An image-processing-based technique for detecting quantitative fluorescence in plant cells

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Method Article

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Abstract

Biologists can now solve complex environmental problems by fabricate practical plant organisms, difficulties related to characterisation of cellular architectures of plant cells are often encountered which constrained the application of plant cells in synthetic biology. The objective of this study was to develop a automated, accurate and high-throughput quantitative analysis method ACFVA for single plant cell identification. ACFVA can address a variety of biological questions quantitatively of large number of plant cells automatically including standard assays (for example, cell localization, count and size) and complex morphological assays (for example, different fluorescence in cells). These assays can be used in a wide range of synthetic biology directions.

Introduction

With the development of modern technology, more and more problems such as cleaning hazardous waste in inaccessible places (Cases and Lorenzo, 2005), sensing chemicals dynamically and responding accordingly (Antunes et al., 2006; Bowen et al., 2008) and producing clean fuel (Savage et al., 2008) need some novel and convenient approaches for solving these complex problems. Synthetic biology has the potential to combines the investigative nature of biology with the constructive nature of engineering which can meet the above requirement (Feng et al., 2022; Purnick and Weiss, 2009). Especially, when plant cells have applied into creation and perfection of genetic devices and small modules which would bring significant advances in synthetic biology (Brophy, 2022; Zhu et al., 2021). Thus, efforts focusing on characterisation of cellular architectures of plant cells is important for the development of synthetic biology and fabricate practical organisms to solve problems.

Examining plant cells by microscopy has long been a primary method for studying plants’ cellular function, visual analysis can reveal biological mechanisms with the help of fluorescent light (Carter et al., 2013; Schweizer, 1980). Advanced microscopes can now, easily collect thousands of high resolution images of plant cells in a single day (Carpenter and Sabatini, 2004). However, a bottleneck exists at the image analysis stage. Several pioneering large screens have been scored through visual inspection by expert biologists (Kiger et al., 2003; Kim et al., 2005), whose interpretive ability will not soon be replicated by a computer. Still, for most applications, cell image analysis is strongly preferable to analysis by eye. It was much more labor-intensive and lower-throughput that may require months of tedious visual inspection to finish. Thus, there was a pressing need in appropriate software which can produces reliable results from a large-scale microscopy picture in hours. Till now, there are several commercial (for example, Imaris (Gilda et al., 2021), Volocity (Perkel, 2002) and Amira (Stalling et al., 2005)) and open-source (for instance, ImageJ (Collins, 2007), CellProfiler (Carpenter et al., 2006), Vaa3D (Peng et al., 2014), BioImageXD (Kankaanpää et al., 2012), Icy (de Chaumont et al., 2011), and Konstanz Information Miner (KNIME (Berthold))) software or platforms designed for the analysis of biological microscopy images. Among these, commercial platforms/software often focused on ease of use and broad coverage of image-processing tasks which were targeting relatively unprofessional assignments. And almost invariably the principal details of the image-processing algorithms are hidden, which is undesirable for
use in biological research. Conversely, these details are transparent in open-source platforms such as ImageJ, whose long existence, wide adoption and extensible plugin architecture has made it a tool of choice for scientists from a broad range of disciplines. But ImageJ was developed primarily by biologists for biologists, and its architecture does not follow modern software-engineering principles which made ImageJ is unable to process automatically (Collins, 2007). To adapt to modern technology in computer, several open-source image analysis platforms have developed such as Vaa3D21 which can make visualization in three dimensions (Peng et al., 2014), CellProfiler which can achieve trainable segmentation of animal cells (Carpenter et al., 2006) and Fiji which can assembling diverse image-processing steps into complex pipelines with the use of scripting languages(Schindelin et al., 2012). However, none of these above software is designed for plant cell characteristics which were different from animal cells (Liu and Stewart Jr, 2015), the results of image analysis of plant cells by this software were inaccurate. In practice, therefore, researchers still need to identify the characteristics of plant cells by humans. However, human-scored image analysis is qualitative, usually categorizing samples as 'hits' (where normal physiology is grossly disturbed) or 'non-hits which would cause subtle samples missed(Carpenter and Sabatini, 2004; Kiger et al., 2003). By contrast, automated analysis rapidly produces consistent, quantitative measures for every image (Stöter et al., 2013). This is very important for synthetic biology whose emphasis was engineering and conformity to obtain systems-level conclusions from the quantitative measures a large number of features even features undetectable by eye for every image (Feng et al., 2022; Schweizer, 1980).

In summary, while existing software enables particular assays for particular cell types, high throughput image analysis for plant cells has, to this point, been impractical unless commercial packages are used with their built-in algorithms for a limited set of cellular features and for a limited set of plant cell types such as the whole stain leaves or roots (Cai et al., 2015; Sekulska-Nalewajko et al., 2016). There exists a clear need for a powerful, flexible, open-source platform for high-throughput plant cell image analysis. Here we describe our new method, our effort to develop such a software system for analysis plant cell microscopy images. We called it Automated Cell Fluorescence Values Analysis method (ACFVA). ACFVA simultaneously measures the localization, size, shape and texture of a variety of plant cell types in a high throughput manner. ACVFA is therefore well suited to the needs of synthetic biology research as it allows the phenotypic characteristics of a large number of individual plant cells to be homogenised by a uniform standard and then presented as quantitative data. Note that we focus in this paper not on the technical details of the software (which are described in the method), nor computational validation of the mostly published algorithms, nor on a mechanistic study of any particular biological finding. Rather, we describe the system, validate the new method for a variety of real-world biological problems, demonstrate the breadth of its utility (including on various plant cell types and assays), and hope to stimulate ideas within the biological community for future applications of this new method especially in synthetic biology.

Materials And Methods

Plant materials and vector construction
The Arabidopsis ecotype Colombia (Col) were used as plant materials for plant protoplast isolation. After surface-sterilization in 30% bleach, Arabidopsis seeds were kept at 4 °C for 48 h before sowing. The seeds were grown in vitro on half-strength Murashige Skoog with 0.5% sucrose media (pH 5.7, 1.2% agar) in a growth chamber (20 °C) under white light (120 µmol m⁻² s⁻¹ photons) in 16 h light/day photoperiods for 10 days and then collected for protoplasts isolation. To prove the widely application of our method, the full-length cDNA of AtVDAC3 was amplified and cloned into p1301-35S-EGFP vector (F: 5' ATGGTTAAAGGTCCAGGACTCT 3'; R:5' GGGCTTGAGAGCGAGAGCAATC3') as well as approximately 1kb rd29A promoter (F:5'CATTAGACCTTATCGGAATT3'; R:5' TTTCCAAAGATTTTTTTCTTT 3') linked with full-length cDNA of AtRD29A (F: 5' ATGGATCAAACAGAGGAACC 3'; R:5'AAGCTCCTTTCTGCACCGG3') was amplified and cloned into p1301-EGFP vector. Dunaliella cells was cultivated according to the protocol as previous described (Hosseini Tafreshi and Shariati, 2009).

Arabidopsis protoplast isolation and transfection assays

Arabidopsis young leaves were cut into small pieces (width: 0.5 cm; length: 2 cm) and then completely submerged into digestion enzyme buffer (1.5% Cellulase R10, 0.4% Macerozyme R10, 0.4M Mannitol, 20mM KCl, 20mM MES, pH5.7), 10mM CaCl2 and 0.1% BSA) for 6 h without light. After protoplasts released, added enough W5 solution (154mM NaCl, 125mM CaCl2, 5mM KCl, 2mM MES, pH5.7) to stop the digestion reaction. The protoplasts were centrifuged at 100 × g for 10 min washed twice with 25 mL of pre-chilled W5 solution and incubated on ice for 30 min. The protoplasts were then centrifuged and resuspended in MMg solution (0.2 M mannitol, 15 mM MgCl2, and 4 mM MES, pH 5.7) to a final concentration of 2 to 5 × 105 cells/mL.

Protoplasts were transfected by a modified method from Yoo's report (Yoo et al., 2007). Approximately 5 × 10⁴ protoplasts (2 × 10⁴ to 1 × 10⁵) in 0.1 mL of MMg solution were mixed with approximately 10 (10 to 20) µg of plasmid DNA at room temperature. An 0.11 mL of a freshly-prepared solution of 40% (v/v) PEG (MW 4000; Fluka) with 0.1 M CaCl2, enough carrierDNA and 0.2 M mannitol was added, and the mixture was incubated at room temperature for 5–15 min. After incubation, 0.8 mL of W5 solution was added slowly, the solution was mixed, and protoplasts were pelleted by centrifugation at 100 × g for 10 min. The protoplasts were resuspended gently in 1 mL of W5 and were incubated in 6-well plates coated at room temperature for 12–16 h in light.

Confocal Laser Scanning Microscopy

Protoplasts were observed with a Leica TCS SP8 laser scanning confocal microscope using HC 10×/0.40 CS Plan-Apochromat, HC 20×/0.7 CS Plan-Apochromat, 40×/0.85 CS Plan-Apochromat or 63×/1.4 Oil Plan-Apochromat in multi-track channel mode. Excitation wavelengths and emission filters were 488 nm/band-pass 505–530 nm for GFP, and 488 nm/band-pass 650–710 nm for chloroplast auto-fluorescence.
ACFVA analysis pipeline

The software is programmed in Python and has been packaged, the user does not need to download any other attachments. The software is easy to use and the source code and comments are included in the accompanying manual. The software has a user-friendly and convenient interface. 3-D image recognition is not involved for now, but can be further developed. ACFVA was originally developed for the detection of a single cell type. High-throughput detection can be completed just by selecting a folder and clicking a single button. The threshold is set precisely for this type of cell detection, so the impurities can be more accurately detected and filtered out.

The software identification module contains the algorithm based on image recognition – contour algorithm. The algorithm is not limited to identifying cells based on fluorescence. This algorithm is applicable even if the image contains non-fluorescent cells. In most biological images, cells touch each other, causing the simple, fast algorithms used in some commercial software packages to fail. The Python-integrated OpenCV2 module can “Erosion” and “Dilation” the image to separate contact cells easily. When the pixel standard of the image is sufficient, this software has no requirements on the dispersion of the main object. The only limitation of using this software is that the pixels of the images must achieve the requirements.

ACFVA is based on the image recognition algorithm. First, the image will be gray processing during equation XX. The RGB color mode is a color standard in the industry. It obtains various colors by changing the three-color channels of red (R), green (G), and blue (B).

\[
Gray = 2^{\frac{2.2}{2}} \left( \frac{R^{2.2} + (1.5G)^{2.2} + (0.6B)^{2.2}}{1 + (1.5)^{2.2} + (0.6)^{2.2}} \right)^{2.2} \quad (XX)
\]

After gray processing, the amount of computation is reduced, but the RGB information remains in the grayscale. Preprocessing of the image includes but is not limited to Gaussian denoising, flood filling, etc. The functions are: filtering and denoising, contrast enhancement, impurity filling, etc. The identification of cells in unprocessed color images has been avoided. Second is to binarize the pre-processed grayscale image. The function is to make the image simpler, to further reduce the amount of computational data, and to highlight the contours of the target of interest. As a final step of cell identified, the cell contours will be detected in the binary image by the contour recognition module of OpenCV2. A series of eigenvalues of cells can be correspondingly captured in the original image according to the identified contours, such as fluorescence value, cell area, cell relative position, etc (Figure1). There are also modules that can be added during the software development. For new types of cell images, the threshold and other parameters can be simply adjusted to achieve the purpose of improving the adaptation of new types of cells. A user-friendly module for adjusting the threshold value can also be added during further development of the software.

Results And Discussion
Validation of ACFVA for different plant cells

We first demonstrated that ACFVA could accurately measure many different biologically important features of suspensions cells using several plant cell types, including live Arabidopsis protoplasts and Dunaliella cells because these cells are particularly challenging to identify by automated image analysis (Lobet et al., 2013; Sekul ska-Nalewajko et al., 2016), and they enable rapid chemical substance detection such as pollutants in the environment or generation of large quantities of active ingredients such as Plant-derived therapeutic proteins using living cell after genetic modification (Feng et al., 2022; Liu and Stewart Jr, 2015). Using the basic cell-culture methods described previously (Hosseini Tafreshi and Shariati, 2009; Yoo et al., 2007), we prepared Arabidopsis protoplasts and Dunaliella cells for experiments shown in Figs. 2 and 3. Direct comparison of image analysis method is difficult because results from image analysis can be heavily skewed by how the software or method is tuned and commercial software packages are numerous and expensive. Furthermore, the algorithms in commercial software are proprietary and so cannot be directly compared apart from the entire software package, including image preprocessing methods. The best practical comparison, therefore, is for image analysis method to release the crucial results of these method on the same image. For plant live-cell imaging analysis, in which living cells are imaged over a period of time using phase contrast and/or fluorescence microscopy, an important and necessary procedure is image segmentation of single cells which give crucial results of microscopy images such as cell counts, cell localization and cell size which was also important in animal cells microscopy images analysis (Chupeau et al., 2013; Stöter et al., 2013).

Cell count which is a straightforward phenotype is used to probe cell proliferation/apoptosis/death in cytological research. As Arabidopsis protoplasts is a novel and convenient chassis cell in synthetic biology (Zhu et al., 2021), we chose three concentration (low/medium/high) Arabidopsis protoplasts as plant living cells for photoing using Confocal microscopes. Their microscopy images under brightfield were used for microscopy images analysis comparison because it is the most convenient way to understand the state of cells. For methods used to analysis cell microscopy images, we choose manual statistics methods using ImajeJ software by two testers and automatic statistics method using CellProfile software, a free, open-source system designed for flexible, high-throughput cell image analysis (Stöter et al., 2013) comparison with our new automatic statistics method. For small amount of cells (low concentration), there is no obvious difference among various methods in cell count (30, 29, 45, 29 cells per image). However, the cell numbers counted by CellProfile were much higher (> 2 fold of medium and > 20 fold of high cell concentration) when the amount of cells is not small. In both medium and high concentration cells, our new automatic method obtain similar results as manual methods (Fig. 2A). Object identification is the most challenging step in automatic microscopy image analysis and its accuracy determines the accuracy of the resulting cell measurements that has, nonetheless, proved challenging for many existing software due to their poor ability of separating target cell and contaminants when objects in picture are numerous and complex such as Arabidopsis protoplasts microscopy image of medium and high concentration used in this study. ACFVA was originally developed for the detection of a single cell type whose threshold was set precisely for automatic distinction single
cells from numerous contaminants and accurately filtered out impurities. It made our new method can be as accurate as manual cell identification, but less time consuming than manual identification.

Beside cell count, cell size is also an important phenotype for determining cell healthy and the ability of measurement of cell size is a direct indicator of the accuracy of a method to identify individual cells. In all cell concentrations, there was no difference in cell size of Arabidopsis protoplasts (20-40nm) determined by our automatic method and manual method (Fig. 2B), which were both consistent with previous reports (Yoo et al., 2007). Because only in low concentration, the identification of plants cells by CellProfile was accurate. We only used this method measure the cell size of low concentration according to its software introduction. Unlike our method which give the information including cell localization and cell diameter of single cell, CellProfile just gave us three results: 10th pctile diameter (the average diameter of top 10% small cells) was 13.6 µm, medium diameter (average diameter of all the cells) was 20.1 µm and 90th pctile diameter (the average diameter of top 10% large cells) was 31.3 µm. To make comparison between these two automatic methods, we obtain the same three results: 22.92 µm, 28.66 µm and 34.48 µm using our new method. Although there was no significant difference (< 2 folds) among diameters measuring by two methods, the average diameter of top 10% small cells of CellProfile was smaller than the standard diameter of Arabidopsis protoplasts. It may cause by some small concomitants were counted as cells by CellProfile method. Moreover, the correlation coefficient R² also proved the diameters obtained by our new method were more stable and accurate than CellProfile (Fig. 2C).

In order to test whether ACFVA can automatically identify a wide range of plant single cells, a microscopy image of Dunaliella cells and a low resolution microscopy image of plant single cells randomly downloaded from Internet (Chupeau et al., 2013) were also analyzed using different methods. In terms of cell counts as same as the result above, the cell numbers counted automatically by our new method in two types of cells were almost identical to manual methods (< 1.1 folds). Because the cell numbers of two pictures were not small enough (> 50), the cell numbers of CellProfile were much higher (> 100 folds of Dunaliella cells and > 20 folds of other plant cells) than other methods (Figure D and E). The large errors of Dunaliella cell numbers counted by CellProfile may be due to the irregular cell shape of Dunaliella. The cell diameter of these two types of cell ranged from 10.26–11.48 µm and 11.13 to 23.69 µm, which were both in line with the previous reports (Hosseini Tafreshi and Shariati, 2009). Consistent with the results above, the correlation coefficient R² of cell diameter automatically measured by CellProfile of these two types of cells were lower than our new method. Taken together, our new method can automatically (no complex parameter setting), rapidly (the analysis was run on a desktop computer at a rate of > 1 image/minute) and precisely (including cell localization, cell size, cell shape etc) identify various single plant cell.

**Broad applicability of ACFVA with plant cells**

Plant cells such as Arabidopsis protoplasts are ideal as chassis cells in synthetic biology; they are eukaryotic, allowing exogenous DNA molecules to enter the chassis cell and be encoded correctly; they
are cellularly totipotent, with complete energy and metabolic pathways; and they are simple and economical to prepare compared to animal cells. Therefore, we applied ACFVA to perform transfection efficiency calculation and detecting environmental change (high-salinity stress) —two important and useful analysis in molecular and synthetic biology using Arabidopsis protoplasts after having demonstrated its ability to accurately identify plant cells and measure a large number of relevant phenotypes.

Gene transfection is a widely used technique for molecular studies, which could make a huge impact on subsequent experiments. Therefore, accurate calculating the transfection efficiency of the cells is a necessary and important pre-requisite for for most biological research. Counting the ratio of cell with positive fluorescent light among sufficient cells is a directly methods for calculating transfection efficiency. In this study, we applied ACFVA to compare the transfection efficiency of two systems both using Arabidopsis protoplasts. It was easy and convenient to identify more than 50 cells per picture of two systems using our new method, which ensure a sufficient number of cells are counted. In addition, the fluorescent light of chloroplast which were easily measured by our new method were used to evaluate the cells’ activity of two systems. Unlike animal cells, healthy plant cells including Arabidopsis protoplasts have strong and stable chloroplast's fluorescent. This property not only helps to identify cells but also to determine their activity, making plant cells more versatile as chassis cells for synthetic biology. In this study, we compared the transfection efficiency of with-carrierDNA and without-carrierDNA systems using the same vector 35S::AtVDA3-EGFP in Arabidopsis protoplasts. AtVDA3 was reported to involved in metabolite exchange between the organelle and the cytosol which are prominently localized in the outer mitochondrial membrane, chloroplast and nucleolus. With the help of our new method, there were 54 cells in with-carrierDNA and 84 cells in without-carrierDNA systems identified both in bright and chloroplast's fluorescent field. It confirmed that our new method can automatically and precisely found single healthy Arabidopsis protoplasts again. There were no significant differences in the fluorescent light of chloroplast measured in two systems (Fig. 3A), which suggested the cells in these two systems were both healthy and appropriate for gene transfection. Cells with fluorescent light of both EGFP and chloroplast were set as positive cells in this study. There were 49 positive cells in with-carrierDNA and 12 positive cells in without-carrierDNA systems identified both in chloroplast and EGFP fluorescent field, and this positive cell can also be found in BF and EGFP fluorescent field. There is also no significant difference in EGFP fluorescent light of positive cells between two systems (Fig. 3A), which indicated that the host cell activity and positive cell activity of these two Arabidopsis protoplast transfection systems are consistent and the transfection efficiency were their main difference. In this study, the ratio of positive and hots cells was calculated as transfection efficiency. The transfection efficiency was 90.74% and 14.29% in with-carrierDNA and without-carrierDNA systems, respectively. It was consistent with previous reports, all suggesting that carrierDNA can improve gene transfection efficiency(Uherek and Wels, 2000). All the above data suggested that using ACFVA with Arabidopsis protoplasts can analysis gene transfection efficiency quickly and accurately.

Increasingly, synthetic biology research requires biological models that can rapidly and accurately sense changes in the external environment such as chemical stress. The development of modern gene editing
techniques and fluorescent tags enable the use of fluorescent signals from biological models to detect external environment changes (Bennett et al., 2008). As we all known, various external stress such as NaCl, dehydration, ABA and cold treatments can lead to synergistic activation of Responsive-to-Dehydration 29A (RD29A), which encodes a hydrophilic protein (Yamaguchi-Shinozaki and Shinozaki, 1994) of unknown function (Msanne et al., 2011). In this study, we construct a biological model using Arabidopsis protoplasts with high transfection efficiency as chassis cells, Arabidopsis RD29A as sensor and fluorescent tags EGFP as reporter to detect the exist of NaCl in external environment (250µm NaCl). The results of chloroplast fluorescent which were also detected to evaluate the healthy of chassis cells showed no significant differences between two treatments (Fig. 3B). It proved that the this biological model was strong enough to detection of external NaCl signals, and the change of reporter fluorescent was caused by NaCl not cell activity. The reporter fluorescent (EGFP) was significantly increased when NaCl was present in the external environment (Fig. 3B), which confirmed the accurate and rapid response to NaCl of our biological model combining with ACFVA. High-salinity water poses hazards for the environment as well as affecting agriculture, infrastructure and communication under seawater. Salinity is one of the most important variables for ocean monitoring, marine environment, seasonal weather forecasting, aquaculture and solar engineering. Therefore, an effective method for sensing salt solution has been much sought for application in many fields, such as agriculture (Li and Kang, 2020), public health (Lugli and Lutz, 1999), and environmental management (Zhao et al., 2003). Many techniques have been proposed to measure salt concentration such as optical techniques (Yin et al., 2018), infrared attenuated total reflection spectroscopy (Rauh and Mizaikoff, 2016), microwave sensing(Harnsoongnoen et al., 2018) and bio-chemical sensing(Kabaa et al., 2019). ACFVA allowed rapid and accurate identification of individual plant cells and the measurement of relevant phenotypic indicators such as fluorescence values which can. Thus, compared to other methods mentioned above, using our method, on the one hand, we can make full use of the easy preparation of plant cells and detect a sufficiently large volume of sample data (billions of samples) to increase accuracy; on the other hand, it allows an increasing wealth of molecular mechanisms and fluorescent labels to be used in practice.

Conclusion

In this study, we presented a new, automated and quantitative image analysis method for plant cells. The automated image analysis for plant cells via ACFVA are faster than previously described methods that rely on manual calibration and more accurate than computational methods that were only suitable for animal cells. Together, the methods developed in this work should facilitate quantitative analysis of plant cell microscope images to solve a variety of synthesis biological questions.

Declarations

Ethical Approval

Not applicable
Competing interests policy

The authors declare that they have no conflict of interest.

Author Contributions

JF & ZC performed most of the experiments, CB, ZL, BC, BW & YZ participated in experimentations; BC, YZ & BW designed the experiments and analyzed the data, ZY wrote the paper.

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Availability of data and materials

Not applicable

References


Figures
Figure 1

The analysis pipeline of Automated Cell Fluorescence Values Analysis (ACFVA)
Figure 2

Validation of ACFVA for different plant cells

A. Counts of Arabidopsis protoplast in different concentration by different methods. For Manuual1, An experienced experimenter counts directly against the images. For Manuual2, An experienced experimenter
counts the cells with the help of ImageJ software. For CellProfiler, the cell counts were analyzed according to its manual. The healthy Arabidopsis protoplasts were marked by red using ACFVA in each image.

B. The cell size of Arabidopsis protoplast in different concentration measured by Mannual1, Mannual2 and ACFCA. AMVOA tested were performed to analyze the significance and NS means no significant difference.

C. The cell size of Arabidopsis protoplast in low concentration measured by CellProfiler and ACFVA. The 10th percentile diameter (the average diameter of top 10% small cells), medium diameter (average diameter of all the cells) and 90th percentile diameter were measured by two methods. The correlation coefficient $R^2$ was also calculated.

D and E The analysis of Dunaliella cells microscopy image and low solution Arabidopsis protoplasts microscopy image by different methods. The Dunaliella cells microscopy image were obtained under 10×/0.40 CS Plan-Apochromat microscopy. And the low solution Arabidopsis protoplasts microscopy image was downloaded from reports.
Figure 3

Broad applicability of ACFVA with plant cells

A. Transfection efficiency assay using plant cells with ACFVA. The transfection efficiency of with-carrierDNA and without-carrierDNA systems using the same vector 35S::AtVDA3-EGFP in Arabidopsis protoplasts were compared by EGFP fluorescent value quantified by ACFVA. The chloroplast fluorescent
value were also quantified by ACFVA to evaluate the cells’ activity of two systems. The line connected the fluorescent value of chloroplast and EGFP of each cells. NS means no significant difference according to AMVOA analysis.

B. Detection environmental change (high-salinity stress) using plant cells with ACFVA. RD29A promoter::AtRD29A-EGFP with Arabidopsis protoplasts were used to detecting environmental NaCl change. The constructed biological model were cultured in NaCl (250μm)+DMSO and DMSO for 45 min, and then the fluorescent value of chloroplast and EGFP were photoed by Confocal and quantified by ACFVA. The chloroplast fluorescent value were determined to evaluate the cells’ activity, and the EGFP fluorescent value were used to display the environmental NaCl change. AMVOA tested were performed to analyze the significance and ** means p<0.01.