60	0.68	0.05	0.04	0.05
R9P16	13.97	1.15	2.55	2.55	0.08	0.18	0.18
R8P23	13.46	20.18	20.82	26.29	1.50	1.55	1.95
P1	19.54	0.00	4.69	4.69	0.00	0.24	0.24
Hayward K	15.8	0.00	0.50	0.88	0.00	0.03	0.06
Hayward 8	16.9	0.00	0.00	0.34	0.00	0.00	0.02
Soreli	21.5	3.18	6.32	10.82	0.15	0.29	0.50

Table 5

Genomic DNA extracted from leaves samples of *Actinidia spp.* with foliar symptoms caused by *Pseudomonas syringae pv. actinidiae*

Samples	Size (bp)	Conc.(ng/µl)
R8P1*	15	2.61
R8P1**	25	2.61
R9P16*	50	2.61
H8*	75	2.61
Soreli**	100	2.61
R9P18*	150	2.61
Soreli*	200	2.61
R9P18**	250	2.61
R10P10**	300	2.61
HK*	400	2.61
R8P23*	500	2.61
P1**	600	2.61
Total		**31.32**

* - without injury

** - with injury

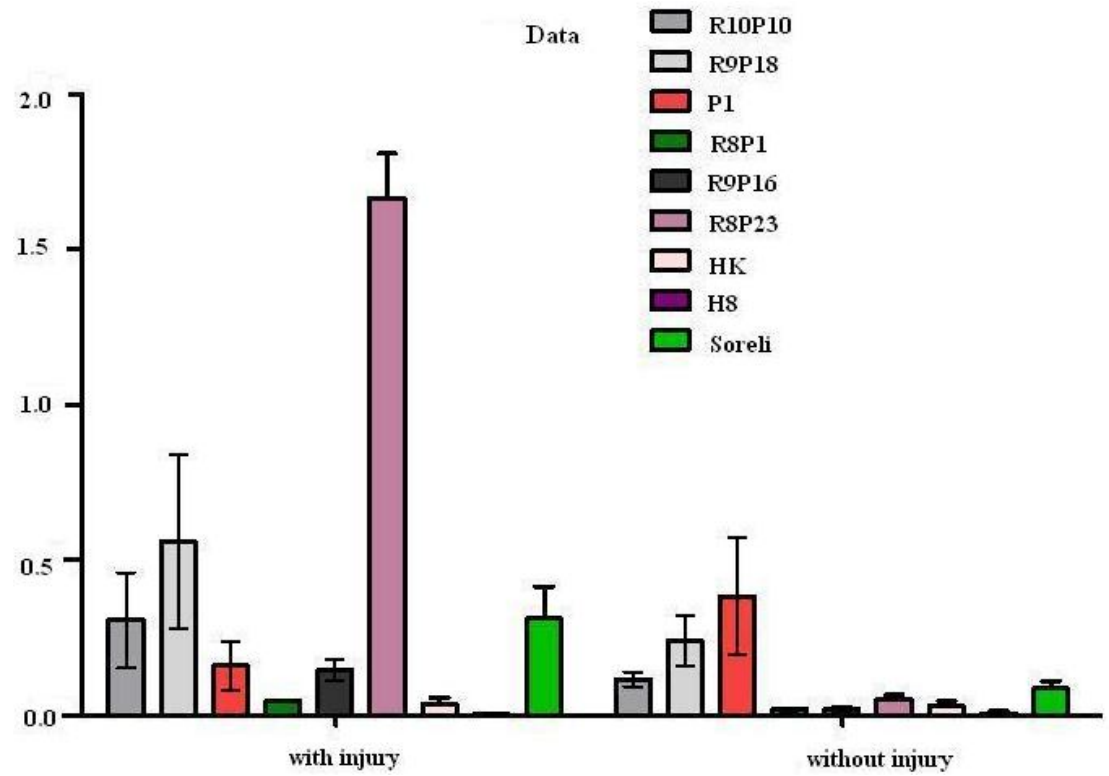

Figure 4. **Number of spots per total number of leaves on *Actinidia spp.* with and without injury (spots/cm²)**

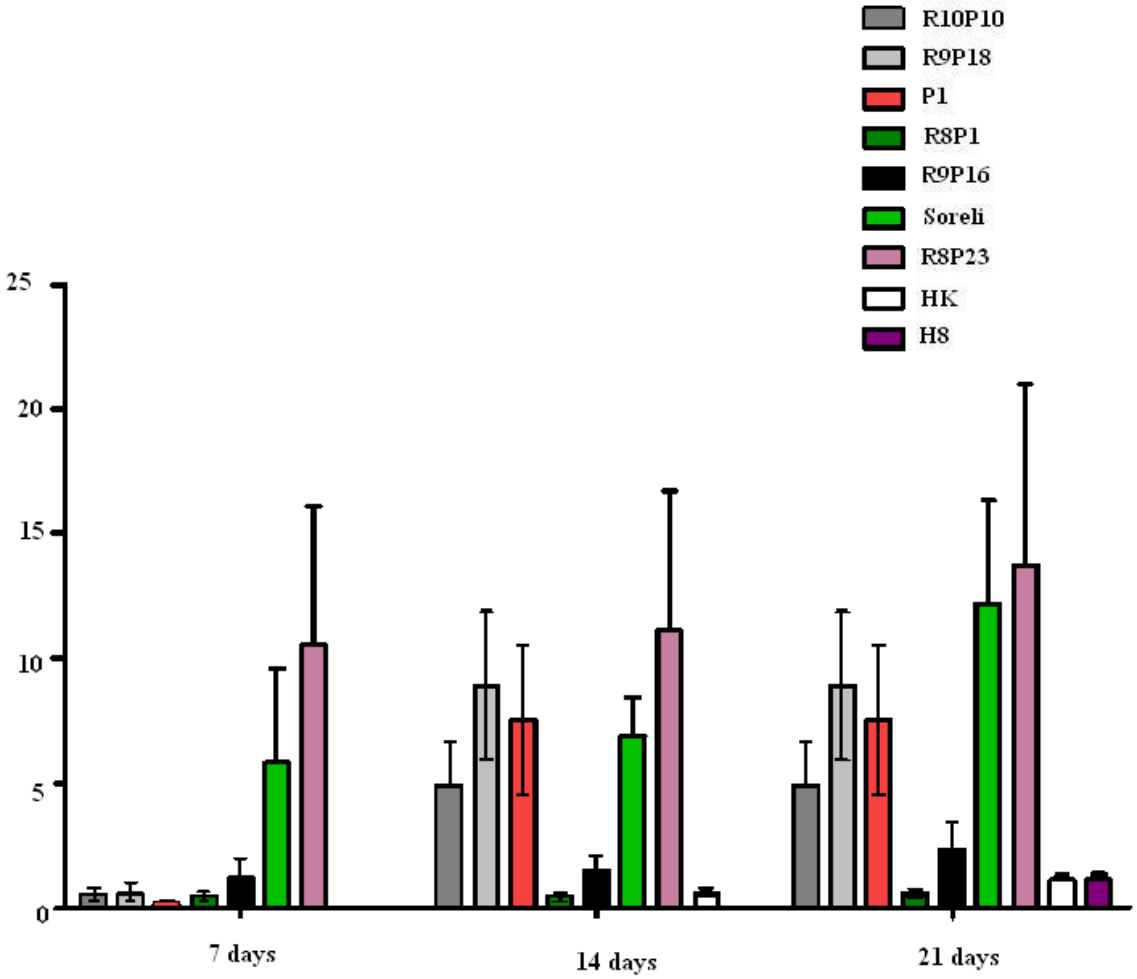

Figure 5. **Number of spots per total number of leaves on *Actinidia spp*, as they appear after 3 consecutive weeks**

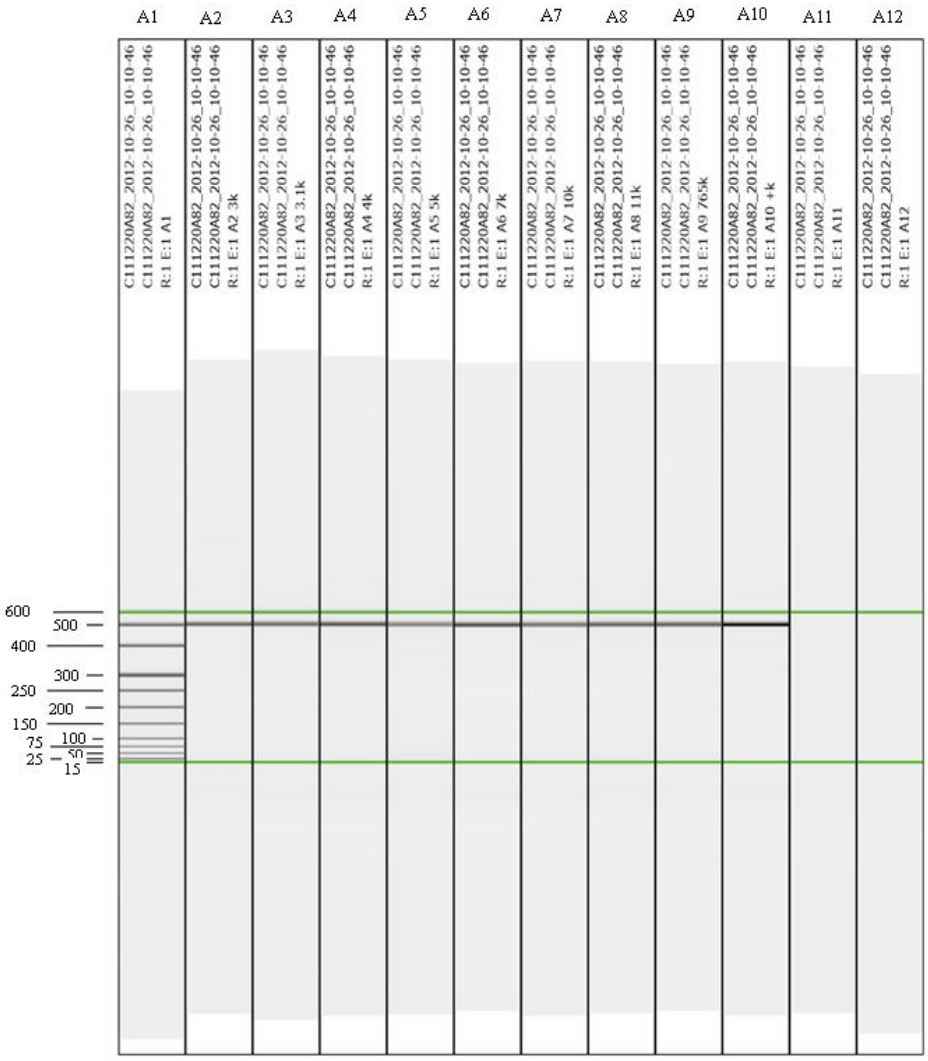

Figure 6. ***Actinidia spp*** **PCR amplification by capillary gel electrophoresis using the QIAexcel DNA fast analysis. (Generated by QIAxcel ScreenGel 1.0.0.0 26/10/2012 11.04.42)**

Higher allelic diversity at immune genes could help hosts recognize a greater diversity of pathogens. If the additional genome copies are expressed, then polyploids may generate higher amounts of gene products related to immune function (King *et al.,* 2011*).* Relationships between ploidy and disease resistance are certainly complex, and selection on resistance to plant pathogens is not necessarily positively linear (Raberg and Stjernman, 2003). To determine if ploidy-level variation can affect disease spread and resistance evolution, several key points are still to be discussed: if increased ploidy is associated with a higher allelic diversity at resistance genes; if organisms with higher ploidy levels have higher expression levels of immune genes; if the resistance affected by host conditions is mediated by polyploidization and how.

CONCLUSIONS

Our results demonstrate that difference level in Psa susceptibility between the diploid and polyploid population, is due to the increased frequency of heterozygous genotypes present in tetraploid versus diploid populations. When the parental diploid host population is nearly monomorphic (low variance), the tetraploid, hexaploid population do not contain a significantly greater proportion of heterozygous genotypes, and therefore the difference in resistance is minimal (Oswald and Nuismer, 2007).

This study brings novel insights to the role played by pathogens in promoting the establishment of polyploid populations, and reinforces the long-standing debate over the genetic basis of pathogen resistance (Frank 1994; Parker and Gilbert 2004).

The present study carried on using different polyploid population of *Actinidia*, respect to *Pseudomonas syringae* pv. *actinidiae* point out that difference in pathogen susceptibility existing among diploid, tetraploid and hexaploid populations. The polyploids *Actinidia arguta* selections used here showed a lesser susceptibility than the diploids *Actinidia arguta* individuals. This effect can be quite strong and potentially providing a new understanding about the relation of novel polyploid lineages and disease resistance, provide a novel and showing a fruitful path to unraveling the genetic basis of pathogen resistance in plants. Like previous studies mentioned, we know that even if selection is strong, evolution will occur only if there is genetic variation in resistance for selection to act upon. Pathogens evolve in response to the host,

which may exhaust genetic variation for resistance more rapidly (Parker & Gilbert 2004).

We need to understand the genetic and evolutionary factors influencing shifts to the target hosts. As we know, even if the selection is strong, the evolution occurs only if there is genetic variation in resistance for selection to act upon. Pathogens evolve in response to the host, which may exhaust genetic variation for resistance more rapidly (Parker & Gilbert 2004). In addition, it is necessary to understand the genetic and evolutionary factors influencing shifts to the hosts. Specifically, in order to determine if ploidy-level variation can affect the level of susceptibility to pathogens, is it important to ask if the host can develop susceptibility over time.

If higher allelic diversity at resistance genes can be associated with increased ploidy? Or do organisms with higher ploidy levels have higher expression levels of immune genes? How susceptibility can be affected by host conditions mediated by polyploidization?

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REFERENCES

Adams, K. L., Wendel, J. F., 2005 - *Polyploidy and genome evolution in plants*. Current Opinion in Plant Biology 8, 135-141.

Balestra, G.M., Taratufolo, M.C., Vinatzer, B.A., Mazzaglia A., 2013 - *A multiplex PCR assay for detection of Pseudomonas syringae pv. actinidiae and differentiation of populations with Different Geographic Origin*. Plant Disease 97 (4), 472-478.

Bierzychudek, P., 1985 - *Patterns in plant parthenogenesis*. Cellular And Molecular Life Sciences 41, 1255–1264. (doi:10.1007/ BF01952068).

Frank, S. A., 1994 - *Recognition and polymorphism in host–parasite genetics*. Phil. Trans. R. Soc. B 346, 283-293 (doi:10.1098/rstb.1994.0145).

Galbraith, D.W., Harkins, K.R., Maddox, J.M., Ayres, N.M., Sharma, D.P., Firoozabady E., 1983 - *Rapid flow cytometric analysis of the cell cycle in intact plant tissues*. Science 220: 1049–1051.

Gerstein, A. C., 2012 - *Mutational effects depend on ploidy level: all else is not equal*. Biological Letters 9: 20120614.

Greilhuber, J., 2005 - *Intraspecific variation in genome size in angiosperms: identifying its existence.* Annals of Botany (London) 95, 91–98. (doi:10.1093/aob/mci004).

Kearney, M., Wahl, R., Autumn, K. 2005 - *Increased capacity for sustained locomotion at low temperature in parthenogenetic geckos of hybrid origin*. Physiological and Biochemical Zoology 78, 316–324. (doi:10.1086/430033).

King, K. C., Seppala, O., Neiman, M., 2011 - *Is more better? Polyploidy and parasite resistance*. Biological Letters doi:10.1098/rsbl.2011.1152.

Koh, Y. J., Nou, I. S. 2002 - *DNA markers for identification of Pseudomonas syringae pv. actinidiae.* Mol. Cells 13:309-314.

Leitch, A. R., Leitch, I. J., 2008 - *Genome plasticity and the diversity of polyploid plants*. Science 320, 481–483. (doi:10.1126/science.1153585).

Nuismer, S. L., Otto, S. P., 2004 - *Host–parasite interactions and the evolution of ploidy*. Proceedings of National Academy of Science USA 101, 11 036–11 039. (doi:10.1073/pnas. 0403151101).

Nuismer, S. L., Thompson, J. N., 2001 - *Plant polyploidy and non-uniform effects on insect herbivores.* Proceedings of Royal Society London Biological Sciences 268, 1937–1940. (doi:10.1098/rspb.2001.1760).

Oswald, B. P., Nuismer, S. L., 2007 - *Neopolyploidy and pathogen resistance*. Proceedings of Royal Society London Biological Sciences 274, 2393–2397. (doi:10.1098/rspb.2007.0692).

Otto, S. P., Whitton, J., 2000 - *Polyploid incidence and evolution*. Annual Review of Genetics 34, 401–437. (doi:10. 1146/annurev.genet.34.1.401).

Parker, I. M., Gilbert, G. S., 2004 - *The evolutionary ecology of novel plant–pathogen interactions*. Annu. Rev. Ecol. Evol. Syst. 35, 675–700. (doi:10.1146/annurev.ecolsys. 4.011802.132339).

Raberg, L., Stjernman, M., 2003 - *Natural selection on immune responsiveness in blue tits Parus caeruleus*. Evolution 57, 1670–1678.

Rees-George, J., Vanneste, J. L., Cornish, D. A., Pushparajah, I. P. S., Yu, J., Templeton, M. D., Everett, K. R. 2010 - *Detection of Pseudomonas syringae pv. actinidiae using polymerase chain reaction (PCR) primers based on the 16S-23S rDNA intertranscribed spacer region and comparison with PCR primers based on other gene regions.* Plant Pathology 59:453-464.

Renzi, M., Copini, P., Taddei, A.R., Rossetti, A., Gallipoli, L., Mazzaglia, A., Balestra, G. M 2012 - *Bacterial canker on kiwifruit in Italy: Anatomical changes in the wood and in the primary infection sites*. Phytopathology 102, 827-840.

Stover, R. H., 1986 - *Banana breeding: polyploidy, disease resistance and productivity*. Fruits (France) 41, 175–191.

Testolin, R., Cipriani G., 2010 - *Soreli - A yellow-fleshed kiwifruit UNIUD 'Soreli'* Bulletin Università degli Studi di Udine – Ripartizione Ricerca

Weider, L. J., 1993 - *A test of the 'general-purpose' genotype hypothesis: differential tolerance to thermal and salinity stress among Daphnia clones.* Evolution 47, 965–969. (doi:10.2307/2410201).

Watanabe, K., Takahashi, B., Shirato, K., 1990 - *Chromosomes numbers in kiwifruit (Actinidia deliciosa) and related species*. J.Jpn. Soc. Hort.Sci. 58:835-840.

Woodhouse,M., Burkart-Waco, D., Comai, L., 2009 - *Polyploidy*. Nature Education 2(1).

Yan, G. J., Ferguson, A.R., McNeilage, M. A., 1994 - *Ploidy races in Actinidia chimensis*. Euphytica 78: 175-183.