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Competency of *alpha-D-glucopyranosyl* Regulates Cells Death and Functions in Senescence Inhibition

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Abstract— *Senescence is a key process determining the life interval of several cut flowers and sugars added to the nutrient are known to increase vase life in several flowers, very little is known about endogenous variations in* ***glucopyranosyl*** *levels in several floral tissues, predominantly beyond petals, during natural flower development. In present study, we had evaluated the* ***glucopyranosyl*** *efficiency at different concentrations and different time intervals. We had found that the efficiency of* ***glucopyranosyl*** *slightly affect the senescence approaches and played a significant role in inhibition of senescence. Furthermore, it also played a key role on increasing the cells size and enlargement of cells at growing stages of petals. Further we had randomly investigated the content of sucrose in treated petals and control. Interestingly we had found increasing amount of sucrose in treated petals compare to that of untreated. Sucrose contents increased with flower development reaching 50% to 74% higher levels at later stage compared to that of early stagein excised petals.*

Keywords: Senescence, Glucopyranosyl, Petals, Cells, Competency, Sucrose.

I. INTRODUCTION

Beauty is the main reason why cut flowers are sold, much effort has been put on lengthening flower lifespan, one of the major problems in the floricultural commercial sector. In this regards, various studies have been conducted to unravel the pathways that lead floral organs to death so that the resulting scientific and technological advances can be used to extend flower longevity **[1,2]**. Although, senescence is a programmed cells process that does not occur in all floral organs at the same time. According to its specific biological function petals are the first tissues showing signs of senescence, while the gynoecium, particularly the ovary, remains functional throughout all phases of flower development to ensure seed development. In this way, since tepal senescenceis the limiting factor for flower longevity, the main objective off loral senescence studies relies on the maintenance of a viable and visually healthy corolla for a long time **[3].**

Many studies have shown how exogenous sugar added to the vase solution delays the onset of visible signs of flower senescence in several cut flowers, although its role in petal senescence seems to be indirect **[4]**. However, petals can also act as a source during flower senescence **[5]**. Furthermore, cutting the stems leads to a complete reduction of sucrose import to sink tissues of cut flowers and an altered carbohydrate metabolism. Therefore, depending on the floral tissue and stage of development sucrose on the vase solution may have a complex effect on endogenous levels of sucrose and the physiological status of different floral tissues. Exogenous sugar effects on flower longevity may also differ between ethylene sensitive and

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insensitive species. In the former, sugars seem to delay petal senescence by reducing ethylene sensitivity **[6,7,8,9]**. In contrast, it has been suggested that in ethylene-insensitive flowers sugars might prevent a decline in osmotic pressure and delay celldeath **[10]**.

In view of the facts stated above, the present study was carried out to evaluate the effect of *Glucopyranosyl* on vase life of rose on controlled conditions with the objectives; to examine the effect of *Glucopyranosyl* at various concentrations on the growth attributes ofthe rose and to inhabit the senescence approaches.

II. MATERIALS AND METHODS

2.1 Plant materials and growth conditions

For infiltration of rose petals, about 1.8-stage cut roses flowers (Samantha) were collected from a greenhouse and brought into the Genomic laboratory. For the treatment of rose flowers, 1.8 stage flowers were collected and stem being cut about 20 to 25 cm under water, and placed in deionized water, more the flowers were placed into solution containing different concentration of *Glucopyranosyl*, and observed the phenotype.

2.2 Infiltration of flowers and petals

The petals were immersed in different concentration of *Glucopyranosyl* infiltrated at vacuum of 0.8 atm. After the release of the vacuum, the petals were washed by deionized water and kept vertically in deionized water for 3 days at 8^{o}C, then transferred to 23^{o}C for 5 days. Photos were taken for the petals every day, and petals were picked for RNA isolation on the 8thday after infiltration. Petal size was measured according to the photos by using the ImageJ software. Statistical testing was performed by using the SPSS software.

2.3 Preparations of different concentration *glucopyranosyl*

The each solution was prepared with supplementation of *glucopyranosyl* at different concentrations i.e. 1%, 3% 5% and 7% and (control) without any *Glucopyranosyl* concentration. Solution was stirred on magnetic stirrer and remaining sterilized distilled water was added to final volume. After the next day, petals were then infiltrated with different concentrations. With treatment of these concentrations, the samples of petals were also been infiltrated with normal water as negative control.

2.4 Measurement of cell size by Scanning Electron Microscopy

Measurement of cell size and encountering the number of cells was performed as described by (Ma et al., 2008). In short petal samples were taken as 0.5-cm 3 0.4-cm slices from regions of petal length from the top. For Scanning Electron Microscopy (SEM), slices of the petal middle region (50% of the length) of fully opened flowers were selected and then fixed and processed according to a standard protocol **[11]**. The slices were flat mounted on either their adaxial or abaxial surface, or fractured to reveal internal anatomy transverse to the petal longitudinal axis. Scanning electron microscopy was performed using a Philip S3400N apparatus. AbsE cell photography and cell counting were performed as described by **[12]**. Petal samples were taken as 0.5-cm 3 0.4-cm slices at 25%, 50%, and 75% of the petal length from the petal top. The slices were fixed in formaldehyde and then cleared in ethanol. AbsE cells of the slices were photographed using a Nikon IX-71 camera. The traces were drawn using Photoshop 7.0 software. Fifteen flowers were used in each treatment. Numbers of AbsE cells were counted using Image J software in a visual field of 1,360 3 1,024 mm

2.5 Experimental procedure

Experiment was performed by according the method reported **[13]**. The plant materials were prepared, about total of 30 petals were used on each treatment supplemented with concentrations of *glucopyranosyl* i.e. 1%,3%,5% and 7% and petals were also immersed in water as a control. The observations were recorded at two days intervals. Further, about 15 whole flowers were collected and placed into solution containing different concentration of *glucopyranosyl*, as described above. For tissue culture plant about 12 plants were used for each treatment applied 3% of *glucopyranosyl* solution at 1:1 interval with water. Experiment was replicated in three biological repeats.

2.6 Statistical analysis

Data representing the mean±standard error (SE) of three replicates were analyzed by one-way ANOVA procedures using the SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Significant differences between the treatment means were evaluated by Duncan's multiple range test at $p<0.05$.

III. RESULTS

3.1 Competency of *glucopyranosyl* on inhibition of excised petal senescence

As we all know and it is well reported that flower opening and senescence are key processes determining the vase life of several cut flowers and sugars added to the vase solution are known to increase vase life in several cut flowers, little is known about endogenous variations in sugar levels in

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several floral tissues, particularly beyond petals, during natural flower development. In uncut flowers, endogenous sucrose contents varied throughout flower development in all floral organs. However in present study, we had evaluated the *glucopyranosyl* efficiency at different concentrations and at different time intervals. We had found that the efficiency of *Glucopyranosyl*s lightly affect the senescence approaches and played a significant role in inhibition of senescence. Furthermore, it also played a key role on increasing the cells sized and enlargement of cells at growing stages of petals. However, further we had randomly investigated the content of sucrose in treated petals and control(Figure 1).Interestingly we had found increasing amount of sucrose in treated petals compare to that of untreated. Sucrose contents increased with flower development reaching 50% to 74% higher levels at stage5 compared to that of stage1 in excised petals(Figure 2).

3.2 Efficiency of *glucopyranosyl* accelerates flower opening

Further we also had investigate the efficiency of sucrose in whole tissue culture plants, it was interestingly to observed that the flower were opened very quickly in *glucopyranosyl* treated plant compare that of control which took normally more time to open the flower.It is of interesting to note that, *glucopyranosyl* levels increase with flower development in petal from different cut rose flower, reaching higher amounts from second stageto fifth stage of flower opening(Figure 3). On the other hand, it seems to be an association between petals sucrose content and flower longevity, where in normal flower it has less sugar content and have a shorter lifespan. Thus, exogenous *glucopyranosyl* might expand vase life of rose cut flowers by counteracting that possible lack of carbohydrates due to stem cutting. In present study, flower durability from early stage to later stage was not affected by sucrose addition to the vase solution. However, results showed an acceleration of flower opening days.

3.3 Effectiveness of *glucopyranosyl* involved in delaying senescence

It is well known reports that, the process of senescence is the final stage of flower development that significantly precedes the termination of the floral organ of any plant. However, in present work we had investigated that *Glucopyranosyl* drastically delayed the senescence of flowers(Figure 5). However during our first parameter we found the specific efficiency of sucrose on excised petals. In present study we had applied about 3% of *Glucopyranosyl* concentration on each of plants, further we applied the water about 1:1 intervals with *Glucopyranosyl* (Figure 6). Interestingly we found significant effect of *Glucopyranosyl* concentration on delaying durability of rose flowers compare to that of control (plant grow without *Glucopyranosyl* treatment).

3.4 *Glucopyranosyl* involved in increasing of cells enlargement

Amusingly further we found that the efficiency of *Glucopyranosyl* drastically increased the cell density and as well as the number of cells. However after treatment at two days intervals we had investigated the microscopic examination of sucrose treated and without addition of *Glucopyranosyl*. Surprisingly from the early stage to later stage, the sizes of cells were outstandingly increased compare to that of normal grow plants and excised petals as well(Figure 7). The results showed that the rose treatedwith *Glucopyranosyl* concentration produced flowers with maximum diameter (7.61 cm);while, the lowest average flower diameter (2.23 cm) was observed in control without addition of any *Glucopyranosyl* treatment.

IV. DISCUSSIONS

Flower opening and senescence are key processes of determining the vase life of several cut flowers and sugars added to the vase solution are known to increase vase life in several cut flowers, little is known about endogenous variations in sugar levels in several floral tissues, particularly beyond petals, during natural flower development. In uncut flowers, endogenous sucrose contents varied throughout flower development in all floral organs. The treatment comprised of sucrose at 3%concentration prolonged the time period required for opening the flower; these results are in line with those of **[14]** who reported that treatment of cut flowers with sucrose potentially affected the physiological factors of roses and with application of these solutions at higher rates the days to open flower were increased.

Although it was found that, the *Glucopyranosyl* efficiency at different concentrations and at different time intervals affect the petals of flowers in many aspects. Further had found the efficiency of *Glucopyranosyl* slightly affects the senescence approaches and played a significant role in inhibition of senescence, these results remarkably supported with the evidence of **[15]** who reported the role of sugar in senescence of cut flowers. Furthermore, it also played a key role on increasing the cells sized and enlargement of cells at growing stages of petals. However,

further we had randomly investigated the content of sucrose in treated petals and control. Interestingly we had found increasing amount of sucrose in treated petals compare to that of untreated. Sucrose contents increased with flower development reaching 50% to 74% higher levels at stage-5 compared to that of stage-1 in excised petals. Furthermore, it is worthy to note that the major decreases in sugar levels were observed between stages IV and V in normal petals compare to that or treated petals. In any case, cytokinins are known to delay flower senescence in several species irrespective of their sensitivity to ethylene **[16]**, therefore, sucrose-mediated increases in cytokinins may be responsible for the delay of senescence**.**

***Glucopyranosyl* accelerates flower opening and delaying of senescence**

It was noticing to observed that the efficiency of sucrose in whole tissue culture plants, was interestingly showed significant effect on flower opening as it was opened very quickly in *Glucopyranosyl* treated plant compare that of control which took normally more time to open the flower. Morover, *Glucopyranosyl* levels increase with flower development in petal from different cut rose flower, reaching higher amounts from stage 2 to stage 5 of flower opening stage. On the other hand, it seems to be an association between petals sucrose content and flower longevity, where in normal flower it has less sugar content and have a shorter lifespan. However, results showed an acceleration of flower opening days**[16]**. While, the process of senescence is the final stage of flower development that significantly precedes the termination of the floral organ of any plant**[17]**. However, in present work it as investigated that *Glucopyranosyl* drastically involved in senescence of flowers. Further found that about 3% of *Glucopyranosyl* concentration on each of plants showed significant effect, compare to that of control without *Glucopyranosyl* treatment. Interestingly we found significant effect *Glucopyranosyl* concentration on delaying durability of rose flowers compare to that of control, these work have been supported with the work of **[18]**. Further, it was amusingly found that the efficiency of *Glucopyranosyl* drastically increased the cell density and as well as the number of cells. However, microscopic examination was done at two days intervals and revealed cells enlargement from the stage 2 to stage5 of treated rose petals, the sizes of cells were outstandingly increased compare to that of normal grow plants and excised petals as well. The flower opening is one of the key factors that possess economically consequence that influences the common performance of a flowering prickle**[19]**. In rose plants treated with *Glucopyranosyl* at 3% concentration, the number of dropped flowers maximally reduced to 1.32;although, the highest number of dropped petals (5.32) was recorded under controlled plants where only distilled water was supplied without addition of *Glucopyranosyl* **[20]**.The results showed that the rose treated with *Glucopyranosyl* at 3% concentration produced flowers with maximum cells size.

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CONFLICT OF INTEREST

All authors disclosed that they have no any conflict of interest.

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Figures

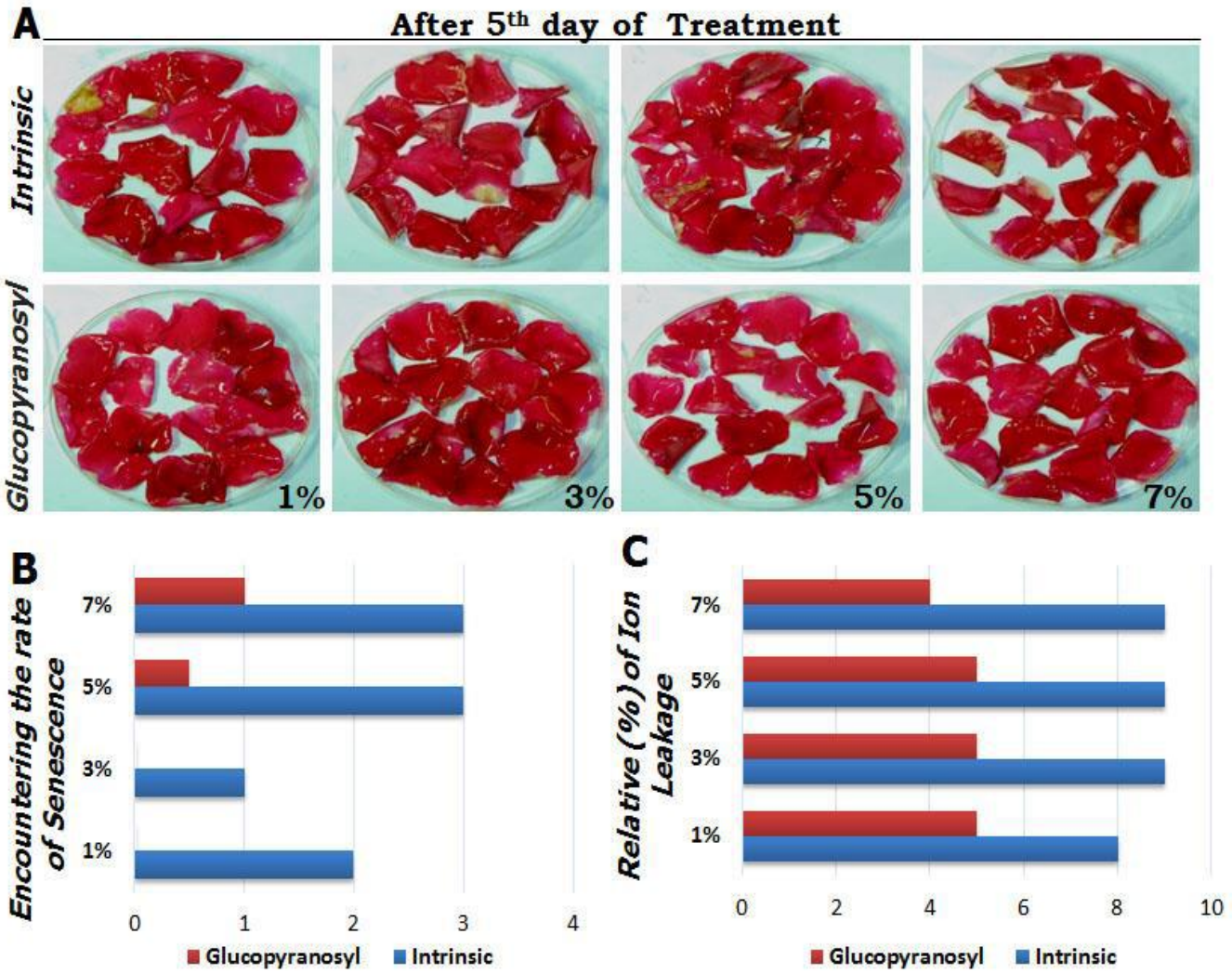

Fig.1: Efficiency of glucopyranosyl on inhibition of excised petal senescence. (A) Phenotypic analysis of after 5 days of glucopyranosyltreatment. (B) Encountering the rate of Senescence. (C) Relative percentage of Ion Leakage. Various concentrations of glucopyranosyl at different time intervals applied. Observation is the concise with three biological repeats.

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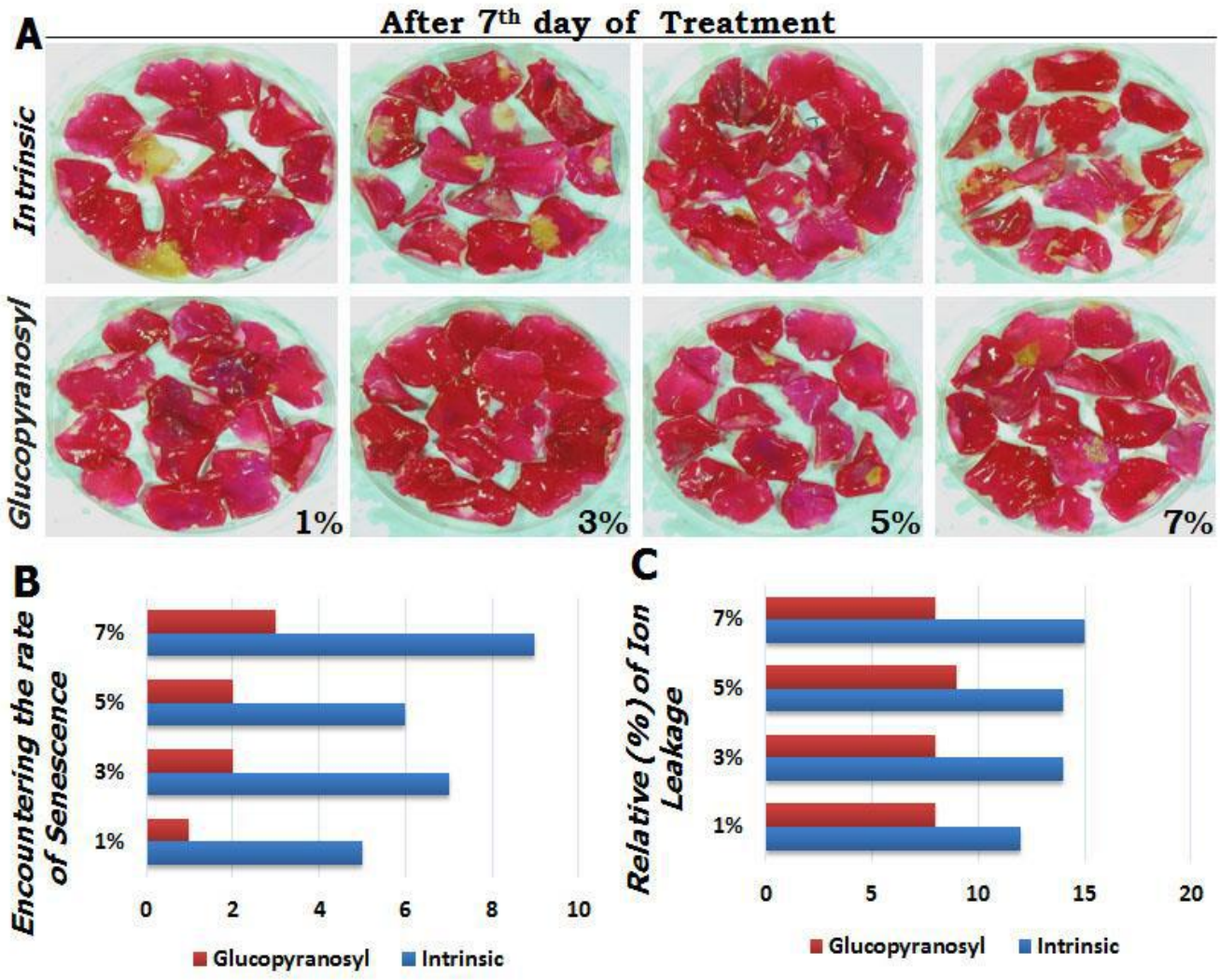

Fig.2: Glucopyranosyl inhibited the excised petal senescence. (A) Phenotypic analysis of after 7 days of glucopyranosyl treatment. (B) Encountering the rate of Senescence. (C) Relative percentage of Ion Leakage. Various concentrations of glucopyranosyl at different time intervals applied. Observation is the concise with three biological repeats.

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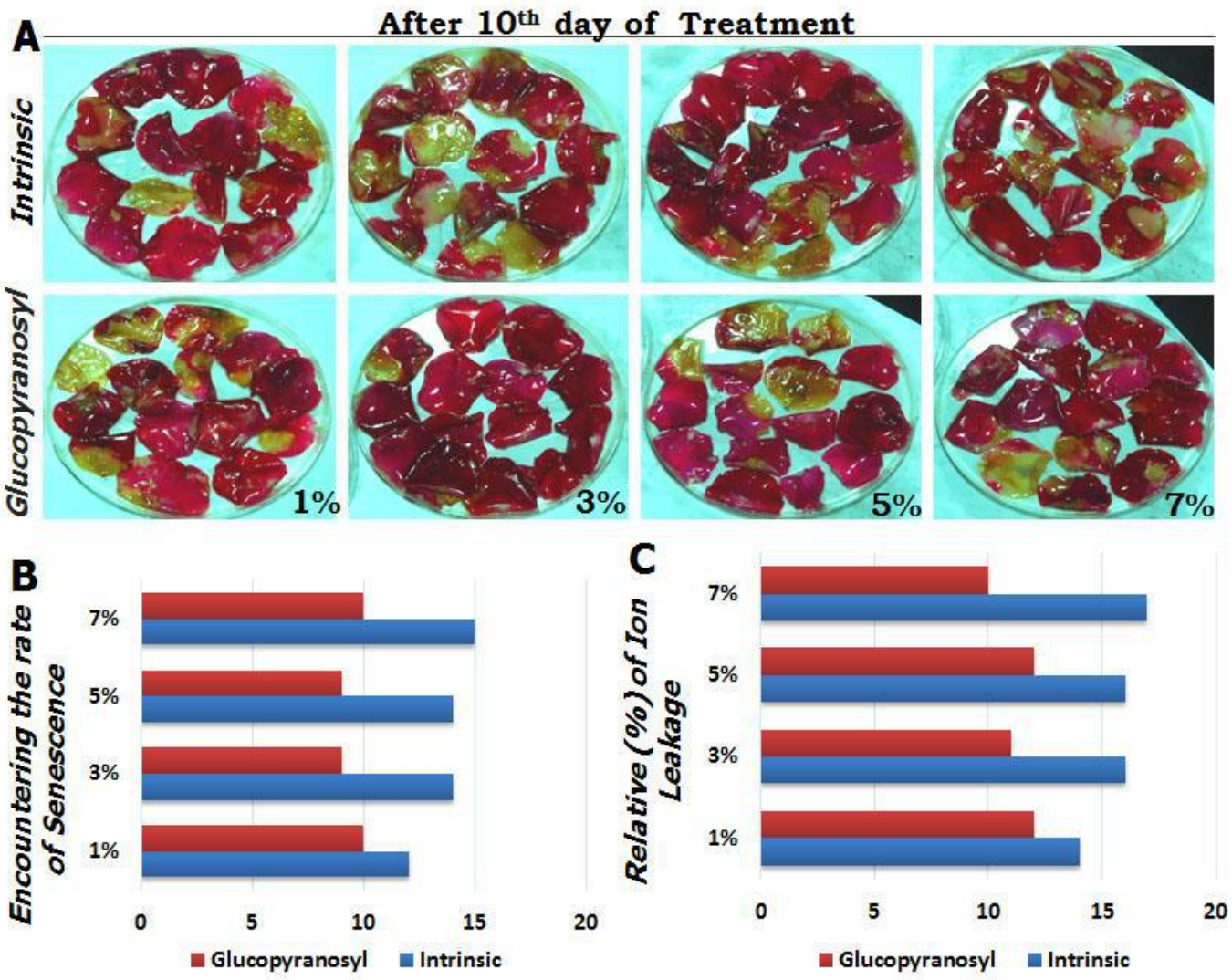

Fig.3: Glucopyranosyl inhibited the excised petal senescence. (A) Phenotypic analysis of after 10 days of glucopyranosyltreatment. (B) Encountering the rate of Senescence. (C) Relative percentage of Ion Leakage. Various concentrations of glucopyranosyl at different time intervals applied. Observation is the concise with three biological repeats.

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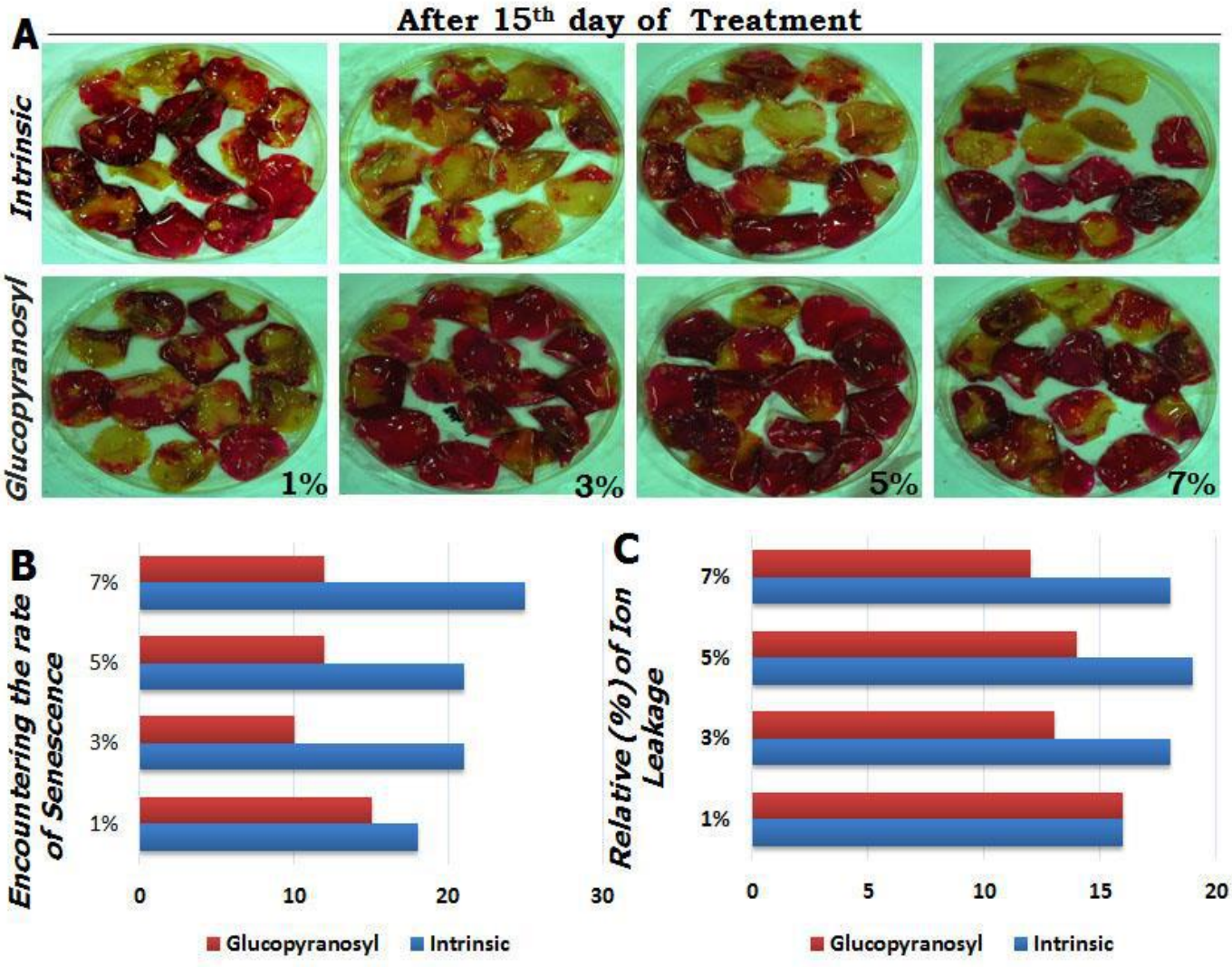

Fig.4: Effectiveness of Glucopyranosyl involved delaying of senescence. (A) Phenotypic analysis of after 15 days of glucopyranosyl treatment. (B) Encountering the rate of Senescence. (C) Relative percentage of Ion Leakage. Various concentrations of glucopyranosyl at different time intervals applied. Observation is the concise with three biological repeats.

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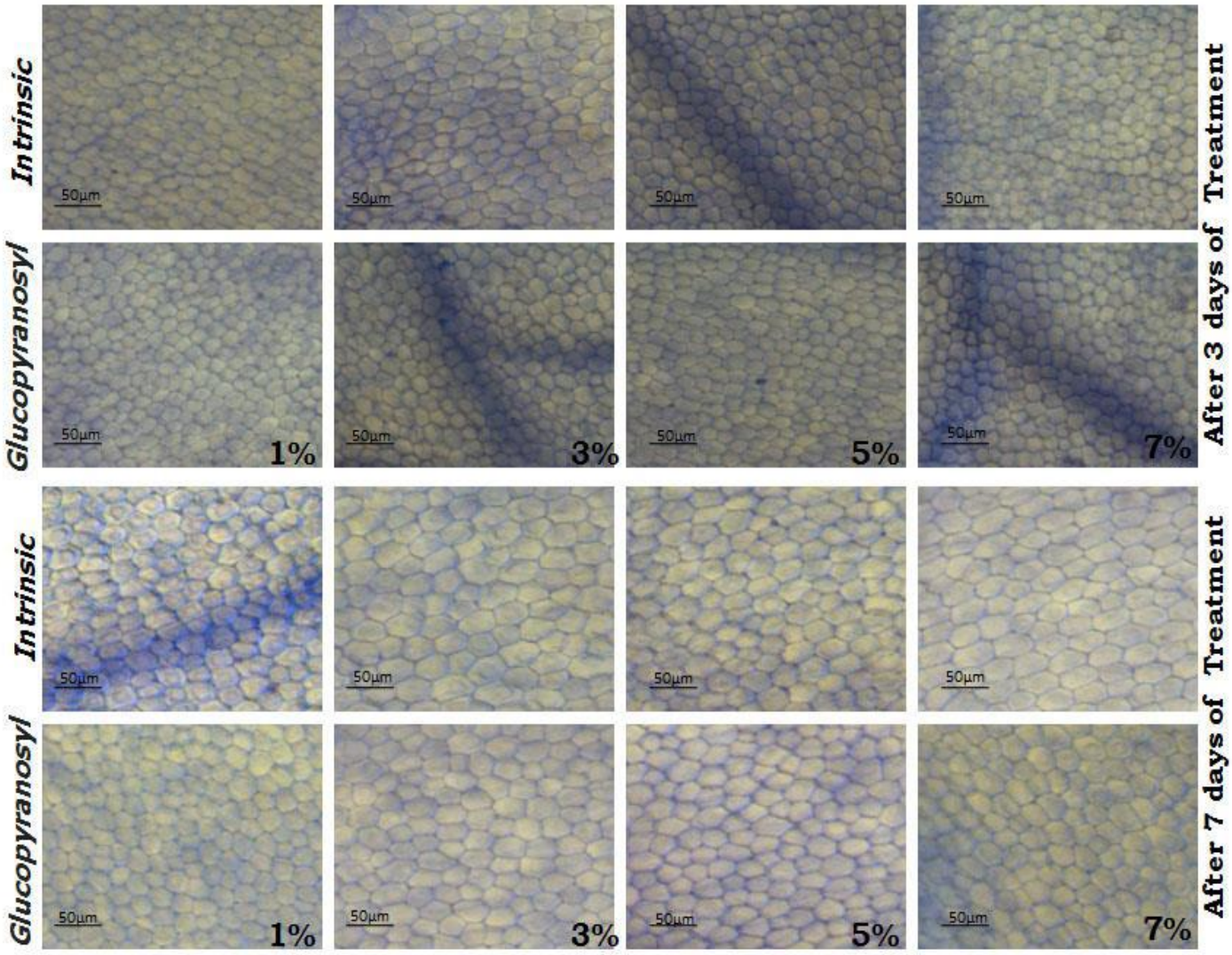

Fig.5: Histological examination of glucopyranosyl treated petals of rose after a week interval. Scanning Electron Microscopy (SEM) was performed using a Philip S3400N apparatus. AbsE cell photography and cell counting were performed as described by (Dewitteet al., 2007). Photograph was performed using a Nikon IX-71 camera. Numbers of AbsE cells were counted using Image J software in a visual field of 1,360 3 1,024 mm. Various concentrations of glucopyranosyl at different time intervals applied. Observation is the concise with three biological repeats.

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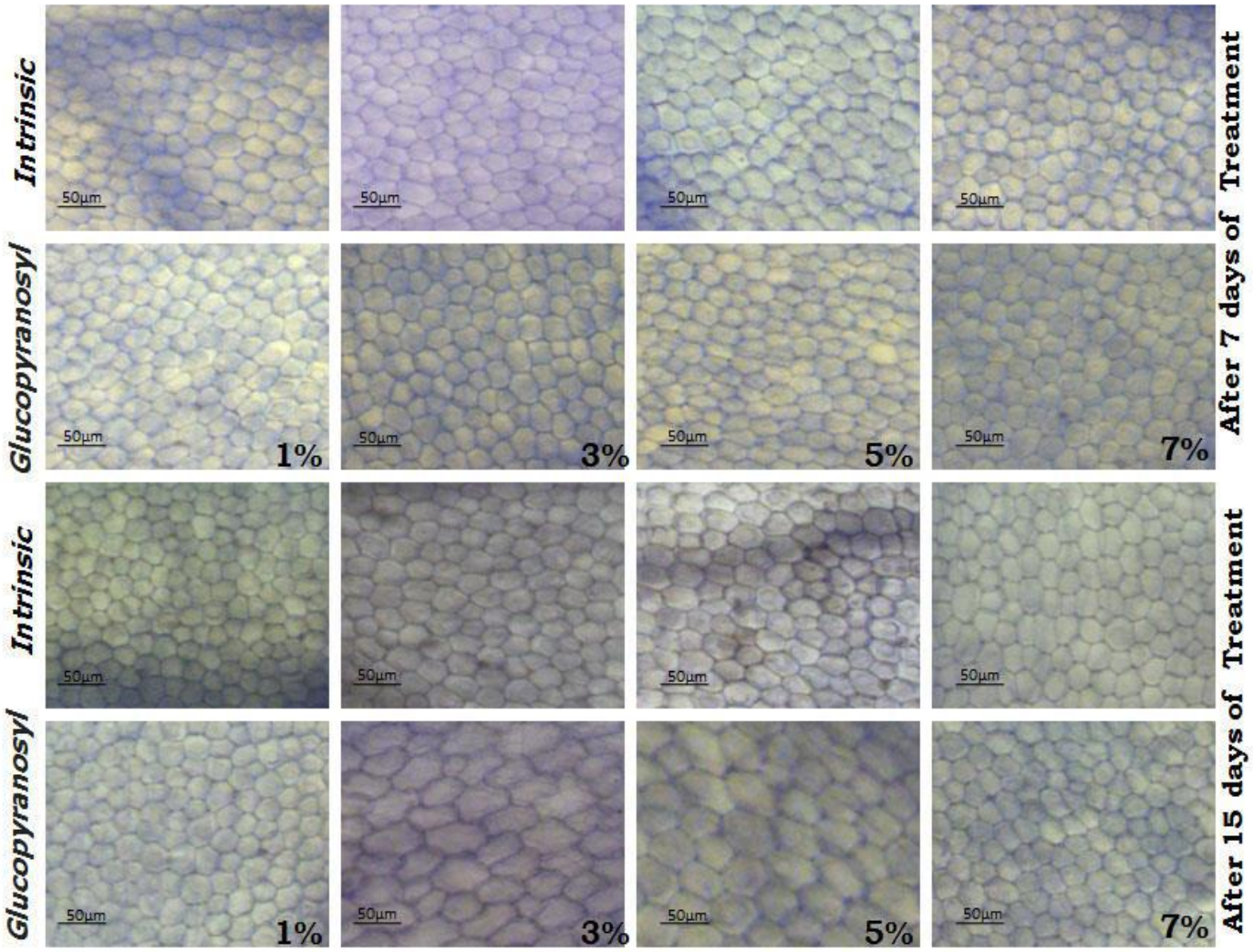

Fig.6: Histological examination of glucopyranosyl treated petals of rose after two weeks. Scanning Electron Microscopy (SEM) was performed using a Philip S3400N apparatus. AbsE cell photography and cell counting were performed as described by (Dewitteet al., 2007). Photograph was performed using a Nikon IX-71 camera. Numbers of AbsE cells were counted using ImageJ software in a visual field of 1,360 3 1,024 mm. Various concentrations of glucopyranosyl at different time intervals applied. Observation is the concise with three biological repeats.

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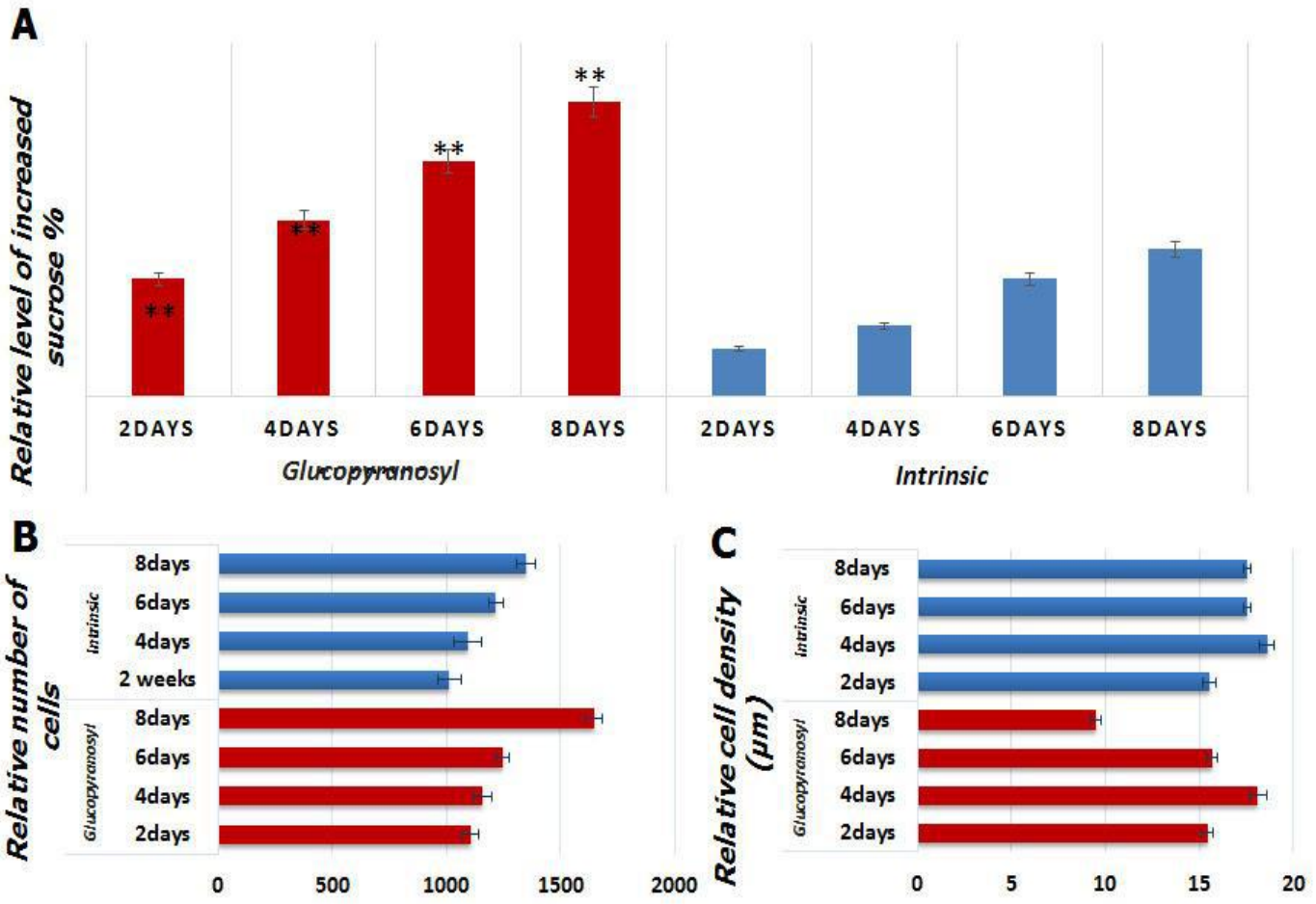

Fig.7: Glucopyranosyl elaborate to increase the cells size. (A) Increase the level of sucrose content. (B) Relative number of cells. (C) Relative cells density. Observation is the concise with three biological repeats.