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Dynamical scaling analysis of plant callus growth

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Abstract. – We present experimental results for the dynamical scaling properties of the development of plant calli. We have assayed two different species of plant calli, *Brassica oleracea* and *Brassica rapa*, under different growth conditions, and show that their dynamical scalings share a universality class. From a theoretical point of view, we introduce a scaling hypothesis for systems whose size evolves in time. We expect our work to be relevant for the understanding and characterization of other systems that undergo growth due to cell division and differentiation, such as, for example, tumor development.

Introduction. – Pattern formation in biological systems has been investigated since the nineteenth century [1]. Pigmentation of sea shells, mammalian coating, and even hallucination patterns illustrate the richness and variety of the spatio-temporal structures that may appear [2, 3]. Complementary to the discovery and understanding of mechanisms responsible for pattern formation, fractal growth in natural processes has recently been a subject of interest. Scaling analysis has become a powerful tool to characterize the properties of the structures that develop in a variety of biological systems such as DNA *walks*, and bacteria and fungi colonies [4–8]. A particularly interesting example deals with cancer tumors [9]. Recent analyses have shown that fractal methods may be used for diagnosing pathologies and cancer research [10].

Plant *calli* are undifferentiated tissues that develop on, or around, an injured or cut plant surface or in a tissue culture. One of the most important features of the *in vitro* culture of calli is that it constitutes a necessary step for obtaining genetically modified plant species. Moreover, the relative simplicity of the experimental setups for growth control makes it an excellent benchmark to test and characterize the morphological and dynamical properties of the evolution of these tumor-like systems.

In this letter we present experimental results that reveal a common scaling behaviour in the callus growth of two different plant species. By studying different species under different

growth conditions, we show that they present a self-affine structure and share a universality class. Then, according to the basic mechanism governing the growth of calli, we give a hint about what kind of models would be able to explain and reproduce the observed phenomenology. We conclude that the basic mechanisms governing the growth of calli are the same.

Plant callus growth presents two differential characteristics that have led us to introduce a new methodology to study scaling behaviour. The first one is the complex geometry of the initial surface. The second one is the fact that the system size changes in time. To solve the first problem we develop a method to identify and separate the general trend from the fluctuations. To address the second characteristic we introduced a new corrected, and generic, scaling hypothesis for analyzing rough growth in systems whose size is changing in time. This methodology can be applied to other problems such as *in vivo* tumor growth or cloud evolution.

Opposite to previous works in similar systems, such as the growth of bacterial colonies, where a low nutrient substrate is used to study a diffusion-limited aggregation (DLA) process [7], we are interested in characterizing callus growth at optimum nutrient conditions, which results in a rough compact growth.

Experimental design. – We designed an experimental setup to monitor the growth of plant calli, and focused our study on two particular species: *Brassica oleracea* and *Brassica rapa*. First, we germinated seeds within an aseptic environment to obtain small plants that were used as the initial material to develop the calli. We then cut rectangles (typically $4 \times 4\,\mathrm{mm}^2$) out of the leaves and placed them on a substrate in a Petri dish. It is well known that to develop a callus it is necessary to use a nutrient medium with a high concentration of auxin or a high auxin/cytokinin (a/c) ratio [11]. Cytokinins promote cell division, while auxins facilitate cell growth [12]. In order to find the best growth regulator combination for callus formation and growth, we tested concentrations of 0, 1, 2, 3 and 4 mg/l 2,4-D (auxin) and 0 and 1 mg/l BAP (cytokinin). The most favorable combinations were 1, 2, 3 and 4 mg/l 2,4-D along with 1 mg/l BAP. The basal nutrient medium was that of Murashige and Skoog [13]. This initial process to induce and develop plant calli took about four weeks. Once the calli were obtained, they were divided into fragments of typically 5 mm that were placed on a fresh nutrient medium with the same concentration of regulators used for callus induction. This study is focussed on the growth of these calli. Our experiments were monitored for four weeks since within this time period the substrate is considered to maintain rich basal nutrient conditions providing rough growth [14].

The growth process was monitored using image analysis techniques that make it possible to investigate the evolution of calli without a destructive interaction. We captured the images directly from the output of a CCD black/white camera, obtaining a spatial resolution of $9\,\mu$m/pixel. From captured images, we mapped the interfaces into Cartesian coordinates.

We tested the influence of the image projection method in the scaling properties of the profiles. The calli grow upon the substrate and therefore develop in a quasi-bidimensional mode. The projection technique *flattens* their shapes into bidimensional structures. We rotated the samples around the plane defined by the substrate and measured the scaling properties as shown below. The lack of variation of these properties ensure the reliability of the method.

Static and dynamic analysis. – In order to make an initial static characterization, we calculated the fractal dimension, d_f, by means of the box-counting method [15]. We present the results obtained for the two species aforementioned. Both of them show averaged values $d_\mathrm{f} = 1.18 \pm 0.02$ (see fig. 1). This analysis was performed to other calli and similar values were obtained within the error bars. We stress that d_f does not depend on time and/or on the species.

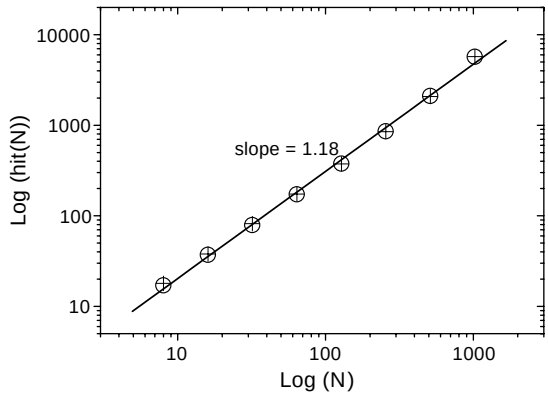

Fig. 1 – Fractal dimension of *Brassica oleracea* (circle) and *Brassica rapa* (cross) calli, on the same time ($t_0 + 30$ days), with an a/c concentration ratio 2 : 1, and 1 : 1, respectively. The slope indicates a fractal dimension $d_{\mathrm{f}} = 1.18 \pm 0.02$.

The principal problem to obtain the dynamic scaling of the callus interface is because the system size is changing in time. Plischke and Racz [16] dealt with that problem when they studied an Eden model whose size showed a time evolution. However, in their work the interfaces were circular and the curvature effects were negligible. In this case, it is possible to subtract the mean radius of the interface obtaining the evolution of the fluctuations. On the contrary, our problem lacks rotational symmetry and the curvature effects are not negligible. Note that the substraction of the mean radius in these cases leads to a wrong characterization, introducing a *spurious* wave-like behaviour in the interface. Therefore, our analysis starts by choosing a complex early interface of a callus as the initial reference for the subsequent growth. By computing the center of mass of the profiles we appropriately subtract the radial coordinate of the reference from the actual interfaces obtaining the *effective* growth of the callus in time. The interfaces, with the exception of a small number of non-representative points, do not present overhangs and then that procedure is well defined.

Once we obtain the effective coordinates, we compute again the center of mass of the profiles, and transform the angle-radius coordinates into arc-radius s, R, where s is the arc length measured from a particular angular origin. The interface is then a one-dimensional function $R(s)$. These coordinates are more convenient for subsequent analysis.

Figure 2 shows the shapes of the calli as they evolve. The shadowed regions indicate the aforementioned initial reference calli that determine our time origin, t_0, and the solid, dotted, and dashed lines their time evolutions. Although we captured images every day, the slow callus growth allowed us to select representative images at irregular intervals of time. Time periods of the images in that figure are: solid line, $t_0 + 21$ days for both species, dotted line data were taken on $t_0 + 23$ days for *B. oleracea* and on $t_0 + 25$ days for *B. rapa*. Finally, dashed lines stand for $t_0 + 30$ days in the case of *B. oleracea* and $t_0 + 28$ days for *B. rapa*.

Notice also that the growth of the calli is not isotropic, and therefore the effective interfaces may contain protuberances that correspond to the parts of the calli that grow faster than others, or even concavities due to natural dehydration processes. The lack of uniformity can be explained by the irregular pattern of cell differentiation. Calli are composed of a main body of large prismatic undifferentiated cells and a series of nuclei with small meristematic cells, responsible for cell division, interspersed in the outer layers. The callus grows as a result of cell division and enlargement of some of the divided cells to form the large undifferentiated cells. Thus, the areas of growth correspond to the activity of the meristematic nuclei. Callus

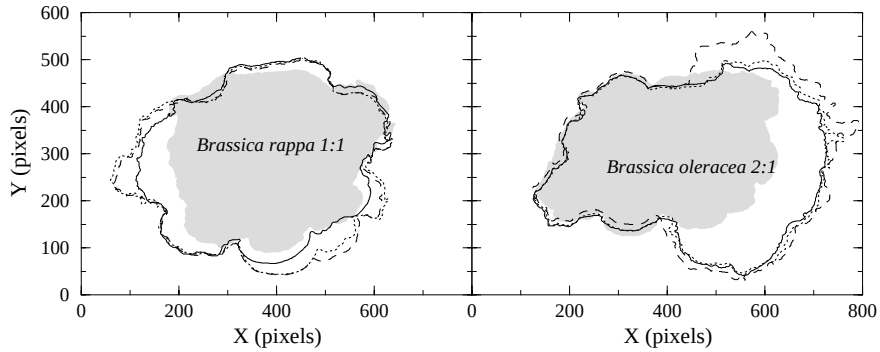

Fig. 2 – Profile time evolution of *Brassica rapa* and *Brassica oleracea* calli. The a/c concentration ratio is noted next to the name of each species. The shadowed regions point out the early stages in the evolution that are taken as a reference for the growth (see text). Notice the anisotropy of the growth process. The time sequence from the initial reference image is: solid line, then dotted line, and finally dashed line.

tissue does not have an outer layer of epidermal cells with wax depositions and is therefore very sensitive to dehydration. This explains why parts of the callus do not grow and can actually shrink as time evolves.

These irregularities where curvature effects are important mask the scale properties of the interfaces because they imply length scales beyond which obviously no scale invariance can be found. However, we can conveniently disregard the influence of these anisotropies by means of detrending techniques [5]. In our case, piecewise linear detrending worked well. The detrended interfaces, $h(s,t)$, constitutes finally the data we are going to analyze. We stress that in the absence of a detrending procedure the interfaces still present well-defined scaling properties but only for length scales below the characteristical size of the anisotropies.

Dynamical scaling properties may be revealed by analyzing the behaviour of different functions of the interfaces. Two useful examples are the power spectrum $S(q,t) = \langle \tilde{h}_q(t)\tilde{h}_{-q}(t)\rangle$, where $\tilde{h}_q$ is the Fourier transform of the interface $h(s,t)$, and the correlation function $C_l^2(t) = \langle (h(s+l,t) - h(s,t))^2\rangle_s$, where l is the size of a measurement window and $\langle\rangle_s$ indicates a spatial average over s [4,5]. However, the scaling hypotheses found in the literature for these functions assume a fixed system size. We introduce a corrected scaling hypothesis based on the generic scaling hypothesis proposed by Ramasco *et al.* [17] that applies to systems whose size is evolving in time. For simplicity, we focus the analysis on a one-dimensional case although the generalization to higher dimensions is straightforward.

Let us call L the size of the system at a given initial time. Note that L undergoes a dilatation (or shrinking) transformation, $L \to f(t)L$, where $f(t)$ accounts for the time evolution. The experiments indicate that the dilation transformation affects equally all length scales. Note that if the dilation process depends on the length scale, that leads to quite different results. Therefore, we correct the originally proposed generic scaling behaviour of the power spectrum as follows: $S(q,t) = \left(\frac{q}{f(t)}\right)^{-(2\alpha_g+1)} g\left(\frac{t^{-1/z} f(t)}{q}\right)$, where the scaling function reads, $g(u) \sim \begin{cases} u^{-2(\alpha_g-\alpha_s)} & u \ll 1 \\ u^{-(2\alpha_g+1)} & u \gg 1 \end{cases}$. For the correlation function, $C_l^2(t) = (lf(t))^{2\alpha_g}\chi\left(\frac{lf(t)}{t^{1/z}}\right)$, here the scaling function is $\chi(v) \sim \begin{cases} v^{-2(\alpha_g-\alpha_l)} & v \ll 1 \\ v^{-2\alpha_g} & v \gg 1 \end{cases}$. The symbols z, α_g, α_l, α_s stand for the dynamical, and global, local, and spectral roughness exponents, respectively. According to

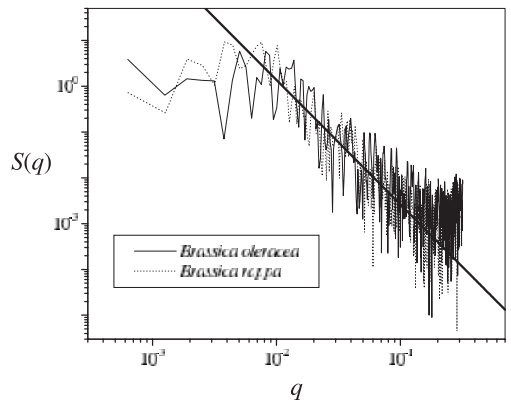

Fig. 3 – Representative cases of the power spectra obtained from interfaces of the species considered. The measured global roughness is $\alpha_g = 0.86 \pm 0.04$ in both cases. The wide solid line is a guide to the eye and has a slope -2.72.

the value of these exponents, different scaling behaviours occur as summarized in the following scheme [17]:

$\alpha_s < 1$ $(\alpha_l = \alpha_s)$	$\alpha_s = \alpha_g$ *Family-Vicsek* [18] $\alpha_s \neq \alpha_g$ *Intrinsic Anomalous* [19]
$\alpha_s > 1$ $(\alpha_l = 1)$	$\alpha_s = \alpha_g$ *Super Roughness* [20] $\alpha_s \neq \alpha_g$ *New Class* [17]

The so-called collapse procedure allows to distinguish between different scaling behaviours by *collapsing* $S(q,t)$ or C_l^2 on the scaling functions $g(u)$ and $\chi(v)$, respectively. In order to test the role of a/c concentration ratio in the scaling properties, we analyzed growths under different a/c ratios. Herein we focus on two representative cases, with a/c ratios: 2 : 1 (*Brassica oleracea*) and 1 : 1 (*Brassica rapa*).

To implement the corrected scaling hypothesis, we chose interfaces showing a well-defined behaviour in the evolution of the system size, that is, the functions $f(t)$ show a clear tendency. In particular, we found evolutions where power law distributions applied to both species, $f(t) \sim t^a$, where $a = 0.25 \pm 0.02$ and $a = 0.07 \pm 0.02$ in the cases of *B. oleracea* and *B. rapa*, respectively. These regimes show a very slow time evolution when compared to tumor cells where $r \sim t$ [9]. This difference might be attributable to the fact that tumor cells responsible for the growth are distributed homogeneously around the tumor surface, whereas in plants meristematic cells, which are the ones that duplicate, only appear in some regions of callus surface [14].

Figure 3 shows the power spectra of the evolutions studied herein. The slope of the power spectrum determines the global roughness exponent, α_g. We obtained a common behaviour, and consequently the same value of α_g, for the two species, $\alpha_g = 0.86 \pm 0.04$. Unfortunately, the power spectra are so noisy that their collapse does not shed light on the type of scaling.

On the other hand, the correlation functions do collapse (see fig. 4), and hence allow us to extract the scaling function and the type of scaling. According to the previous discussion, a Family-Vicsek scaling is obtained and $\alpha_g = \alpha_l = \alpha_s$, that is, the profiles evolve in a *self-affine* way. Note also that, according to the relation $d_f = 2 - \alpha$ [4], there is a perfect agreement between the values obtained for the roughness by means of the static and dynamic analysis. Furthermore, the value of the dynamical exponent obtained by means of the collapse of

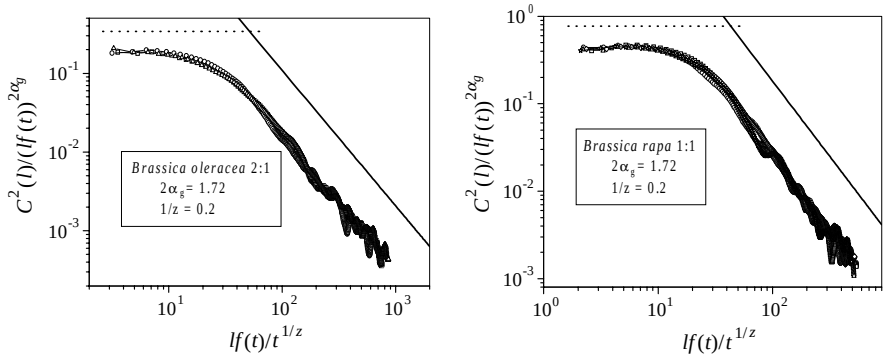

Fig. 4 – Collapses of the correlation functions for the calli shown in fig. 2. The values of the exponents used in the collapse are $\alpha_g = 0.86$ and $z = 5$ in both cases. The growth of the system size is incorporated in the functions $f(t) \sim t^a$ ($a = 0.25$ and $a = 0.07$). The collapses imply a dynamical scaling behavior *à la* Family-Vicsek. The wide solid line has a slope -1.72 and emphasizes the self-consistency of the collapse.

$C_1^2(t)$ is also the same in both cases, $z = 5$, suggesting that *B. oleracea* and *B. rapa* share a universality class.

It is worth pointing out some important differences between previous works in similar systems, such as the growth of bacterial colonies, and ours. Matsushita *et al.* [7] found that colonies of *Bacillus subtilis* followed a DLA model and that nutrient concentration was responsible for this growth pattern. DLA growth was especially evident when suboptimal concentrations of nutrient were used. In their system, the bacterial colony is a simple aggregation of single-cell independent organisms that behave in a similar way. This differs significantly from the callus tissue used in our experiments where cells behave differently due to different degrees of differentiation. Moreover, it is very unlikely that the pattern of callus growth is regulated by the nutrients because the medium we used is very rich and designed to provide optimal growth. Under these rich nutrients conditions, compact rough growth is obtained.

That type of growth has been modelled by means of Eden [21] and reaction-diffusion models [22]. However, the scaling properties obtained in those cases differ from the ones reported herein. In our case, cell differentiation plays a crucial role in the scaling properties. Therefore, a modified Eden model that considers different growth capacities would be appropriate to describe the phenomenology [23].

Conclusions. – In summary, the dynamic scaling properties of plant calli formation have been studied for the first time. In our experiments we analyze and characterize the time evolution of calli of two different species subjected to different growth conditions. By introducing a corrected scaling hypothesis for systems whose size changes in time, we have shown that their profiles evolve in a self-affine way. The experiments suggest that they share a universality class and scaling behaviour *à la* Family-Vicsek, with values of the roughness and dynamical exponents $\alpha = 0.86$ and $z = 5$, respectively.

Since the study covers different species subjected to different growth conditions, and yet they share the *same* scaling properties, we conclude that, as expected, the basic mechanisms governing the ontogeny of calli formation, namely cell division and differentiation, are the same.

In addition to the assessment of an important phase for *in vitro* plant growth, in the present study we introduce a corrected scaling hypothesis for systems whose size changes in

time. This methodology could be applied to a number of problems were fractal growth is obtained. In particular, it is worth remarking that the morphologies of this type of organisms are similar to those that appear in tumor development. Therefore, we expect our methodology to be relevant for the understanding and characterization of the latter.

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