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Three-dimensional distribution of the UV-inducible blue-green fluorescence in leaves

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ABSTRACT Ultraviolet (UV, 290-360 nm) excitation provokes 400-560 nm intrinsic light emission from a vast majority of plant leaves. Using confocal laser scanning microscopy, we show in this study that blue-green fluorescence (BGF) mainly originates in the epidermal surface of *Sorghum vulgare* leaves. BGF was also detected from the cell walls and stomata guard cells. Our experiments support earlier suggestions on the heterogeneous nature of BGF fluorophores.
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KEY WORDS

blue-green fluorescence
laser scanning microscopy
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The phenomenon of UV-inducible blue-green fluorescence (BGF) has been known for almost 20 years (Chapelle et al. 1984, 1985). Since the discovery of the phenomenon, several laboratories confirmed that the emission intensity and spectral distribution of blue-green fluorescenc (BGF) was different in different plant species and was influenced by the physiological status of the plant (Moya et al. 1992; Schweiger et al. 1996; Johnson et al. 2000). A wide variety of sources have been accounted BGF emission, listing basically all plant cell components capable of fluorescence in vitro. In this way, ferulic acid, flavonoids and simple phenols, NADPH and flavin nucleotids have been suggested as emitter molecules (Cerovic et al. 1993; Morales et al. 1996; Lichtenthaler and Schweiger 1998).

The aim of the present work is to describe the cellular localisation of BGF in *Sorghum* leaves. Mechanical damage may alter BGF emission, both biologically – as a wounding stress –, and chemically – by suddenly exposing intrinsic plant substances to oxygen. In this way, the three dimensional study was conducted non-invasively, using confocal laser scanning microscopy (LSM) by making optical sections.

Materials and Methods

Sorghum vulgare plants were grown in a greenhouse, at 20-24 °C, under natural light conditions. Second and third leaves of three weeks old plants were used. For microscopy, 5 x 5 mm leaf cuttings (taken 3-4 cm from the leaf apex) were put between two layers of UV-transparent microscope cover glass (MicroStandard Cover Glass, Matsumi Glass, Japan) and measured using a confocal laser scanning system (LSM 510, Karl Zeiss, Germany) in combination with an inverted microscope (Axiovert 100 M, Karl Zeiss, Germany). Adaxial sides of the leaf segments faced the 351 nm Ar laser excitation (80 mW, ENTCII-653, Coherent Enterprise, Santa California, USA). Fluorescence was imaged from leaf areas excluding the vascular tissues. Fluorescence emission was observed through filters: 385-470 nm for blue, 505-550 nm for green and above 650 nm for red fluorescence. Images were scanned at 0.8 s per frame, averaging 4 images.

Results and Discussion

Typical images of UV-inducible fluorescence from *Sorghum* leaves illustrated that both blue and green light emission was most pronounced at the epidermal surface. This was characteristic to both the adaxial (Fig. 1) and abaxial (data not shown) surface of the leaves. Red ($\lambda > 650$ nm) chlorophyll fluorescence was relatively small at the surface (Fig. 1c) as compared to emission from the photosynthetically active cells located in 10 – 30 mm distance from the epidermal surface (Fig. 1f and i). Contrary to red fluorescence, both blue ($385 < \lambda < 470$ nm) and green ($505 < \lambda < 550$ nm) emission was weaker inside (Fig. 1d,e,g and h) than at the leaf surface (Fig. 1a and b). At the former location, BGF was almost entirely confined to stomata guard cells. In *Sorghum* leaves, guard cells contain only a few chloroplasts (2-2 at each end of the stomata are clearly marked by their red fluorescence emission in Fig. 1f), which did not show BGF emission. Contrary, the interior and cell wall of the guard cells emitted BGF but no red light. Blue light induced, green fluorescence emission has been reported previously in onion guard cells and attributed to flavins (Zeiger and Hepler 1979), which are also excitable by UV radiation (Morales et al. 1994). On the other hand, intense BGF emission from the epidermal surface (data not shown), supports the putative role of cell wall components, such as simple and polyphenols (Chapelle et al 1985; Lichtenthaler and Schweiger 1998). In our *Sorghum* experiments, BGF emission was not observed from chloroplasts, contrary to earlier reports on such emission in other plant species (Chapelle et al. 1991; Latouche et al. 2000). However, in barley leaves under oxidative stress conditions symplastic – presumably chloroplastic – BGF emission was found (Hideg et al. 2002).

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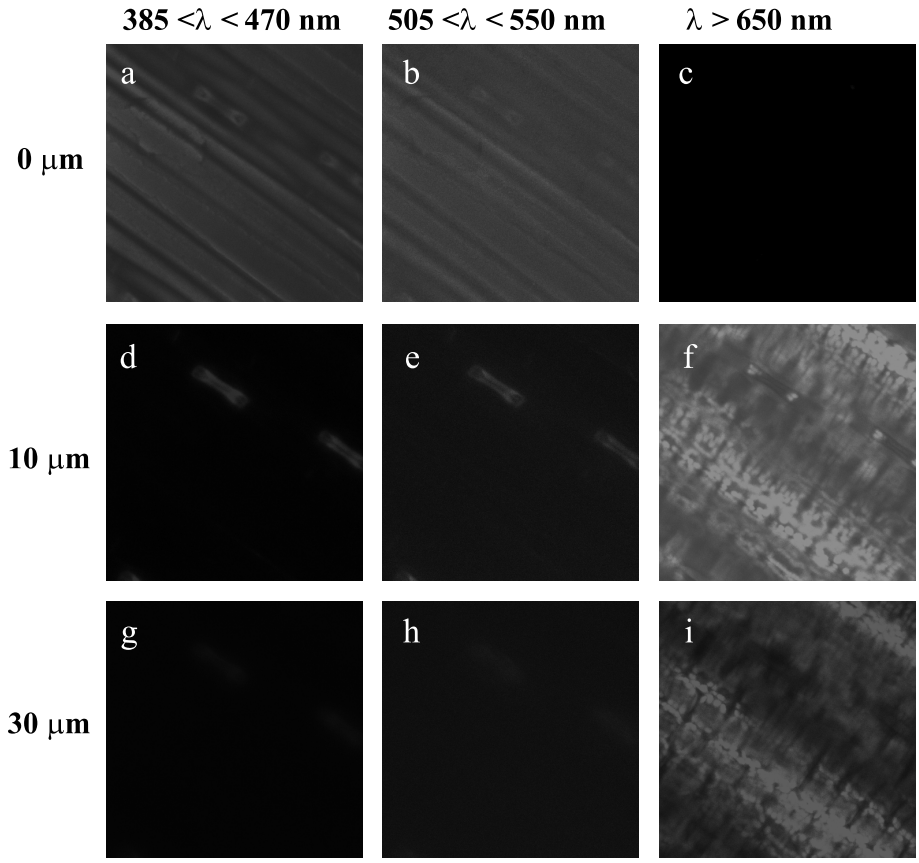

Figure 1. LSM images of 351 nm excited (a,d,g) blue (385 < λ < 470 nm), (b,e,h) green (505 < λ < 550 nm) and (c,f,i) red (λ > 650 nm) fluorescence detected in an untreated *Sorghum* leaf at (a-c) the adaxial leaf surface, or (d-f) 10 μm and (g-i) 30 μm from it. Image size 200 x 200 μm.

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