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Research Article

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New method for detecting *Collectorichum* species found in Korea using image analysis

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ABSTRACT

Colletotrichum acutatum spp. infects various economical crops worldwide and causes massive loss on their yields. Among those, *Capsicum* spp., which is known as chili pepper, is on a critical issue by those pathogens. Due to the lack of their genetic markers in Korea, the unidentifiable various species of *C. acutatum* obstruct the mechanism studies of these pathogens and the selection of disease-resistant breed lines. Therefore, we screened RGB images of the colonization progresses of pathogens to identify the species of Ca40042, K1, NN, AS2, and SW1 by time and temperature. Cultivated pathogens such as Ca40042, K1, and SW1 were detectable on quantified shape and color data of images from specific temperature conditions, while other pathogens were difficult to recognize. Although several limitations exist in the identification results of the current experiment, also, we can expect this method can suggest the possibility to replace the genetic marker methods which are now unavailable in Korea.

KEYWORDS: Chili pepper disease, color analysis, pathogen phenotyping, RGB imaging

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INTRODUCTION

Species of *Colletotrichum acutatum* J.H. Simmonds, cosmopolitan plant pathogen that causes pre- and postharvest anthracnose diseases. Broad ranges of economically important crops, such as strawberry, peach, pepper, almond, citrus, apple, blueberry, tomato, and mango can be infected (Peres *et al*., 2005). Among the huge and extensive losses on crop yield that derived by *C. Acutatum*, chili pepper, which belongs to the genus *Capsicum*, family *Solanaceae*, and is one of the most important food additives used in spicy cuisines worldwide, has the major causes of fruit anthracnose in various countries including Korea, Taiwan, India, and Indonesia (Than *et al*., 2008; Tozze Jr *et al*., 2010).

All *C. acutatum* species isolated from pepper remains unclear whether they are equally pathogenic or host-specific, and their degrees of toxicity. This results in the unprecise trait selection in breeding processes for the cultivation of resistant varieties which is the most effective method to overcome this disease. Due to the absence of genetic markers for identifying the various *C. acuatum* species that spread in Korea (Kang *et al*., 2005), yet, no resistant varieties were developed and provided.

A recent study revealed *C. acutatum* species subsequently penetrates to the cuticle layers of *Capsicum* spp. fruits by forming highly branched, well-differentiated hyphae than the other crops (Liao *et al*., 2011). Moreover, many studies suggest extracting the colors, shapes, and textures from the images of plant segments that are infected and cultured fungal strain samples (Pietrowski *et al*., 2012; Singh & Chetia, 2017). Their experiments were implemented by nondestructive and exact identification methods based on digital images of spectral sensors (Barbedo, 2013; Pujari *et al*., 2015). Their various applications of image-based phenotyping have shown the potential for providing reliable identification of pathogens in a quick, and efficient process.

Therefore, we focused on image analysis of the colonization progresses, which has the possibility of pathogen recognization by using *C. acutatum* pathogen strains CA40042 (*acutatum*) (=*Colletotrichum acutatum*, KACC40042, KACC40042) in Korea. We expect that this identification based digital images might be a meaningful challenge to Korean chili pepper agriculture industries those suffering by anthracnose.

Figure 1: (a) Incubators; (b) eight pathogens of *C. acutatum* cultured in petri dishes

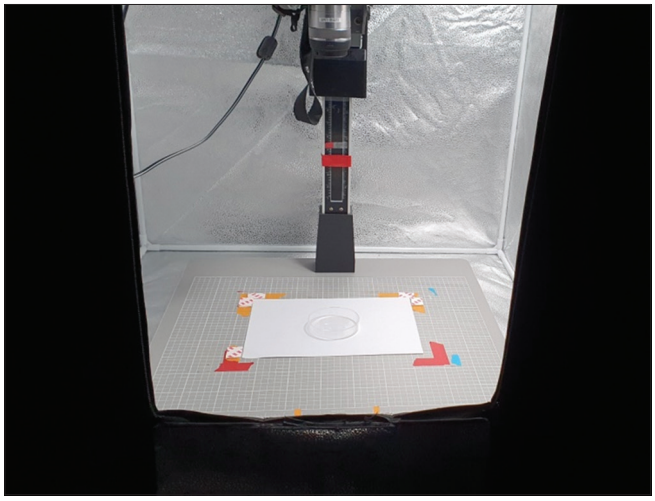

Figure 2: Imaging chamber for image acquisition

MATERIALS AND METHODS

C. acutatum

Eight pathogens (resource names: Ca40042, K1, NN, AS2, SW1) were provided from the Pepper & Breeding Institute Co., and the cultured *C. acutatum* samples were quantified from the vertical images. Images were acquired for 10 days in order to identify the hyphae expression of 8 species of *C. acutatum* that were separately cultured in petri dishes at each condition of 22 ℃, 25 ℃, and 30 ℃ (Figure 1).

Image Acquisition

Images of the three replicates of 8 pathogens in each temperature were collected by a digital camera α6000 (Sony Co., Japan), with 30 mm MACRO lens and wireless remote controller, once a day for 10 days. An external light source, LED lighting, were used for the color uniformity, and the camera was fixed at a 30 cm distance between objectives to adjust equal imaging area (Figure 2).

Image Analysis Procedure

Scale settings and image cropping processes were performed by Matlab (MathWorks Co.) to achieve the constant conditions of image acquisition about hyphae expression of *C. acutatum* at different levels of temperature (Figure 3). The region of interest (ROI) was appointed, edited, and saved for reprocessing.

The background was segregated from petri dishes by noise reduction and binarization (Figure 4) for the quantification of pathogen expressions, due to the color similarity of background, petri dishes,

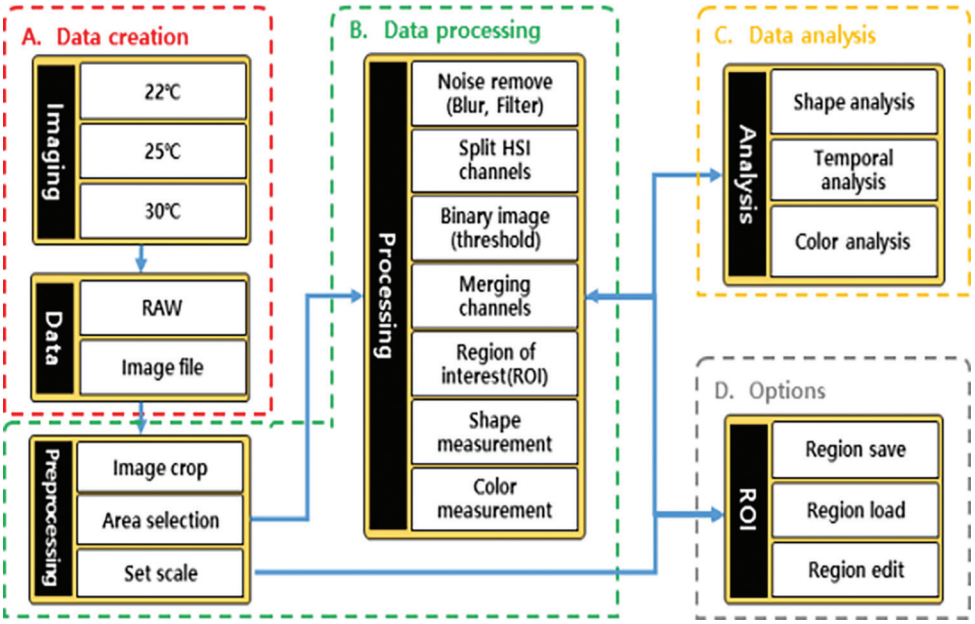

Figure 3: Scheme of image analysis process for qualification of pathogens

and pathogens (Figure 4a). Thereby, pathogen data were extracted from the images (Figure 4b). Shape parameters (area, horizontal/vertical length, convex area, eccentricity, roundness, and solidity) were obtained from the extracted pathogen image data. To verify the color distribution from the images of pathogens expression by time, color distributions were defined as 20 levels of hue area values of hue intensity saturation (HIS) color space (Figure 5). Kruskal-Wallis test was conducted with quantified image data in R (R Core Team) for insuring that there is no difference between replications. Then the parameters which do not reject the null hypothesis of the Kruskal-Wallis test were plotted.

RESULTS AND DISCUSSION

The quantified shape data, parameters such as area, horizontal length, vertical length, and convex area were capable to specify several species. However, eccentricity, roundness, and solidity irregularly differed by their replications.

Results from shape data, Ca40042 and SW1 were separated while other species overlapped indistinguishably (Figures 6-9). SW1 was detectable in four parameters from images of the colony that cultivated at 22 and 30 ℃. It began to differ significantly from other pathogen samples, on 2 days after sampling (DAS) at 22 ℃, and on 6 days after sampling at 30 ℃ rating higher values than the other species. Ca40042 differed only on area, vertical length, and convex area at the 22 ℃.

On the color distribution, only levels 2 and 3 of the hue area have differed throughout its DAS and temperature conditions (Figures 10 and 11). Only SW1 differed against the other *C. acutatum* pathogens species at the hue area level 2, and its

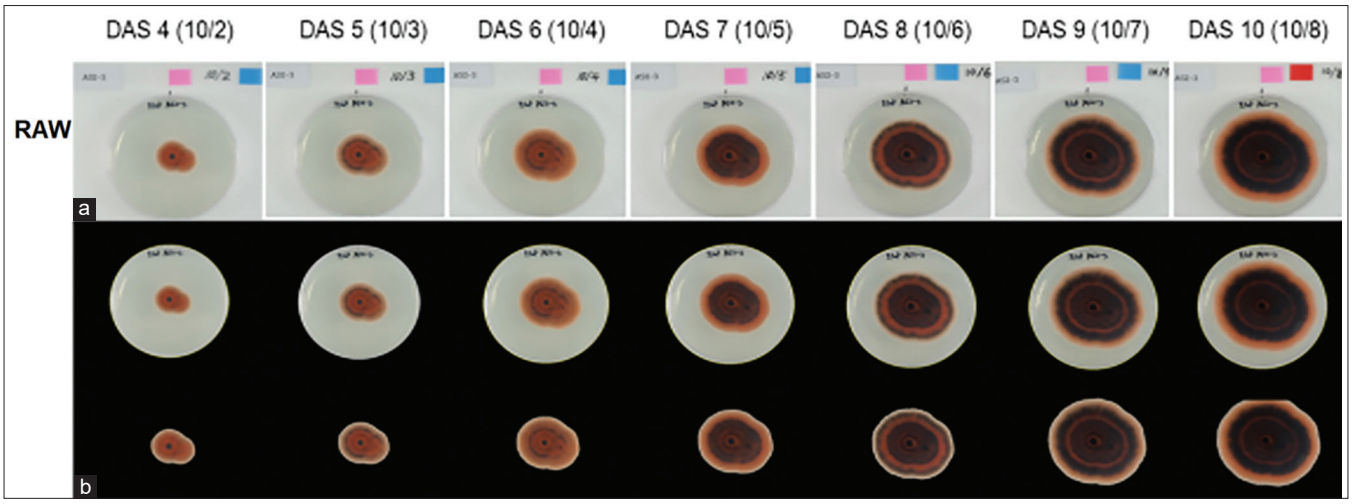

Figure 4: Image of AS2 *C. acutatum* expression at the 22℃ by time.; (a) is the segregation of background and petri dish; (b) is the segregation of petri dish and pathogen

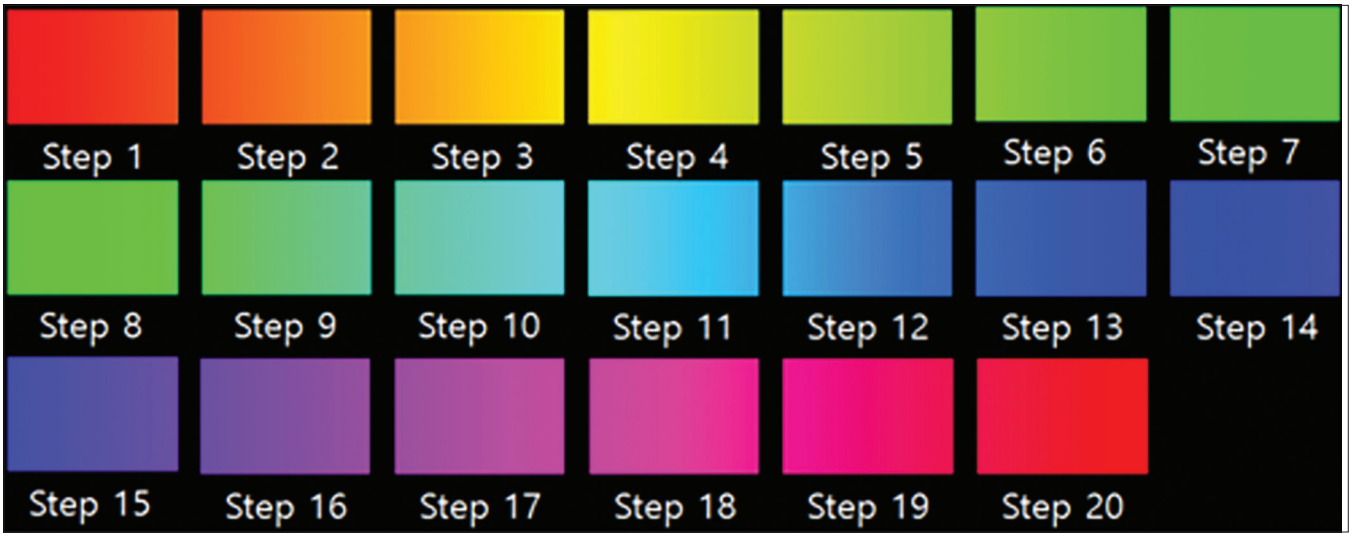

Figure 5: Color table of 20 hue levels

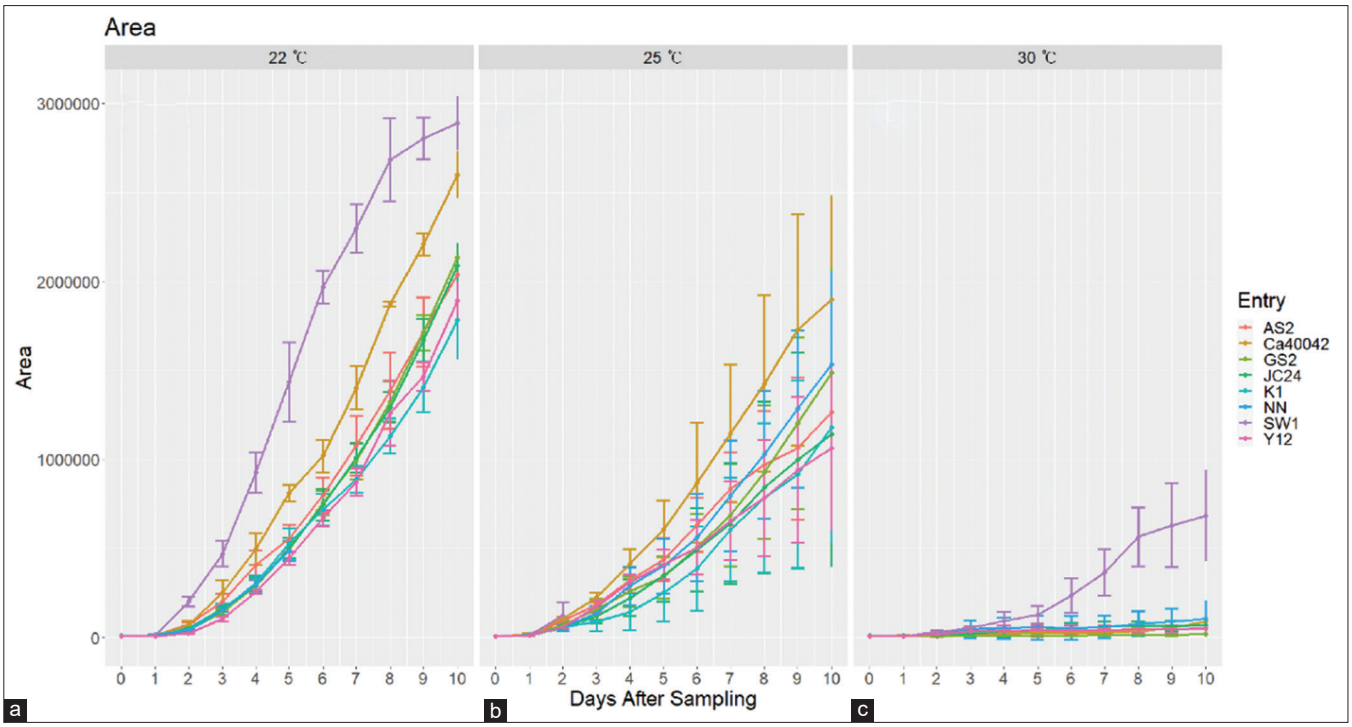

Figure 6: Area of 8 pathogens colonies from DAS01 to DAS10. (a) 22 ℃; (b) 25 ℃; (c) 30 ℃

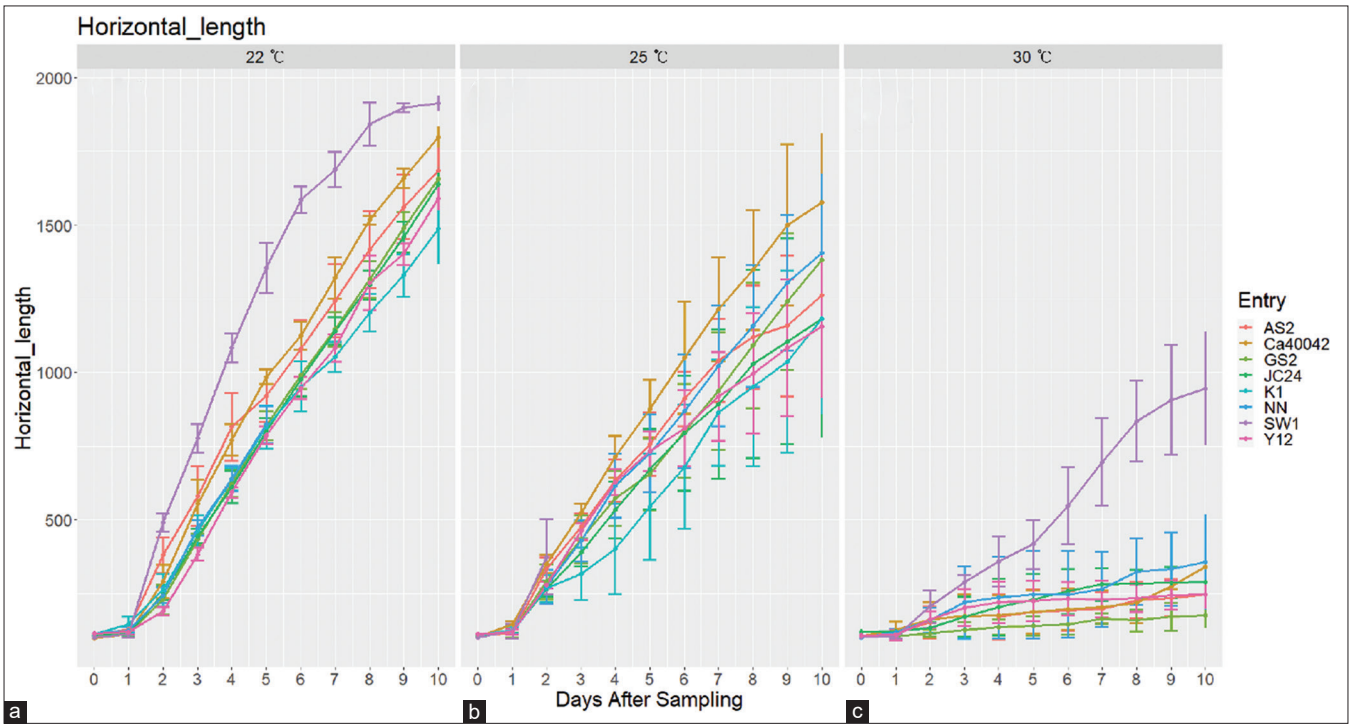

Figure 7: The horizontal length of 8 pathogens colonies from DAS01 to DAS10. (a) 22 ℃; (b) 25 ℃; (c) 30 ℃

identification was possible at the 22 ℃ condition of DAS 7, and after DAS 9. However, values of SW1 rated the lowest in level 2 unlike the other parameters of both shape and color distributions (Figure 10). Notably, two more species were variated in the case of hue area level 3 (Figure 11). Similar to the shape parameters, SW1 were significantly divided from the others at the 22 and 30 ℃ conditions by rating the highest value. Ca40042 was detectable between, DAS 4 to

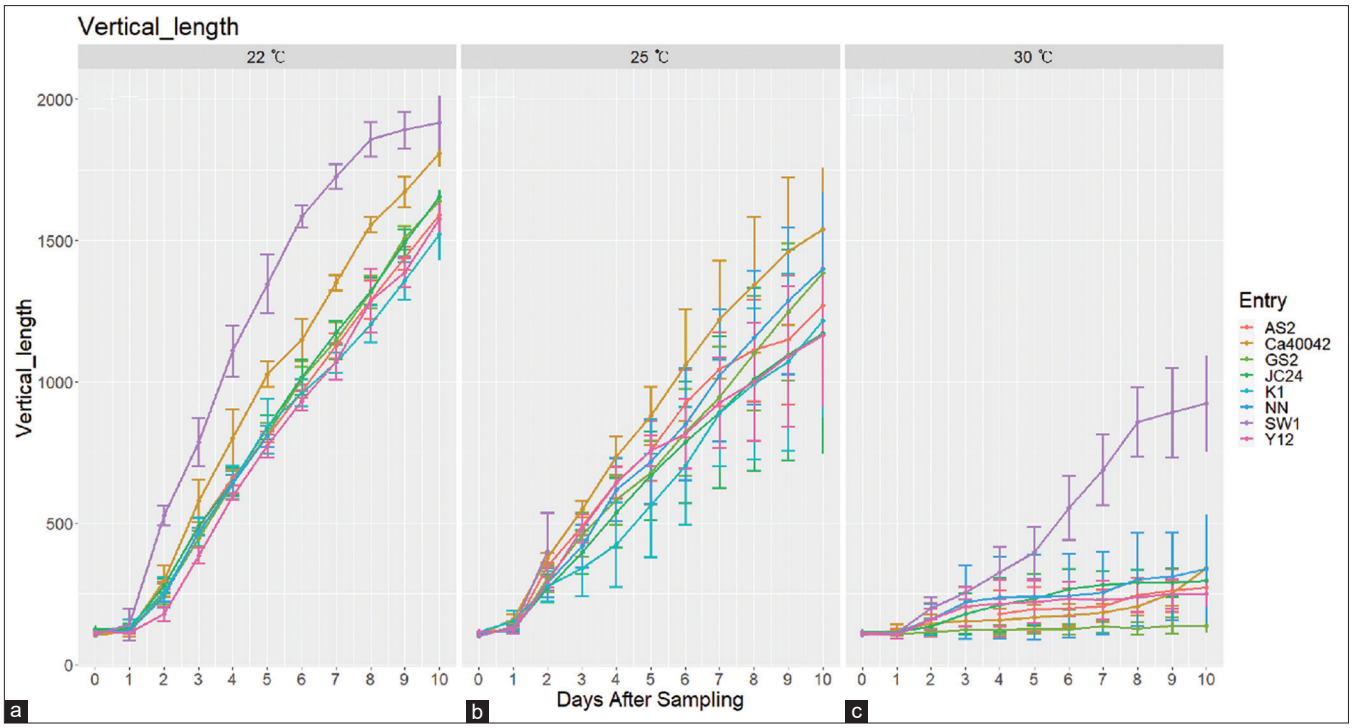

Figure 8: The vertical length of 8 pathogens colonies from DAS01 to DAS10. (a) 22 ℃; (b) 25 ℃; (c) 30 ℃

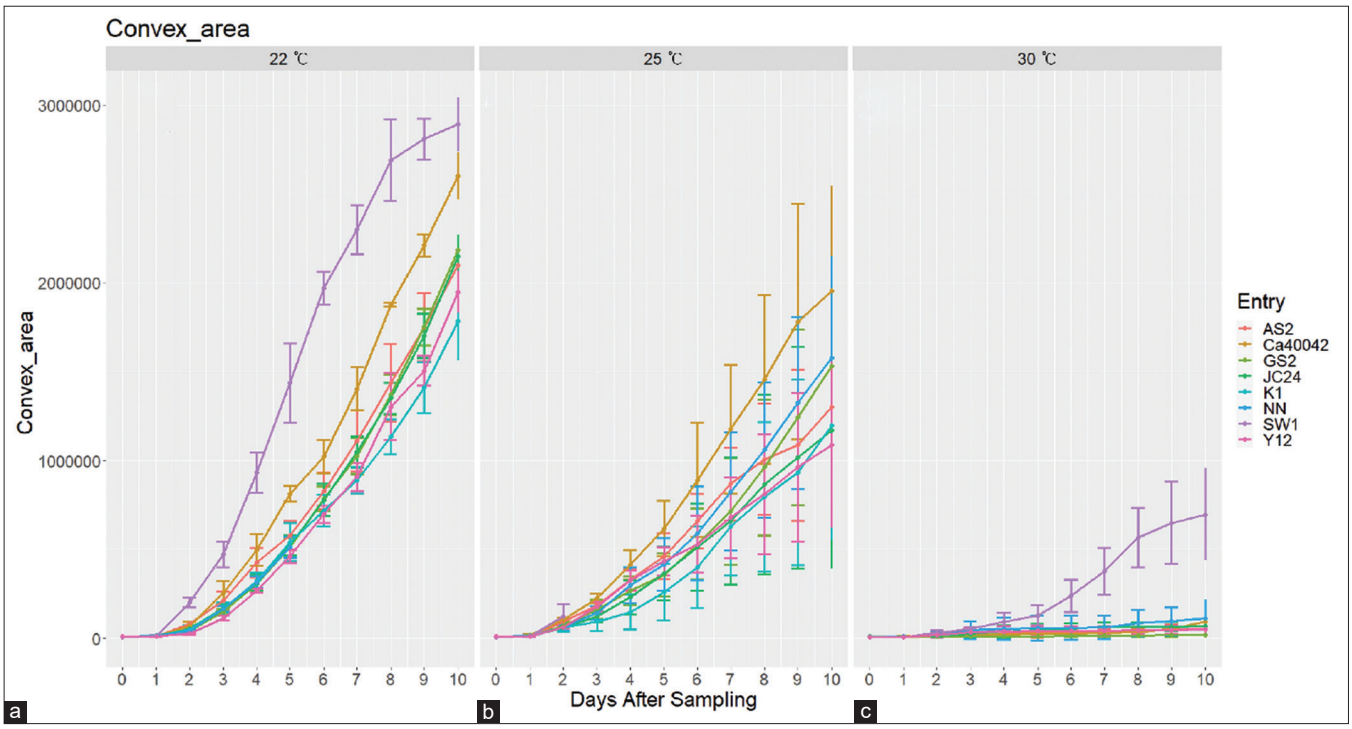

Figure 9: Convex area of 8 pathogens colonies from DAS01 to DAS10. (a) 22 ℃; (b) 25 ℃; (c) 30 ℃

DAS 5 (22 ℃), and DAS 5 to DAS 6 (25 ℃), in a short period at 22 and 25 ℃ conditions. Moreover, the differentiation of K1 was observed after the DAS 8 at the condition of 22 ℃. The present study aims to find the identification criteria based on the characteristics of CA40042 strains, that are feasible to replace the genetic markers.

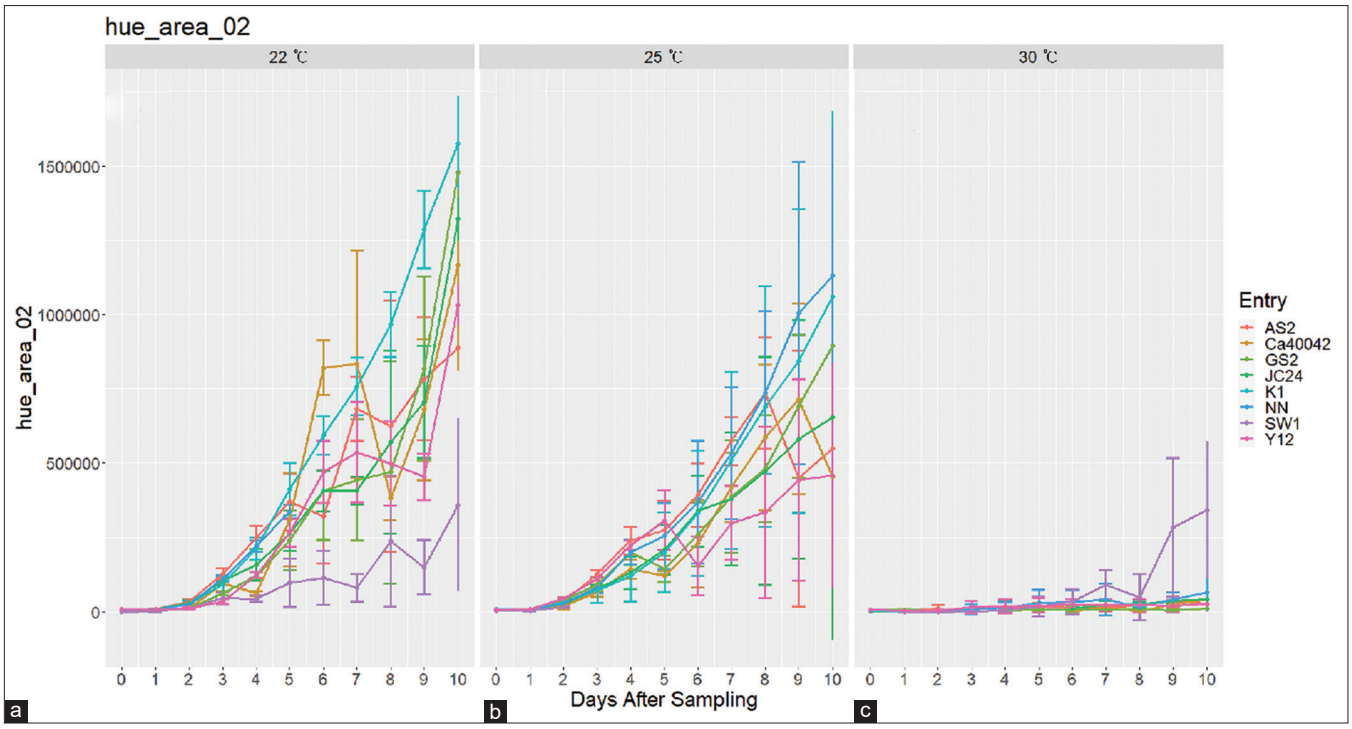

Figure 10: Hue area of 8 pathogens colonies from DAS01 to DAS10 in hue level 2. (a) 22 ℃; (b) 25 ℃; (c) 30 ℃

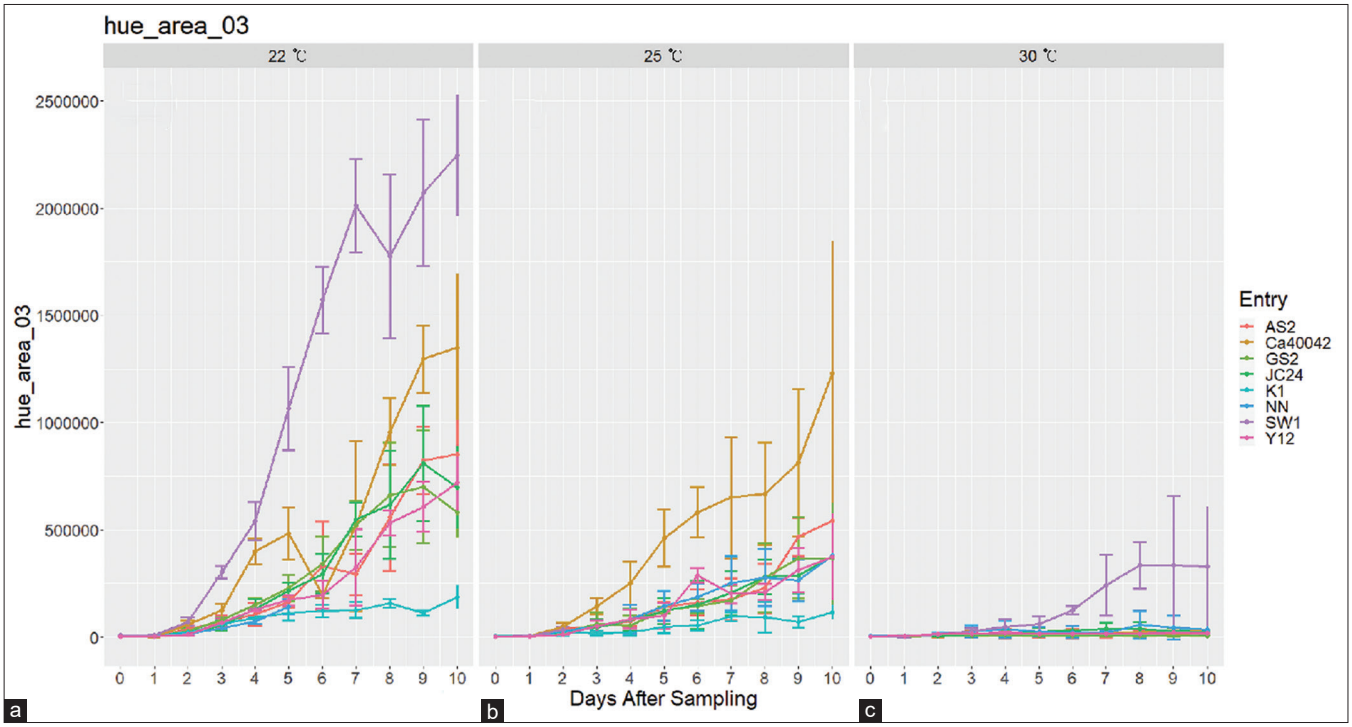

Figure 11: Hue area of 8 pathogens colonies from DAS01 to DAS10 in hue level 3. (a) 22 ℃; (b) 25 ℃; (c) 30 ℃

CONCLUSION

The principal proposal was the phenotype analysis of fungi through the digitalized image data that reported promising results by numerous researchers (Dörge *et al.*, 2000; Puchkov, 2010). We challenged on developing a novel method of identifying *C. acutatum* species from the morphological and color characteristics from the RGB images of fungal colony growth which has benefits on financial and technical issues in Korea. From the results, we could confirm that the quantified phenotypes of pathogen species that differentiate have the potential to allow the identification progress. However,

further studies need to be done for the exact establishment of identification criteria which is due to the less samples and experimental limitations of the present research. We expect our results will be a basis for developing the identification method for C. *acutatum* species.

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AUTHOR'S CONTRIBUTION

J.B. conducted data process and analysis. C.L., J.K., N.K., and Y.K. aquired images and actual measurements. S.L.K., H.J., S.P., and I.C. provided materials and resources; K.-H.K. supervised the experimental design and coordination. J.B. for the writing—original draft preparation, K.-H.K. for the review and editing.

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