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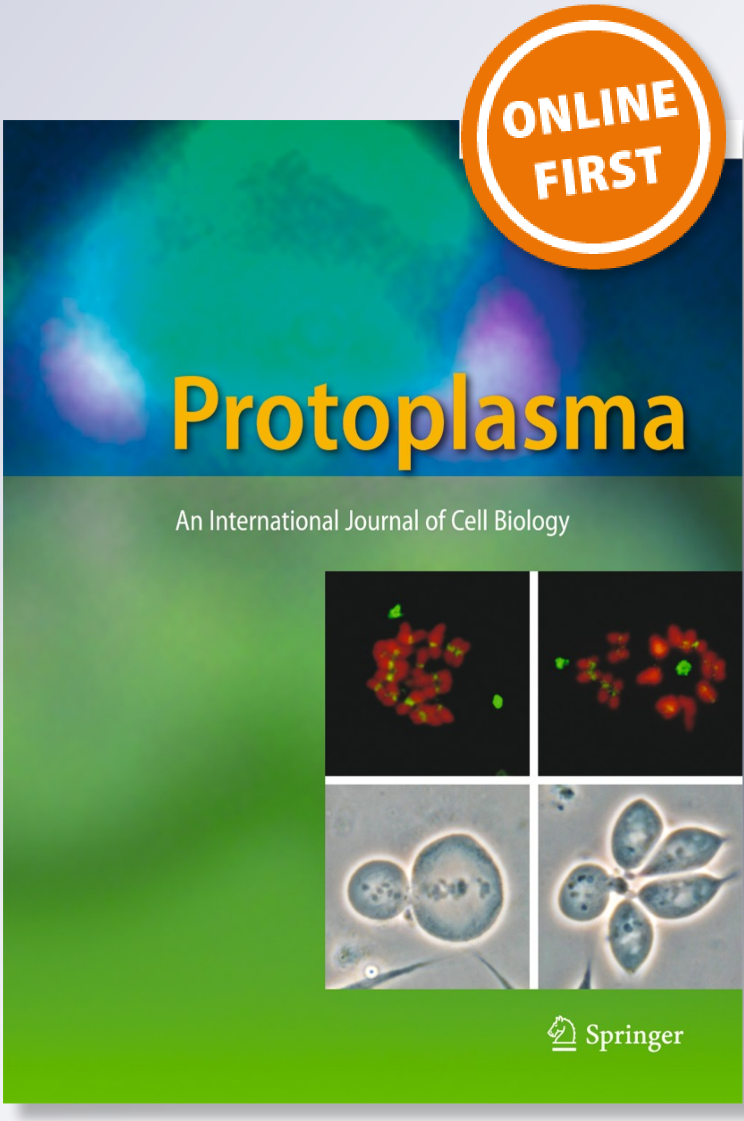

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ORIGINAL ARTICLE

Simultaneous and intercontinental tests show synchronism between the local gravimetric tide and the ultra-weak photon emission in seedlings of different plant species

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Abstract In order to corroborate the hypothesis that variations in the rate of spontaneous ultra-weak photon emission (UPE) from germinating seedlings are related to local variations of the lunisolar tidal force, a series of simultaneous tests was performed using the time courses of UPE collected from three plant species—corn, wheat and sunflower—and also from wheat samples whose grains were transported between continents, from Brazil to The Netherlands and vice versa. All tests which were run in parallel showed coincident inflections within the UPE time courses not only between seedlings of the same species but also between the different species. In most cases, the UPE inflections were synchronised with the turning points in the local gravimetric tidal variation. Statistical tests using the local Pearson correlation verified these coincidences in the two time series. The results therefore support the hypothesis of a relationship between UPE emissions and, in the oscillations, the local gravimetric tide. This applies to both the emissions from seedlings of different species and to the seedlings raised from transported grain samples of the same species.

Keywords Biophoton emission · Chronobiology · Seed germination · Gravimetric tide

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Introduction

Although the behaviour of plants and animals, as well as of humans, was perceived long ago as being regulated in a periodic manner in relation to environmental cues (Sweeney 1969), investigations of the physiology of periodic behaviour have appeared only in the last 300 years (Luce 1971), with the first ideas of an endogenous timekeeper being established by the observations of de Mairan in 1729 on plant leaf movements (Marchant 1729). Advances in biochemistry and genomics in the last few decades have laid emphasis on temporal patterns of gene expression for such endogenous clocks (McClung and Gutierrez 2010), often without acknowledgment of the possibility of other sources of timekeeping, which some workers believed to co-exist with, and even be driven by, endogenous timekeepers (Brown 1970; Palmer 2005; Zhang et al. 2013; Fisahn et al. 2015). In relation to the latter, more subtle aspects of chronobiology, certain circadian-like rhythms of plants are apparently modulated by, and synchronised with, the local gravimetric tidal acceleration. These include the diurnal variations of tree stem diameter (Zürcher et al. 1998; Barlow et al. 2010), of root elongation growth (Fisahn et al. 2012) and of the nastic movements of leaves of bean and other species (Barlow and Fisahn 2012; Barlow 2015). Rhythms associated with the monthly lunar cycle have been found also for the imbibition and germination of bean seeds (Brown and Chow 1973; Spruyt et al. 1987).

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Another feature of plant growth which shows synchronism with the local gravimetric variations is the spontaneous, ultra-weak photon emission (UPE) from developing seedlings (Moraes et al. 2012). Even single sunflower seedlings have been found to present peaks of UPE activity which coincide with the turning points in gravimetric tidal time course (Gallep 2014). Spontaneous UPE from seedlings, a phenomenon first presented by Colli et al. (1955), is related to the activity of metabolic processes during germination and early seedling growth and has thus been found to be a useful real-time, non-invasive diagnostic probe of both viability and vigour (Chen et al. 2003, Gallep 2014) and of stress applied by the environment (Bertogna et al. 2013; Komatsu et al. 2014, Kamal and Komatsu 2015). The emissions are possibly directly related to the production of singlet oxygen ($^1O_2$) species released during enzymatic reactions, such as due to catalases and peroxidases (Miyamoto et al. 2014).

The results of our earlier observations on the rhythmic nature of the UPE (Moraes et al. 2012) were gained from seedlings that were raised and tested in a constant dark environment (free-running conditions). Thus, any rhythmicity recorded from the biological material would have been unlikely to have resulted from entrainment by an endogenous *Zeitgeber*. Instead, it was considered that biorhythms, such as those presented by the UPE time courses, could be due to some subtle exogenous influence, as proposed by a number of authors (Brown 1970; Fisahn et al. 2015; Zakhvataev 2015). Further work explored the coincidence between the gravimetric tidal variation and the rhythm of UPE and used a series of simultaneous trans-continental germination tests performed in Brazil and in Germany (Gallep et al. 2013). Later, when up to three locations, spread around the world in both Northern and Southern hemispheres (Brazil, Japan, Czech Republic) were used, results similar to those reported earlier were obtained: that is, the UPE rhythms from the seedlings were always in coincidence with the local time course of the gravimetric tide (Gallep et al. 2014).

In the present report, new UPE data are shown for Brazilian seed samples transported to The Netherlands and vice versa, with local and transported samples measured in parallel in both places. The novel feature of these experiments is that seeds of three different plant species—wheat, corn (both monocots) and sunflower (dicot)—were used, rather than making use of seeds of a single species (wheat), as previously. It is hypothesised that, even if the different species show different rates of germination and development, synchrony between the UPE and gravimetric oscillations should still be displayed in the three species when each is germinated and grown simultaneously, irrespective of whether the seedlings are raised at a common location (the present observations) or at widely separated locations (as in the previously reported experiments). Such a finding from the former case, i.e. synchrony between different species at a common location, would counter the criticism that the effect initially discovered in wheat was simply either a chance relationship or was a feature peculiar to this species alone.

Materials and methods

Initial tests in Limeira, Brazil (BR), commenced on 14 May 2014 using grain and seed samples of corn (*Zea mays*, standard cultivation), sunflower (*Helianthus annuus*, standard cultivation) and wheat (*Triticum aestivum*, organic cultivation). Native wheat grain samples were also transported by air (BR → NL) for tests in The Netherlands (NL), departing from Campinas (BR) on 29 June 2014 and arriving in Amsterdam (NL) on the next day (30 June 2014). Tests in Leiden (NL) started on 01 July 2014. Samples of native wheat grain harvested in Netherlands (*T. aestivum* L. cv. Demeter, biodynamic cultivation) were used for local (NL) experiments. A portion of these samples was also sent by air to Brazil (NL → BR), departing from Amsterdam on 20 July 2014 and arriving in Rio de Janeiro (BR) the next day (21 July 2012), finally being delivered at Limeira (BR) on 04 Aug 2014, where germination tests started immediately.

UPE measurements in Limeira used two simplified dark chambers (PMT01 and PMT02), each with a photon-counting unit (H7630, Hamamatsu K.K., Japan) (Bertogna et al. 2011) kept at a controlled room temperature between 19 and 21 °C. Each germination test used 25 wheat grains placed on a filter paper in a Petri dish (10-cm diam) with distilled water (10 mL). Tests with corn and sunflower used the same procedure, but with only 10 grains per dish.

UPE measurements in Leiden used a dual dark chamber with two similar photon-counting systems (Electron Tube 9235QA, as described in Van Wijk et al. 2010) running in parallel at room temperature (20–22 °C). Germination tests used 10 wheat grains placed on the filter paper in a smaller Petri dish (6-cm diameter) with distilled water (5 mL).

The placement of grains in the Petri dishes prior to imbibition took place under very weak, indirect illumination; imbibition and subsequent germination took place in total darkness. Photon counting commenced during the imbibition phase and continued throughout germination. The accumulated photon counts were stored by dedicated software.

A common time axis was used for all the various germination tests and UPE data collections run in Limeira. It commenced at 00.00 h on 1 May 2014. This date is day 0. Another time axis was used for tests run in Leiden. It commenced at 00.00 h on 1 July 2014 (day 0).

Raw UPE data—number of photon counts (PC) within a 10-s collecting window (no. of counts/10 s)—were smoothed by the averaging of successive groups of 100 counts. A corresponding time course of the local gravimetric tide, δg, with values estimated every 15 min [Supplementary Information],

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was prepared according to the *Etide* program, based on the equations of Longman (1959).

In order to compare the UPE of the simultaneously tested samples in relation to the local gravimetric tidal oscillations and also to compensate for the growth of the seedlings and the consequent increase in the cumulative photon count, the UPE profiles were normalised using a best-fitting parabolic function (ax^2+bx+c) for each UPE profile, as shown in the Supplementary Information.

The detrended, normalised plots revealed inflections at intervals, which indicated abrupt alterations (both increases and decreases) in the photon emission rate. These data are presented together with the contemporaneous local gravity deviation $\pm\delta g$ (μGal). One microgal (10^{-6} Gal) is an acceleration of 10^{-8} m/s^2 and 9.81×10^8 μGal = 1g.

The local correlation (Pearson) coefficient, r, for the detrended photon count data (d-PC) versus the local gravimetric tide δg was calculated using sliding windows of 1, 3, 6 and 12 h (see the Electronic Supplementary Material). For the tests run with transported grains, the local Pearson correlation was determined between the transported sample's d-PC and the d-PC from the reference local sample of the same species. Overall trends are presented in the main text together with certain examples of the local correlation data. However, details of complete time courses of d-PC together with the complete set of local Pearson correlation values are shown in the Electronic Supplementary Material (EMS).

To analyse the relationship between the PC oscillations and the oscillations of δg, the local peak-to-peak amplitude, Δ, of both time courses was determined. In the case of the dual wheat tests, run in Leiden (NL) and Limeira (BR), the respective d-PC amplitudes were measured and plotted against respective amplitudes of δg.

Results

The detrended UPE (d-PC) and the gravimetric δg plots for the preliminary tests, with different grains or seeds in Brazil, are presented at Figs. 1 and 2. Data from further tests in The Netherlands and in Brazil are shown in Figs. 3 and 4, respectively. The original UPE data (non-detrended) are presented in the Electronic Supplementary Material for germination tests run initially in Brazil with corn, wheat and sunflower (Fig. S1), for corn and wheat (Fig. S2), for tests run in Leiden, The Netherlands (Fig. S3) and for a final test in Brazil (Fig. S4), using both native BR and transported NL → BR wheat samples. However, it is the d-PC version of these data (Figs. 1 and 2) which are now discussed because, in these data, the critical points within the time course profiles are most clearly represented. Then, using these data, a search can be made for corresponding turning points in the δg time course. The two time courses, d-PC and δg, were submitted to statistical evaluation of coincident time profile using the local Pearson correlations.

In all cases, germination rates were >98 %. Total vigour, however, differed between the two wheat samples, BR and NL, probably because of the different cultivation methods used prior to grain harvest. The organically cultivated BR seedlings were three to five times smaller than the biodynamically cultivated NL seedlings, as examined by total sprout elongation, i.e. by summation of lengths of all samples' rootlets and leaflets (see example at Fig. S8, ESM).

Data from non-transported seeds

Data for the first 22 days of tests, run in Brazil with three different grain and seed samples—corn, wheat and sunflower—developing in parallel but with staggered starting dates during this 22-day test period, are shown in Fig. 1, data here being presented from day 14 (14 May 2014). The results from corn (c1) showed that the inflections in the d-PC time courses coincided with the local gravimetric ($\pm\delta g$) minima and maxima (tidal turning points), as expected from previous reports (Moraes et al. 2012, Gallep et al. 2013, 2014, Gallep 2014). The first corn (c1) germination test concluded after 7 days, on day 21 (21 May 2014). At this time, the first test using wheat (w1) was started in chamber PMT01. A second test with corn (c2) commenced on day 19 in PMT02 (19 May 2014) and, thus, was concurrent with test w1. d-PC showed many inflection points in coincidence not only with the d-PC data from the test with wheat (w1), which was running in parallel with c2, but also with the contemporaneous local gravimetric turning points (days 19–22, Fig. 1).

Even though the corn and wheat samples presented quantitatively very different d-PC data, there were many coincident d-PC inflection points, all of them occurring in coincidence with the gravimetric δg turning points (days 22–25, Fig. 1). Not only were the inflections of d-PC coincident in the two samples of wheat and corn but also the general trends in the two d-PC records were also similar, at least on some days. Coincidences in the two time courses, d-PC and δg, occurred, for example, on days 20 and 21 in the corn tests and on days 23–25, in both the corn and wheat tests. The second corn test, c2, concluded on day 25 (25 May 2014).

A simultaneous third test with corn (c3) and a sunflower test (s1) commenced on day 26 (26 May 2014) and the UPE recorded for the next 7 days. Although the d-PC patterns were quantitatively different in each species, they nevertheless show similar trends, namely, coincident inflection points of d-PC, many of which were also coincident with local δg minima or maxima. In these tests, similarity of the paired d-PC curves extended throughout the whole recording period, from days 26 to 32. On day 33 (2 June 2014), a new pair of corn and sunflower tests (c4 and s2) was commenced. Again, their respective d-PC curves showed similar trends, with many

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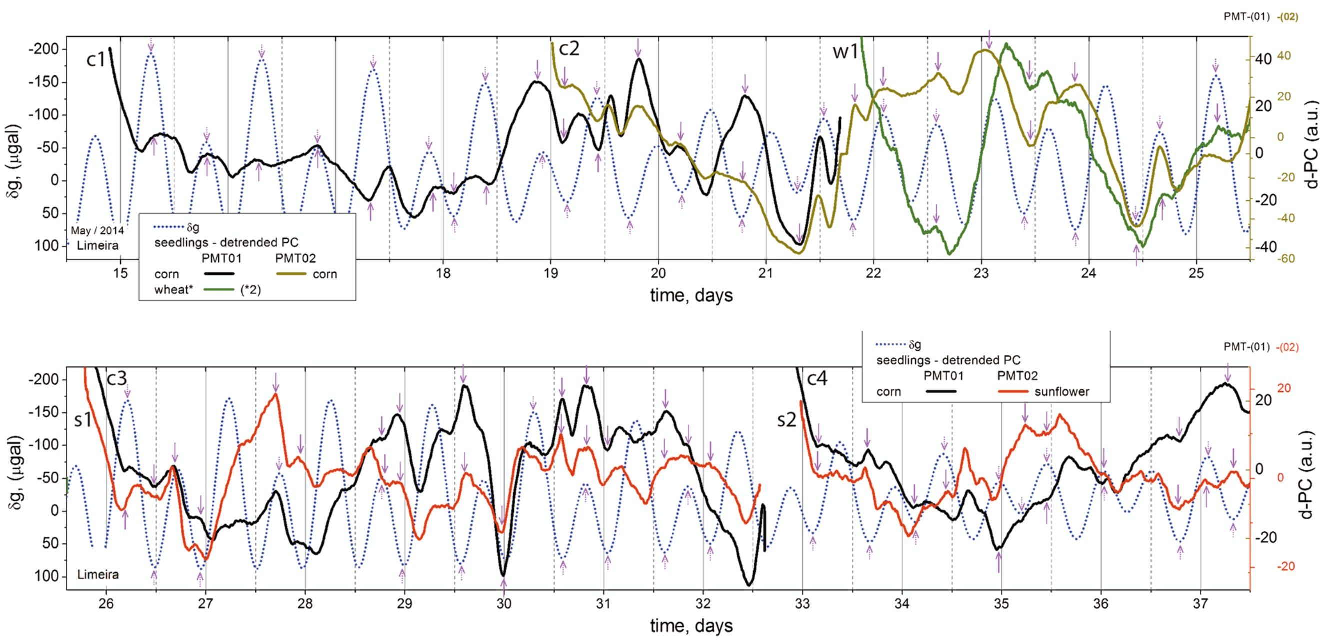

Fig. 1 Local gravimetric tide (δg) and detrended photon count (d-PC) data for germination tests of different grains and seeds, simultaneously run at FT/Limeira (BR), using equivalent PC setups: PMT01 (c1, w1, c3, c4) and PMT02 (c2, s1, s2). Time axis commences on 1 May 2014 (day 0). *Marks* over significant d-PC inflections (*straight-line arrows*) and related δg turning points (*dotted-line arrows*). See full PC curves at Fig. S1 (ESM)

coincident inflection points, these also being in synchrony with turning points of the contemporaneous gravimetric tide.

Figure 2 presents the detrended UPE data of a third corn test (c5) run in parallel with two further wheat tests (w2, w3), at the end of July 2014. The d-PC of corn and wheat, although different, showed similar trends for the first hours of growth—days 82–83 and days 86–87 (21–22 July 2014 and 26–27 July 2014, respectively). During the whole period, coincident

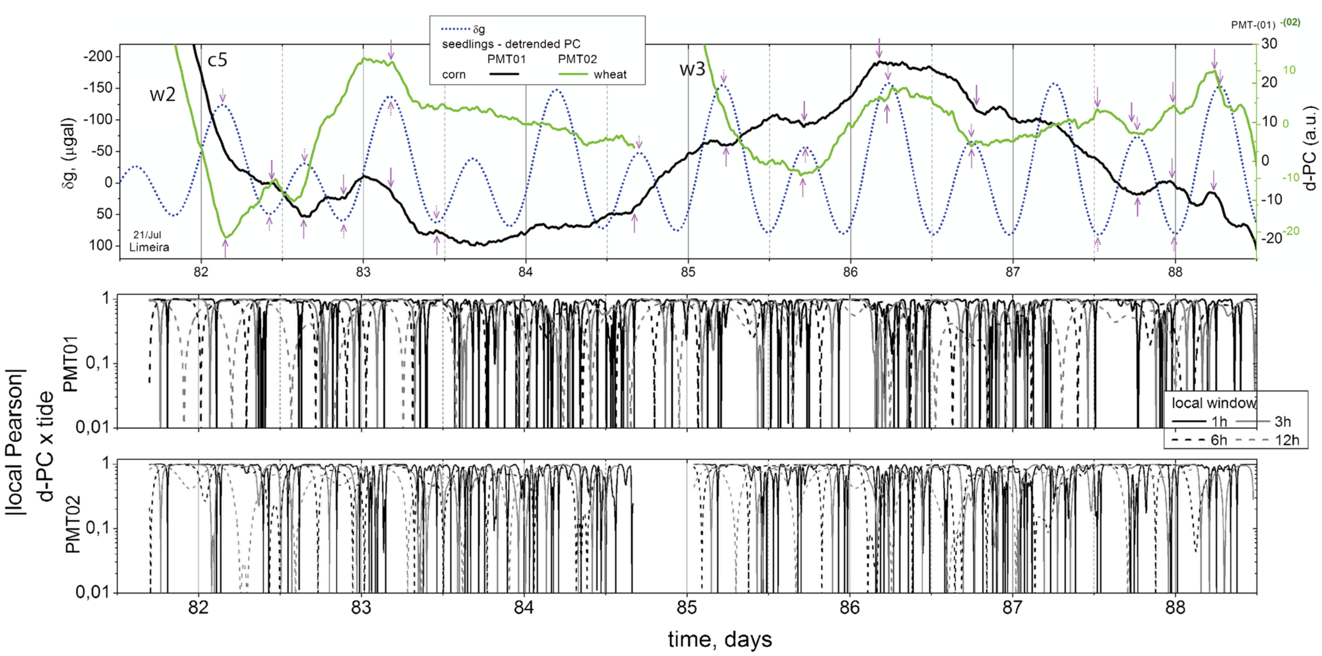

Fig. 2 *Upper panel*: local gravimetric tide (δg) and detrended photon count (d-PC) data for germination tests of different grains, simultaneously run at FT/Limeira (BR), using equivalent PC setups: PMT01 (c5) and PMT02 (w2, w3). Time axis continues Fig. 1. *Marks* over significant d-PC inflections (*straight-line arrows*) and related δg turning points (*dotted-line arrows*). *Lower panels*: absolute value for local correlation (Pearson) factor of d-PC data of PMT-01 and PMT-02 versus δg, for local time windows of 1, 3, 6 and 12 h. See full PC curves at Fig. S2 (ESM)

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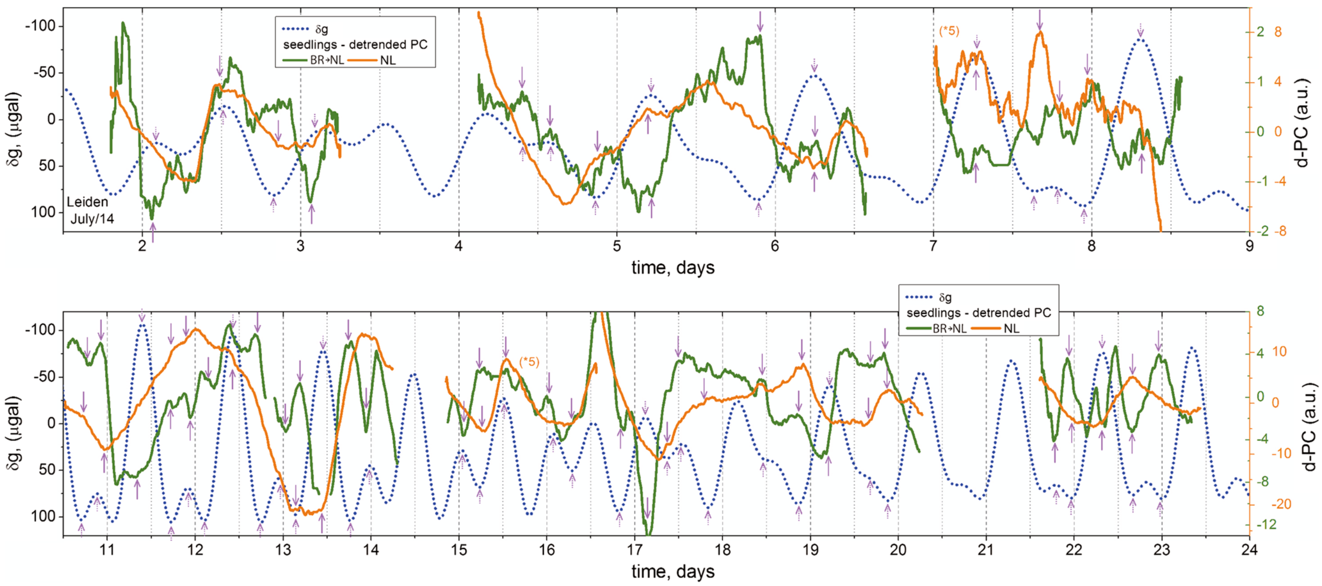

Fig. 3 Local gravimetric tide (δg) and detrended photon count (d-PC) data for germination tests of wheat grains, simultaneously run at LACDR/Leiden (NL), using dual-chamber PC setup: native (NL) and transported (BR → NL) samples. Time axis starts at 1 July 2014 (day 0). *Marks* over significant d-PC inflections (*straight-line arrows*) and related δg turning points (*dotted-line arrows*). See full PC curves at Fig. S3 (ESM)

inflections of d-PC were synchronous with local minima or maxima of the gravimetric tide δg.

Statistical evidence was sought for the coincidence of trends in the d-PC and δg time series: an example of the results from the local Pearson correlation for the mentioned tests is shown in Fig. 2 (lower panel)—the absolute value of the local Pearson factor, for sliding windows of 1 h up to 12 h, was close to 1 (i.e. the trends are highly correlated) most of the time. Steep falls in the correlation to values below 0.01 are the points where PC or, mainly, δg presents inflection points, and so the local Pearson correlation values change rapidly, passing through zero and becoming negative, even though having high absolute values—i.e. the change is to a strongly inverse correlation—as one signal goes up the other goes down, but in a highly correlated fashion.

Other results of statistical testing are presented in the Electronic Supplementary Material. The conclusions from these analyses are similar throughout.

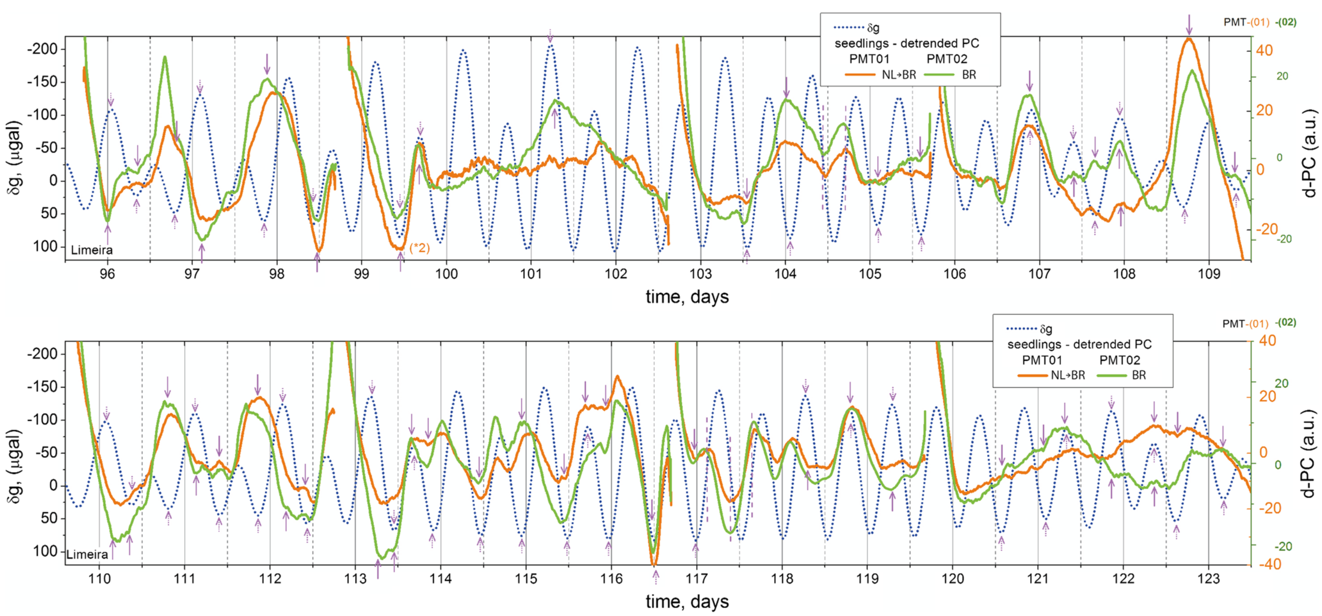

Fig. 4 Local gravimetric tide (δg) and detrended photon count (d-PC) data for germination tests of wheat grains, simultaneously run at FT/Limeira (BR), using equivalent PC setups: transported NL → BR (PMT01) and native BR (PMT02). Time axis starts at 1 May 2014 (day 1). *Marks* over significant d-PC inflections (*straight-line arrows*) and related δg turning points (*dotted-line arrows*). PC inflections related to max ($\delta g/\Delta t$) marked with *vertical dashed lines*. See full PC curves at Fig. S4 (ESM)

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Data from transported grains

At the end of June 2014, BR wheat samples were transported to Leiden (NL). UPE tests of varying duration (2–5 days) with these BR→NL grains were commenced on day 1 (2 July 2014 and using an alternative time axis) using native (NL) wheat samples running in parallel for comparison (Fig. 3). The BR→NL seedlings had a lower vigour than their native NL counterparts, and this may account for their lower UPE intensity (Figs. S3 and S8, ESM). This disparity is reflected in the d-PC curves of the BR→NL samples, which, in many cases, appeared less smooth than those of the native NL samples. Again, in all seven tests, similar trends for the d-PC curves were found, with many coincident inflection points in the d-PC time series in synchrony with the gravimetric δg tidal turning points.

Part of the same stock of NL samples was sent to BR where similar parallel UPE tests were run, commencing on 4 Aug 2014 (day 96) and continuing until 21 Sept 2014 (day 123). Eight sequential tests with these NL→BR grains and native BR grains were performed, and the d-PC data are plotted in Fig. 4. For both samples, NL→BR and BR seeds, the d-PC trends were similar for almost the entire 29-day span of the tests, with many coincidences of inflections of d-PC with turning points of the local gravimetric tide. Prominent similarities appeared in the first of the eight test pairs (days 96–99), in part of the second test pair (days 99–101), in the third test pair (days 103–106), in the fourth and fifth (days 106–109 and days 110–112), in the sixth and seventh tests (days 113–116 and days 117–119) and in the first days of the final eighth test of the pair of seedlings.

In all tests, the pattern of the time courses of both the d-PC and δg changed in synchrony, with coincidences between the respective inflections and turning points. Minor fluctuations in the patterns occurred in cycles of 6 and 12 h, whereas more major trends occurred with an approximate 24-h periodicity. To verify the coordinated trends in the two time courses, local Pearson correlation coefficients, r, were used, choosing time windows (Δt) of 1, 3, 6 and 12 h. The detailed results for the absolute values of r in relation to d-PC versus δg for the time course of the five corn seedling tests and for the two sunflower tests run in Limeira (BR) are shown in lower panels of Figs. S1 and S2 and their relative occurrence for each window size shown in Fig. S5 (ESM). This information is summarised in Fig. 5. Very high local correlation appeared for local window of $\Delta t = 1$ h, with approx. 70 % of the time course presenting Pearson r values >0.9. The value or r decreased slowly as Δt increased, with $r > 0.9$ over approx. 55 % of the time course for $\Delta t = 3$ h and approx. 25 % where $\Delta t = 6$ h and finally becoming very small (<10 %) when $\Delta t = 12$ h. These total occurrences (%) are found by integrating the relative occurrence for $r > 0.9$ in each case.

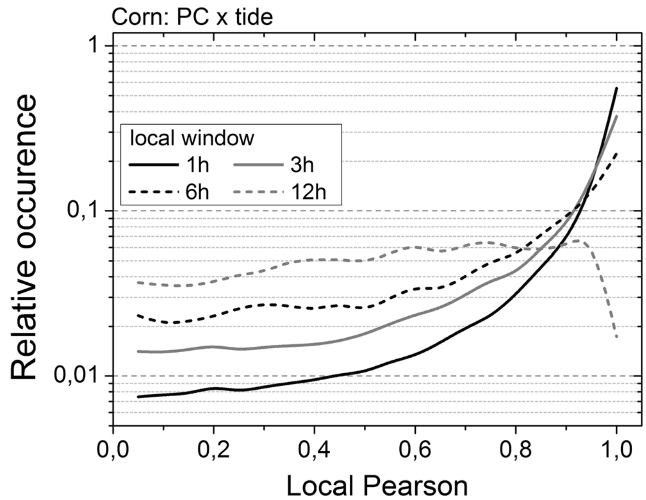

Fig. 5 Relative occurrence of the overall local Pearson factor for (detrended) PC × δg (tide), for the five germination tests of corn (see *lower panels* of Fig. S1 and Fig. S2 (ESM)/Fig. 2), for local windows of 1, 3, 6 and 12 h

A high local correlation was expected on the basis of previous results (Moraes et al. 2012; Gallep et al. 2014) using wheat seedlings. Accordingly, a similar study was performed for the tests run in Leiden (NL) (Figs. 6 and S6) and Limeira (BR) (Figs. 7 and S7). Strong correlations between d-PC and δg were found for the wheat tests run in Limeira (BR), for both the native BR and the transported NL→BR samples. For the intersample series of wheat seedling tests, high correlations between the d-PC time courses appeared when all window sizes were used, being even slightly higher when either $\Delta t = 6$ h or $\Delta t = 12$ h was used (Figs. 7 and S7).

Such strong correlations between d-PC and δg did not appear for the wheat grain tests run in Leiden (Figs. 6 and S6). Here, both the native NL and transported BR→NL samples presented small Pearson r values when $\Delta t = 1$ or 3 h, whereas slightly higher r values emerged when $\Delta t = 6$ and 12 h, with $r > 0.9$ occupying 20–30 % of the time course for the BR→NL samples and 35–45 % for NL samples. The intersample correlation was very small when $\Delta t = 1$ or 3 h ($r > 0.9$ for only 3–5 % of the time course) but appears more relevant (11–25 %) for the longer Δt window periods.

From the d-PC time course profiles at Leiden (Fig. 3), we can see that, although some common long-term similarities appear between the d-PC curves, the local patterns differed greatly, with shorter oscillations occurring for the transported BR→NL samples, a feature which did not appear for the native NL samples. For both native and transported wheat samples, the d-PC values presented inflexions coincident with gravimetric tidal turning points, but the d-PC variations did not follow the tidal pattern in the same way, as observed in the other tests.

To check the relationship between tidal and PC amplitudes for the series of tests run in either Leiden or in Limeira, the local variation Δ(d-PC) and the contemporaneous Δ(δg)

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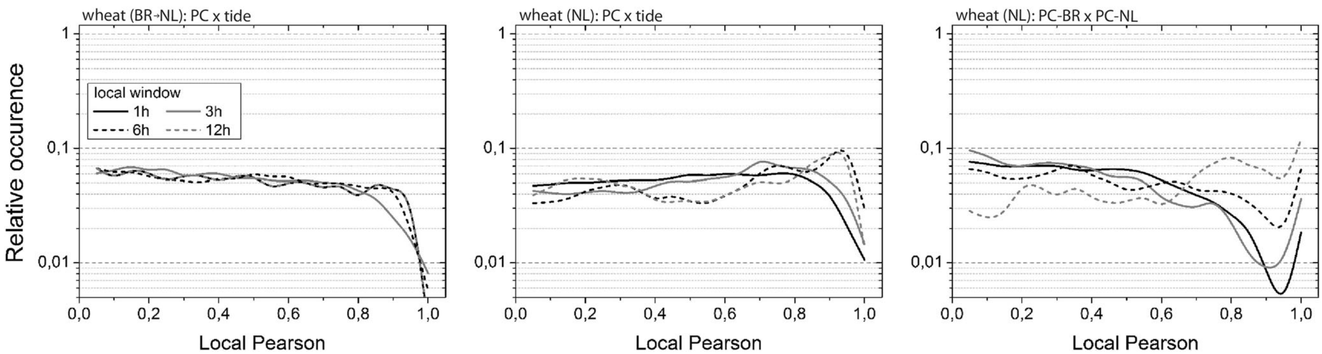

Fig. 6 Relative occurrence of the overall local Pearson factor for (detrended) PC × δg, for the wheat germination tests run in Leiden/NL (*lower panels* of Fig. S3 (ESM)), using transported (BR → NL) and local (NL) grains, and also for PC × PC (intersample correlation, NL), for local windows of 1, 3, 6 and 12 h

variation were plotted in datagrams shown in Fig. 8. For both series of tests, there was a positive relationship, with high tidal amplitudes being associated with high d-PC variations for both local and transported samples. An exception occurred in one single case, in Leiden (third test, days 7–9 of Fig. 3), and was not considered in the linear regression shown. This single case occurred during a period when the local tide presented almost no 12-h components; i.e. only 24-h variations were found on those dates. In both these portions of the amplitude data from Leiden and Limeira, a linear relation was found for the NL samples ($r > 0.7$) and a stronger one ($r > 0.9$) for the BR samples.

Discussion

It is clear from the present data that seedling development, monitored in real time by means of the UPE recordings, appears synchronised with the local gravimetric oscillations. This synchronism also occurs for UPE emission in the tests run in parallel with different seedlings—wheat, corn and sunflower—and for grain samples transported trans-continentally, from South America to Europe and vice versa, and which were tested concurrently with samples native to the locality of testing.

The tests run in Limeira (BR) presented very high local correlation (Pearson) values for d-PC versus gravimetric tidal trends, but the tests run in Leiden (NL) showed much smaller correlation values. Although in all cases, many d-PC inflexions were coincident with the maxima and minima of the gravimetric tide, the amplitude of the tidal variation at Leiden (NL) was, on some days, much smaller than that found at Limeira. Some tests in Leiden coincided with tidal amplitudes of <100 μGal during in a complete day cycle—as can be seen at the beginning of the series, from day 2 to day 7, shown in Fig. 3. Then, when the maximal tidal amplitude (approx. 200 μGal) occurred from day 8 to day 14, the d-PC data also presented a high amplitude. During these days of large tidal swings, the 12 h-cycle components were diminished; i.e. the gravimetric tide tended to oscillate with a 24-h period, with very small secondary peaks. By contrast, this never happened during the Limeira tests, where the peak-to-peak tidal amplitude varied between $\Delta = 150$ and 300 μGal, and the 12-h component was always present; i.e. there were two tidal peaks per day. This might be the basis for the possible tidal effect upon seedling development, since in Leiden, also, the d-PC cycles appeared to have more pronounced 24-h components as compared to the 12-h component, found at Limeira.

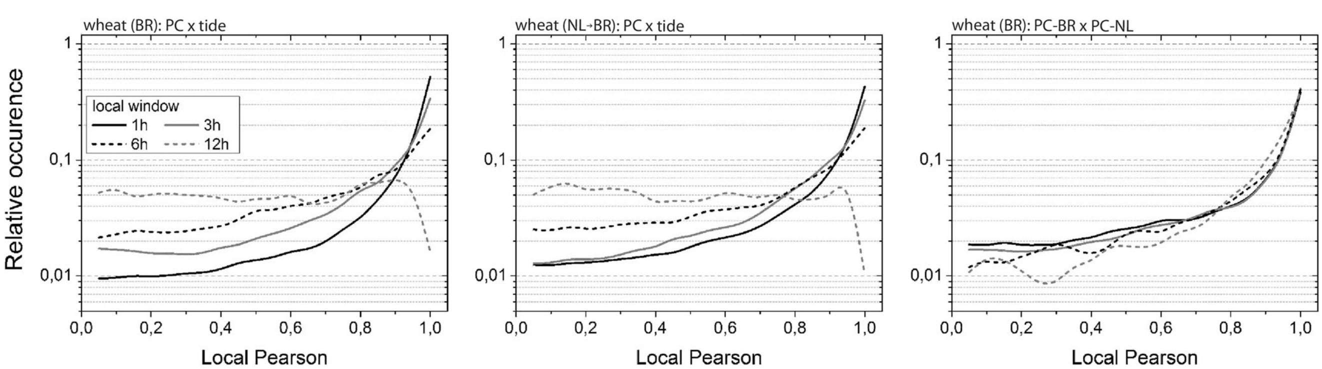

Fig. 7 Relative occurrence of the overall local Pearson factor for (detrended) PC × δg, for the wheat germination tests run in Limeira/BR (*lower panels* of Fig. S4 (ESM)), using transported (NL→BR) and local (BR) grains, and also for PC × PC (intersample correlation, BR), for local windows of 1, 3, 6 and 12 h

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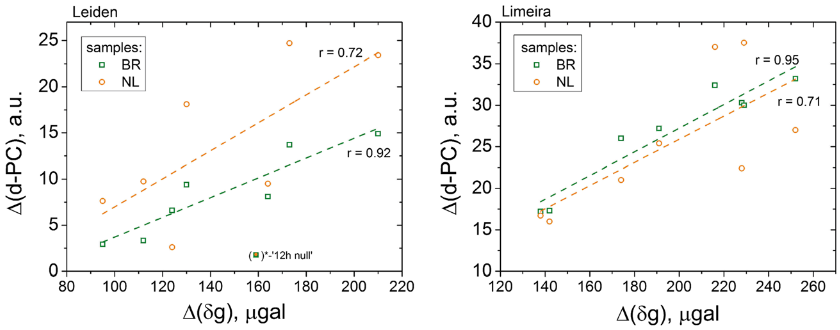

Fig. 8 Local cycle total amplitude (Δ) of d-PC versus the respective amplitude of δg, for the wheat tests run in Leiden (*left panel*) and Limeira (*right panel*), for both local and transported samples, and the linear interpolation for each group of tests (*dashed lines*), with respective total Pearson factor (r) (*asterisk* pair of points of Leiden (third test), outlying from the main trend, not considered for actual interpolation)

It is possible that the gravimetric tidal force acts, together with other periodic exogenous factors, such as geomagnetic variations (cf. Barlow et al. 2013) as a trigger of biological processes, and that these might also involve the generation of reactive oxygen and nitrogen species, mitochondrial permeability transitions, rhythmic gene expression and the modulation of cell signalling, as proposed by Zakhvataev (2015).

For the present study, we checked the fluctuations of some standard geomagnetic indices (i.e. Kp, Ap, Dst—data obtained from OMNIWeb Data Explorer/NASA) during the relevant test period. No particular fluctuations were found which might account for the inflections in the UPE data. Moreover, geomagnetic fluctuations are mostly modulated by Sun–Moon–Earth positioning, thereby making it difficult to dissociate the gravimetric cycling from the geomagnetic variation, as found earlier (Barlow et al. 2010, 2013), because the latter appears to be determined by the lunisolar variations. Because all tests were done in a closed atmosphere, within a metallic dark chamber, possible effects on UPE due to changes in the magnetosphere would be reduced to less than one tenth of that occurring in an open-field situation (Bevington 2015). Nevertheless, such magnetic variations cannot be discounted as possible trigger sources for some of the UPE variation, as exhibited by the inflections in the UPE time courses.

Severe geomagnetic disturbances could not be completely avoided within the recording chambers, although these would be strongly diminished by the metallic walls, but not completely so.

Moreover, during the present time series, some of the δg turning points were not accompanied by abrupt geomagnetic changes but were nevertheless coincident with UPE changes. Conversely and as already mentioned above, some of the geomagnetic variations which occurred were not synchronised with UPE variations. Future tests of UPE should nevertheless consider the geomagnetic environment, particularly at times when strong geomagnetic storms are present and, perhaps, take into account the effect of such storms on seed germination (Neamțu and Morariu 2005).

In the case of the germination process, three main phases may be considered: imbibition (phase I), an inductive phase for biochemical process (phase II) and the start of growth (phase III or post-germination) (Zadoks et al. 1974). Phase I starts with the formation of an aqueous matrix within the seeds; here, the mature kernel roughly consists of 83 % endosperm, 14.5 % bran and 2.5 % embryo, and metabolism is reactivated by enzymes related to both respiration and the utilisation of storage macromolecules (Carver 2009). This is reflected in the early UPE increase during the first 6–12 h. After this imbibition stage, phase II starts, with preparation for biochemical process related to the induction of growth. Contrariwise, phase I, with the absorption of water, and the respiration at phase II are both dramatically reduced at the time when the digestion of reserves—with the help of amylases, lipases and proteases—is providing the energy for growth and is accompanied by renewed synthesis of sugars, triglycerides, amino acids and peptides. These metabolites diffuse towards the embryonic axis, enabling the formation of new cells and tissues. Radicle and coleoptile emerge at phase III.

If the biochemical activity included in these processes is somehow modulating the UPE signal, causing it to increase in jumps, it might be that all activities occur in cycles and that these are triggered, or modulated, by the gravimetric tide. Moreover, if these biochemical changes could be traced in time and UPE be recorded simultaneously, it might be possible to discover the key reactions which relate to these tide-like cycles. Another possibility is that cellular growth is occurring in a rhythmic manner and is modulated by the gravimetric tide, as shown for *Arabidopsis* root growth (Fisahn et al. 2012). Rhythmic cellular growth might itself be modulated by rhythms of water uptake, as described by Barlow and Fisahn (2012). Such rhythms relating to water uptake have not so far been recorded on an hourly basis throughout a day, but have been recorded for seed imbibitions in the course of longer periods, such as the lunar month (Brown and Chow 1973, Spruyt et al. 1987).

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Conclusion

It has been shown that seedlings of different species as well as seedlings grown from seeds transported between continents present patterns of variable rates of photon emissions, the latter being taken to be fine-scale markers of growth, which are in synchrony with the gravimetric tidal oscillations. Time courses of UPE alone, measured by a photon-counting apparatus, revealed many inflections in the data stream that were coincident with tidal turning points. However, additional simultaneous tests using different seedling material also showed UPE inflections coincident not only with the tidal variation but also between the UPE variations in the different seed or grain samples.

Further experiments will be aimed at consolidating the proposal of an exogenous modulation of this particular, growth-linked trait of photon emission. A subsequent step would be to identify the source of the biophoton emissions and the possible significance it may have in relation to plant–environment interactions.

Evident, also, is the problem of how minute changes in the estimated values of the Earthly gravitational acceleration (μGal), as well as the accelerative forces due to the Moon, could act upon the organism, i.e. which specific physiological or biochemical agents are affected by the tides, and where the results of their interaction are cyclic or rhythmic growing process (Fisahn et al. 2015, Barlow 2015). And, more than that, how this "sensing" could be possible, given that the energies involved are much less than the thermal fluctuations at room temperature. This problem is evoked in the so-called kT paradox (Binhi and Rubin 2007). To the best of our knowledge, it might not be possible to provide a solution to this paradox from the data presented or with this particular material.

Nevertheless, it is crucial that this problem should be addressed. It is held that such a paradox could exist at the chemical level—but not at the physical level—taking the assumptions listed below from Binhi and Rubin (2007):

1. Primary energy reception occurs at the atomic/molecular level.
2. The interaction is a single, quantum process.
3. During interaction, the target is in thermal equilibrium.

It is suggested that, in biological systems, the above assumptions do not always apply. Energy absorption (point 1 above) can occur at very complicated interfaces, which may be able to store energy and, hence, be far from thermal equilibrium, at least over the time spans considered for energy absorption. Thus, these assumptions would not be applicable if

- For point 1, structural, macroscopic levels were considered for the energy exchange.
- For point 2, multiple-quantum processes were involved, i.e. non-linear interaction, with more than one quantum of energy exchanged. So far, interactions with quanta of gravity are proposed only as a theoretical possibility (Dorda 2010).
- And for point 3, thermalisation was slow enough to restrain the system from thermal equilibrium. In any case, the critical point with regard to biological responses to the changing lunisolar gravitational tide is that it is not definitely known which processes, parts or physiological systems are involved in such delicate sensing during early plant growth. Nor is it known what kind of system would respond to changes below the kT level. Maybe, the entire biological entity acts in a collective response, and so, no particular part would be found to play the part of a central mechanism (Barlow 2012), unlike in the example of root gravitropism where statocytes with amyloplasts seem to provide mechanisms responsible for detecting deviations from the plumb line (Morita 2010). Nor is it known whether the lunisolar system is affecting classical physical phenomena or whether there are quantum events which are mediating the response process (Barlow 2015).

In the case of electro-magnetic (EM) reception, where this kT paradox is mostly often discussed, the arguments of the paradox have been used to deny any possible interactions between the geophysical and biological systems. Reactions involving free radical pairs would be maybe the only reliable case where EM fields could act to produce metastable states, with so quick response that thermalisation cannot occur. But, such kind of process is ineffective for weak interactions as those in order of the geomagnetic field (Binhi and Rubin 2007).

The level of complexity of biological tissues might itself affect many electrochemical interactions—for example, the surrounding conditions could change the random currents of ionic components in the blood in such a way that a compound in the aorta would obtain 10^{-9} times less energy than in a capillary artery (Kanokov et al. 2010). A specific type of macroscopic agent, or system of complex resonance and energy storage inside the organism, may occur in the case of responses to the lunisolar gravimetric tides, but this awaits elucidation. Apparatus for compensating for the lunisolar tidal acceleration is being developed at present and will help clarify whether or not such small accelerative forces can be detected by plant tissues (J Fisahn, personal communication).

Furthermore, arguments concerning the acceleration thresholds for the gravity response in plants—the absolute or the discrimination thresholds—are probably invalid for the cases where biological processes, such as those that occur in photon emission, are putatively affected by the lunisolar gravitational force. Graviresponses hitherto analysed in terms of thresholds (ca. $10^{-3}g$) have been related to differential organ growth (plant gravitropisms). In such growth responses, minute gravity changes may well fail to be transduced into a

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tropism simply because the signals are dissipated within the complex growth pathways necessary for the realisation of the tropism. Moreover, as pointed out by Hoson and Wakabayashi (2015), plant tissues and organs are actually constructed to resist gravity. It is only by dint of massive gravity susceptors (statoliths) that a gravitropic response is initiated at all. Plant tissues and their development are also responsive to the manner in which gravitational forces are applied (Barlow 1998). By contrast, responses to weak gravitational forces at the level of the molecular makeup of the cell membrane and associated vesicles, say, and perhaps existing in only a limited number of cells (cf. Barlow 2015) could be registered and then be transduced through only a few molecular events, thence initiating photon emission.

Following a careful biophysical analysis of the relevant factors, Persinger (2014) concluded that cells could indeed be affected by minute accelerative forces. For example, the gravitational force acting over a cell is in the order of pico-Newton (10^{-12} N). When applied across the cell membrane (10^{-8} m), this force could lead to a total energy of $\sim 10^{-20}$ J, which is similar to the levels of the action potential relative to the resting membrane potential. So, even without considering specific macroscopic agents that would able to perceive gravity, the direct effect on the cell level might be enough, if not to trigger, at least to drive, certain sensitive physiological processes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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