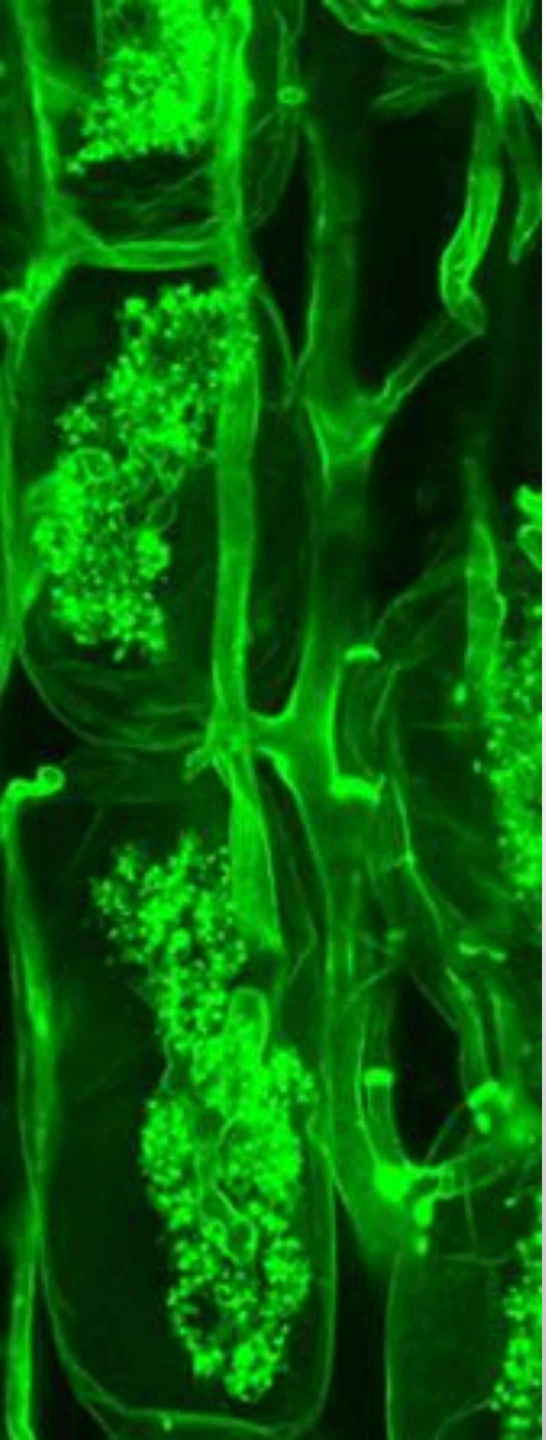

Analysis of the spatial interdependence of nuclear size in confocal microscopy images of plant roots

Analisi della interdipendenza spaziale della dimensione dei nuclei in radici vegetali osservate in microscopia confocale

Ivan Sciascia[1], Andrea Crosino[1], Gennaro Carotenuto[1], Andrea Genre[1]

Keywords: mycorrhized roots, estimated variogram, nuclei size

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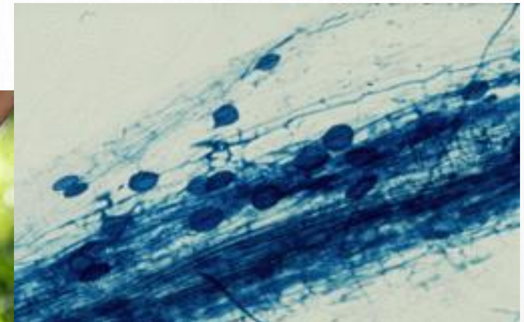
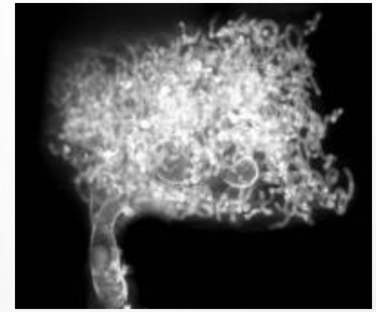
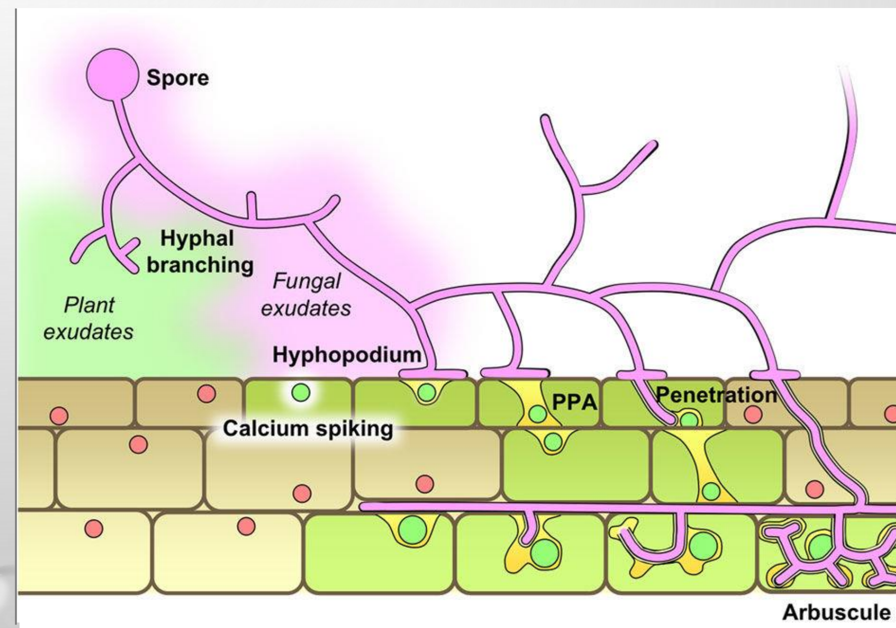
AM symbiosis





AM Symbiosis, beneficial interaction

Arbuscules, the structures devoted to the exchange of mineral nutrients for sugars and lipids

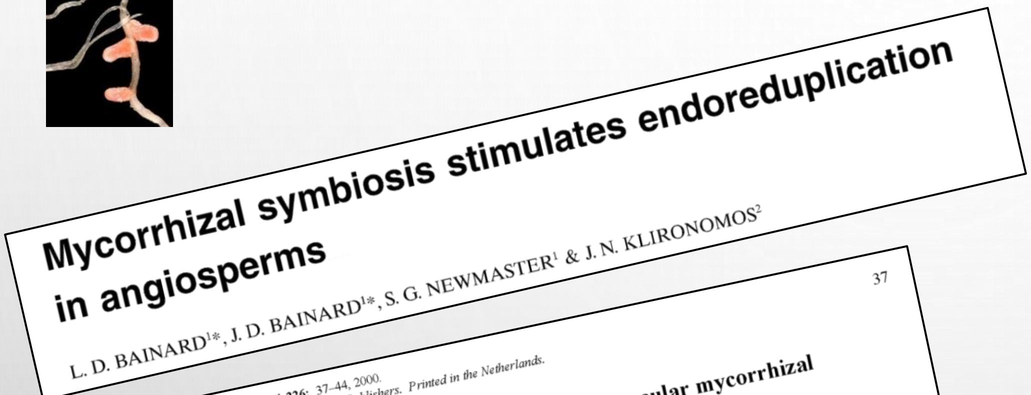


Bonfante & Genre, 2010

AM symbiosis

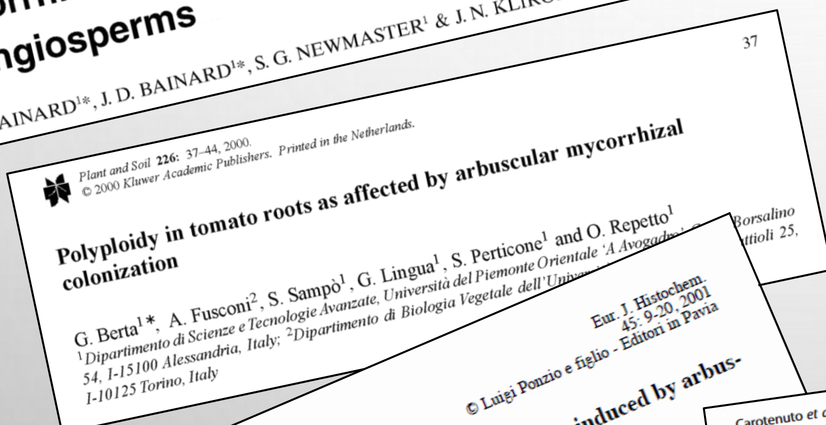
The symbiosis between fungi and plants leads to a mutual exchange of nutrients which brings advantages to the plant in terms of greater growth, resistance to stress and pathogens.

What we already know about endoreduplication and nuclear size



Mycorrhizal symbiosis stimulates endoreduplication in angiosperms

L. D. BAINARD[1*], J. D. BAINARD[1*], S. G. NEWMASTER[1] & J. N. KLIRONOMOS[2]



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Plant and Soil 226: 37–44, 2000.
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Polyploidy in tomato roots as affected by arbuscular mycorrhizal colonization

G. Berta[1*], A. Fusconi[2], S. Sampò[1], G. Lingua[1], S. Perticone[1] and O. Repetto[1]
[1]Dipartimento di Scienze e Tecnologie Avanzate, Università del Piemonte Orientale 'A Avogadro' ... Borsalino 54, I-15100 Alessandria, Italy; [2]Dipartimento di Biologia Vegetale dell'Univ... ttioli 25, I-10125 Torino, Italy

Eur. J. Histochem. 45: 9-20, 2001
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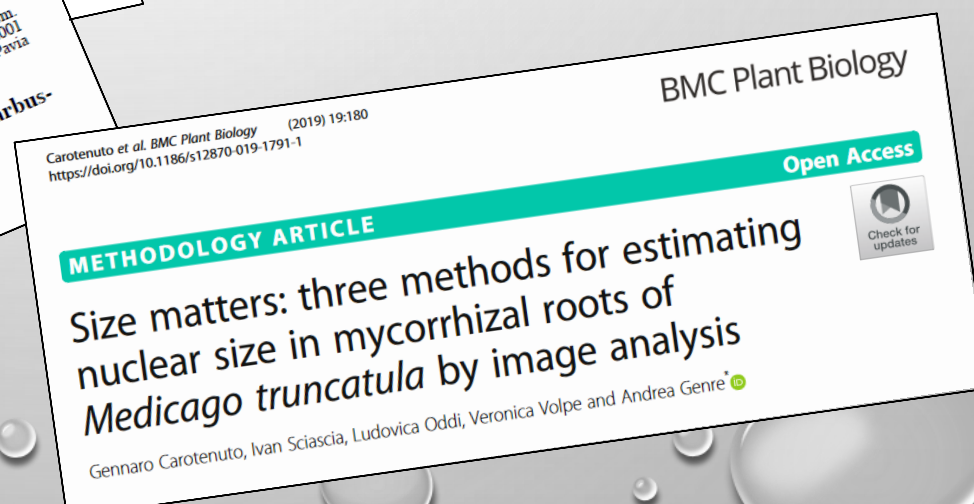
REVIEW

The nucleus of differentiated root plant cells: modifications induced by arbuscular mycorrhizal fungi

G. Lingua[1], A. Fusconi[2] and G. Berta[1]



Carotenuto et al. BMC Plant Biology (2019) 19:180
https://doi.org/10.1186/s12870-019-1791-1

BMC Plant Biology

METHODOLOGY ARTICLE

Open Access

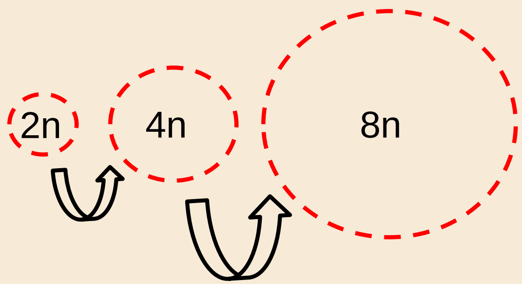
Check for updates

Size matters: three methods for estimating nuclear size in mycorrhizal roots of *Medicago truncatula* by image analysis

Gennaro Carotenuto, Ivan Sciascia, Ludovica Oddi, Veronica Volpe and Andrea Genre*

Previous studies have highlighted endoreduplication in several plant-microbe interaction

Nuclei size and endoreduplication:



Double DNA content without dividing

Endoreduplication events have been analyzed through:

Use of <u>flow cytometry</u> to analyze the occurence of endoreduplication in *M. truncatula* roots.

<u>Gene expression analyses</u> of endocycle initiation markers

<u>Image post-processing</u> from confocal Z-stacks

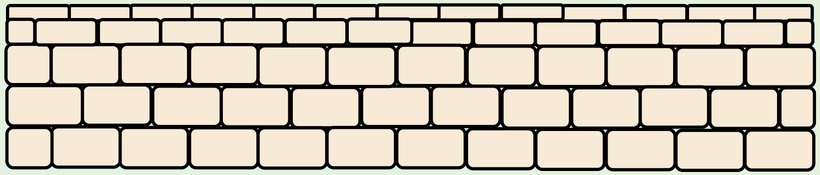
Investigation of ploidy changes in wild-type Medicago truncatula roots

This presentation focuses on post-processing set-up

Medicago truncatula

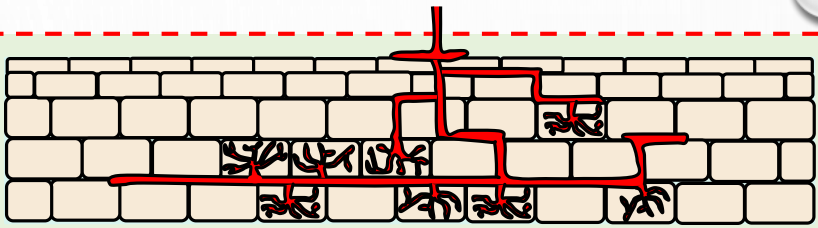
Gigaspora margarita

Workflow:

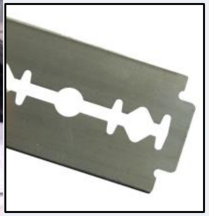


1cm-long Control root segments

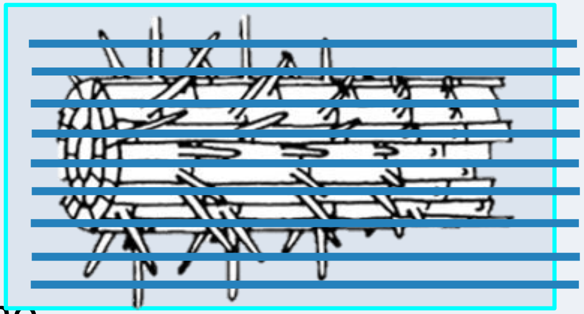
Excision



1cm-long Mycorrhizal root segments



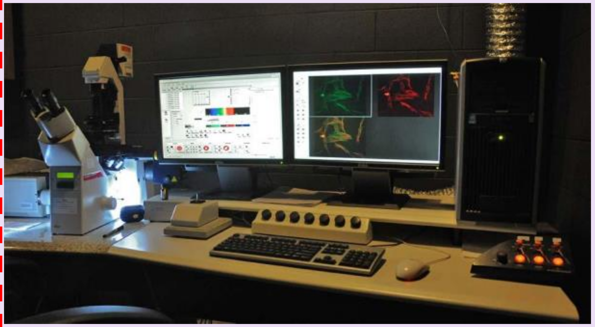
Vibratome sectioning

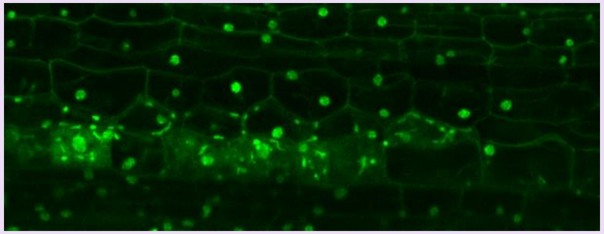


100µm-thick slices

We obtained 100 micrometer thick slices which were prepared with fluorescent dyes for DNA, that label the nuclei

These samples were then imaged on the confocal microscope

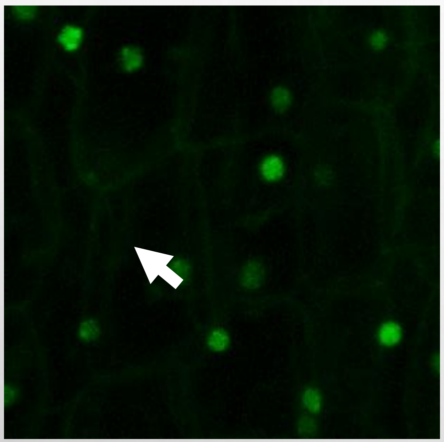


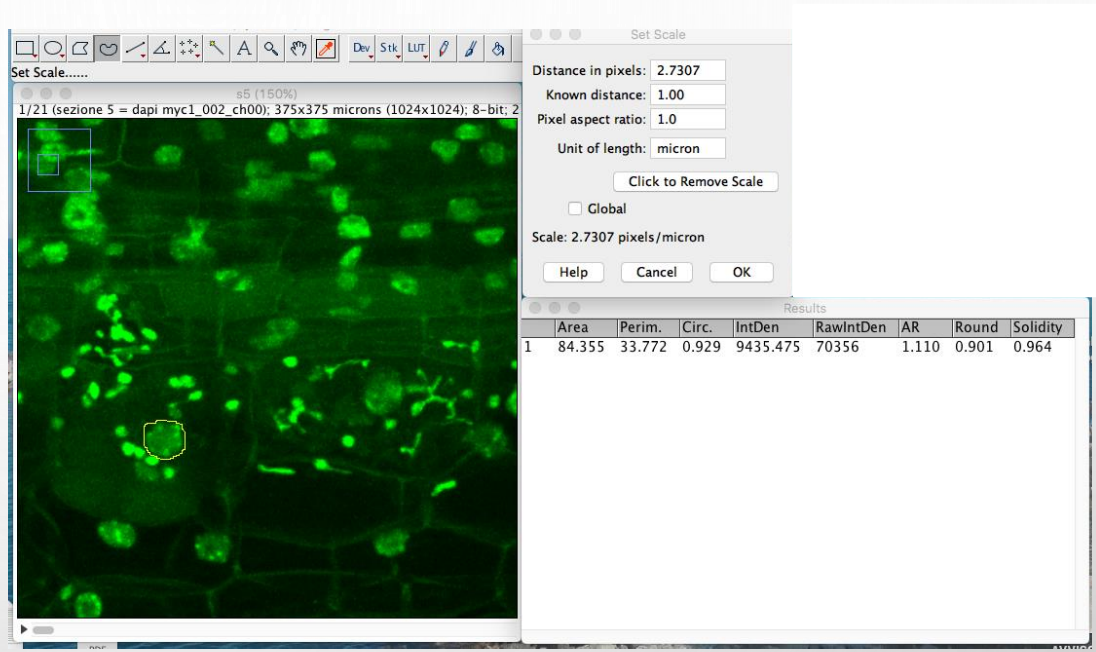


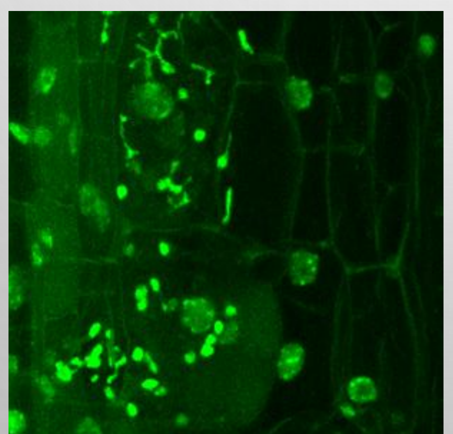
Z-series, taken with 1.5 micrometer Z-step

We observed and measured the size of hundreds of nuclei for every section

Manual measurements with ImageJ:







Spatial statistical analysis: mark variogram

Analysis techniques derived from geostatistics dealing with spatially correlated data

Analysis of the correlation between nuclear size and distances

Mathematical models that describe variability as a function of distance

Mark variogram: areas and distances

To calculate the semivariance as a function of the distance between the nuclei we first calculate the Euclidean distances and then the squared differences between the dimensions of the areas of the nuclei

	Area	BX	BY
1	14,484	76,904	5,127
2	13,947	37,354	31,128
3	34,735	121,216	56,396
4	8,583	58,228	59,692
5	3,219	62,256	80,566

Spatial coordinates

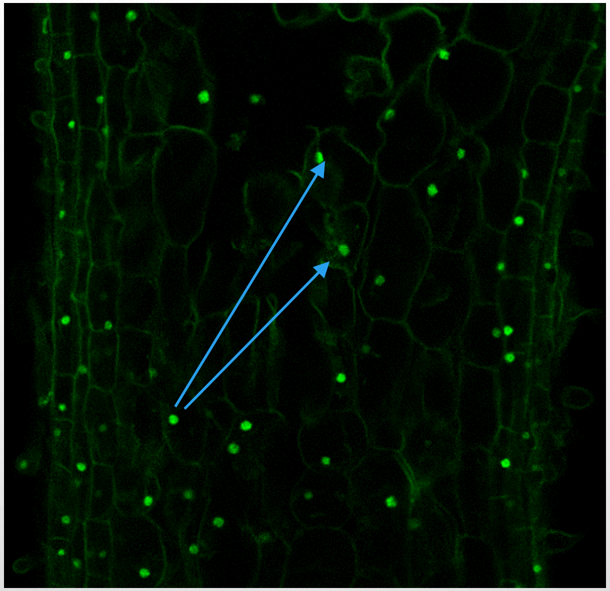
Euclidean distances, above the diagonal of the matrix

	Area	1	2	3	4	5
1	14,484		47,33133	67,76477	57,67263	76,84794
2	13,947	0,288369		87,586	35,37833	55,35545
3	34,735	410,103	432,1409		63,07418	63,72182
4	8,583	34,8218	28,7725	683,9271		21,25908
5	3,219	126,9002	115,09	993,2583	28,7725	

Squared area differences, below the diagonal of the matrix

Semivariance is a function of distance between pairs of objects and the plot experimental function is called variogram:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{(p_{(i)}, p_{(i+h)}) \in S} \left[y(p_i) - y\left(p_{(i+h)}\right) \right]^2$$



If we consider digital images from confocal microscopy with resolution $\delta = 2{,}7307 \ \frac{pixel}{\mu m}$ as in Figure 1 the elements of the formula (1) are:

$\gamma(h)$ is the semivariance in function of the distance h

$N(h)$ are the root nuclei pairs at distance h

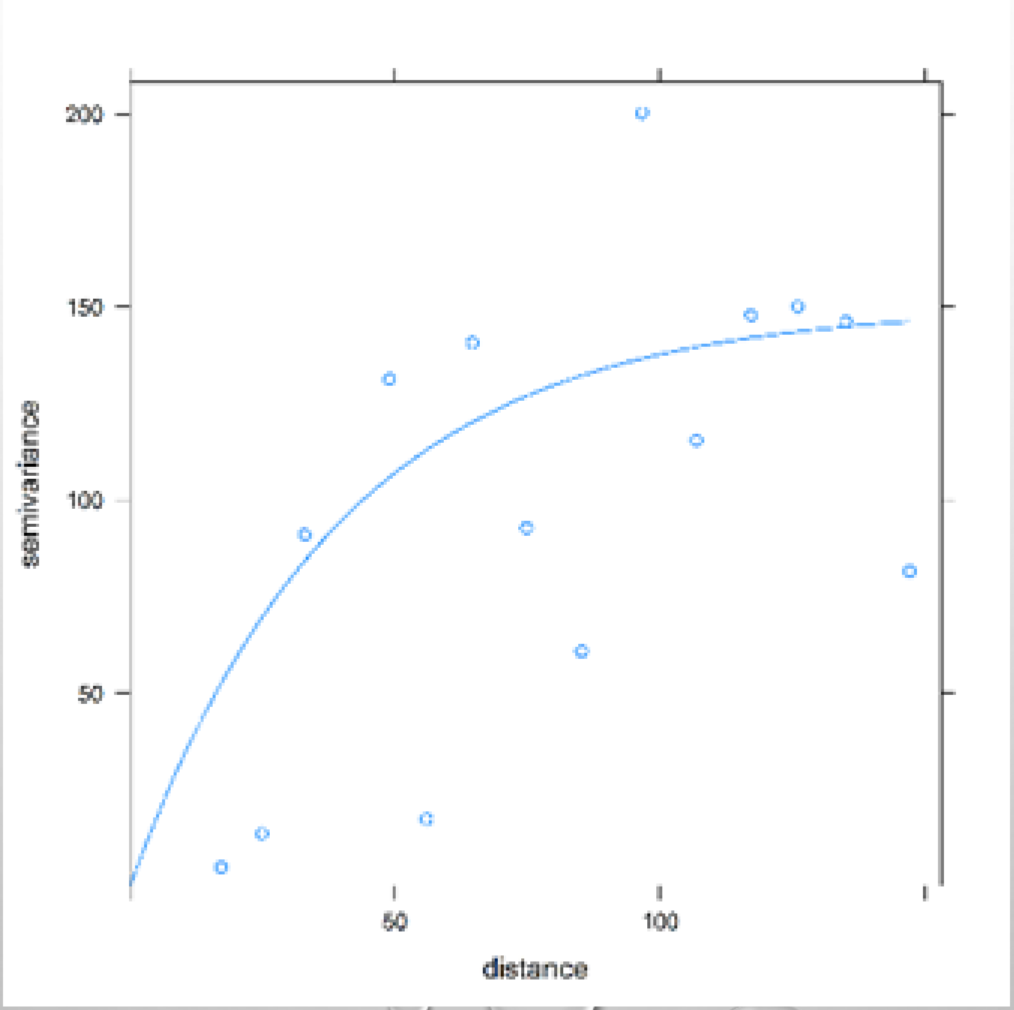
S is the frame sample

$y(p_i)$ is the nucleus area in µm^2 at the coordinates p_i

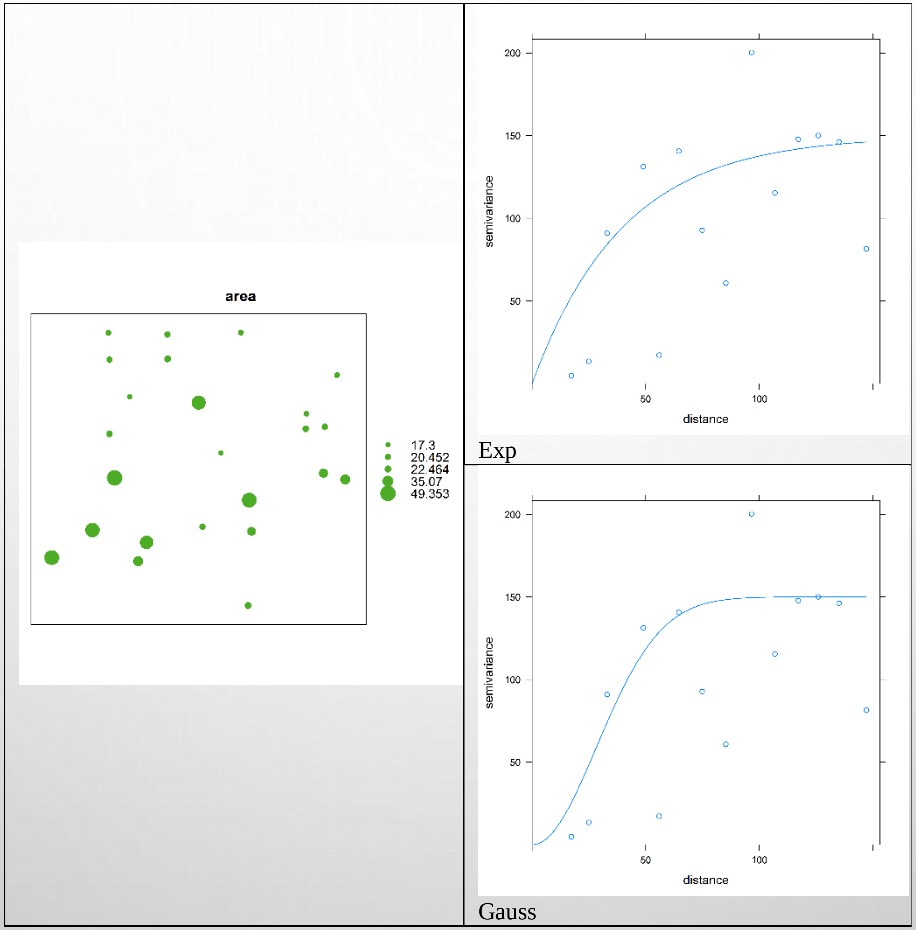
$y\left(p_{(i+h)}\right)$ is the nucleus area in µm^2 at the coordinates $p_{(i+h)}$

Calculation of experimental and theoretical variograms

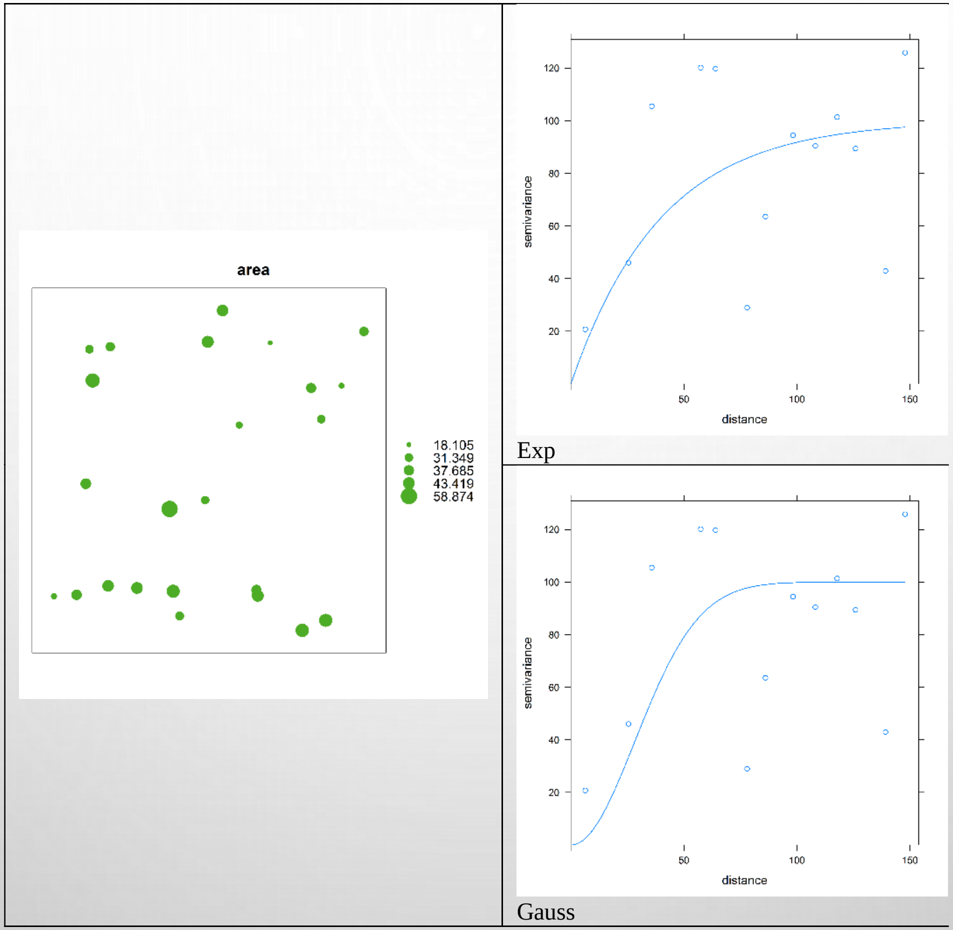
The experimental variogram consists of a cloud of points where the semivariance is a function of the distance. The theoretical variograms are point cloud adaptation curves, we use the exponential and Gaussian models.



Theoretical models have characteristic parameters, the sill, the range and the nugget. The value of the sill of the theoretical graph was set on the basis of the variance of the sample measurements while the range was visually evaluated and set equal for the two data series. The nugget was conventionally set to zero.



Area plot of control nuclei and estimated exponential and Gaussian variograms



Area plot of mycorrhized nuclei and estimated exponential and Gaussian variograms

Performance indexes for the estimation models

Model	*MAD*	*RMSE*
Control Exponential	3,784341	4,735795
Control Gaussian	3,616071	4,948705
Mycorrhized Exponential	2,459242	3,201468
Mycorrhized Gaussian	2,702641	3,368925

From this first study using theoretical semivariance estimation models we observed that the estimation models perform better for the frame sample of mycorrhized roots. This may suggest minor variability in the distribution of nuclei sizes, to be investigated with other research in confocal microscopy.

Thank you for your attention!

Dipartimento di Scienze della Vita e Biologia dei Sistemi

Laboratorio di microscopie avanzate

UNIVERSITA DEGLI STUDI DI TORINO

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