LONGITUDINAL PATTERN OF NUCLEAR SIZE IN BULB SCALE EPIDERMIS OF *ALLIUM CEPA* AND CHANGES IN SIZE IN RESPONSE TO NECKROT

F. B. KULFINSKI AND A. J. PAPPELIS
Department of Biological Sciences, Southern Illinois University, Edwardsville 62025, and Department of Botany, Southern Illinois University, Carbondale 62901.

ABSTRACT.—Nuclear size in onion bulb scale inner epidermis cells was found to vary, increasing in size from base toward the equator and decreasing in size toward the apex of the bulb. The mean nuclear area in basal cells was 3.4 x 10^{-6} cm^2. The nuclear area increased to a maximum of 9.1 x 10^{-6} cm^2 near the equator and decreased to 3.6 x 10^{-6} cm^2 near the apex. Nuclear size may be related to meristematic activity near basal cells, to cell enlargement patterns during bulb development, and to cellular aging and death at the apex following harvest.

Epidermal nuclei adjacent to mycelium of neckrot pathogens responded to infection by decreasing in size. Such nuclei averaged 47 to 81 per cent of normal in area (X.S.), the amount of reduction depending apparently on their initial size.

Nuclear diameter and nuclear dry mass were studied in outer epidermis cells of the equatorial areas of the inner to outermost bulb scales of onion by Courtis (1964). He reported that average cell area, nuclear diameter, and nuclear dry mass increased from inner to outer bulb scales and that these characteristics were closely correlated. These increases were suggested to be age related since outer leaf bases are older than inner ones.

Changes in nuclear size have been reported in a number of studies, a nucleus frequently increasing and then decreasing in response to the same factor. Increases and decreases were reported during cell death by Ogilvie (1962), Cameron (1951), and Bessis (1964), in irradiated cells by Eckert and Cooper (1937), during autolysis by Kasten (1958), and in response to fungus infection by Goodman, *et al.* (1967). Increases have been reported during mitosis by Lyndon (1967) and with differentation of cells by Lorz (1947) and Courtis (1964). Decreases in nuclear size following irradiation have been reported by Schrek (1948). Nuclear size changes involve a gain or loss of water, of dry matter, or of some combination of the two.

This study was designed to determine the longitudinal distribution pattern of nuclear size in bulbs of onion based on a model of cell aging, the youngest cells being at the base of the bulb and the oldest cells at the apex. Since nuclear size and nuclear dry mass were highly correlated (Courtis, 1964), it was considered desirable to characterize longitudinal distribution of nuclear size in leaf bases in order to establish a basis for studies of cell senescence and death using quantitative interference microscopy.

In performing this study, we found that a number of bulbs exhibited fungal neckrots like those described by Walker (1968). In most cases, only the tip of the bulb apex was infected. *Botrytis allii* was easily isolated from many of these bulbs. The study reported herein includes a comparison of nuclear size in infected onions with nuclear size in non-infected ones. We assume the causal organism in the infected bulbs studied to be *B. allii* although no isolations were made from the tissue observed with the interference microscope.

MATERIALS AND METHODS

Inner (adaxial) epidermis of mature white onions, *Allium cepa* L., (approximately 10 cm in diameter) was used in a study of nuclear size

distribution. The outer dry papery scales were removed, and the first exposed turgid scale was used in each of six onions. Ten samples were taken from a 5 mm wide longitudinal strip extending from the stem at the base to the dead tissue at the apex of the bulb. Each sample was one-tenth of the longitudinal strip (approximately 1 cm long), and these were numbered 1 through 10 from base to apex. The inner epidermis was removed from each location, mounted in tap water, and microscopically studied. Ten to twenty nuclei were selected in each location and photographed using a Leitz Orthomat camera on a Leitz interference microscope at a magnification of 500x. No attempt was made to reduce variation among nuclei such as selecting all the nuclei from the same longitude or latitude within the sample area. Only those nuclei lying against tangential faces of cells were studied since nuclei at the radial faces were seen in edge view rather than face view. Nuclear sizes were determined by tracing nuclear images from prophotographic negatives and measuring the tracings with a planimeter. Nuclear size observed in face view will be called nuclear area (NA) throughout this paper.

Fungal infections were confined to apical locations. Since the nucleus and cytoplasm of cells in contact with mycelium were usually disintegrated, host nuclei in the intact cells just ahead of the mycelial front were studied as well as those well in advance of this front. Average NA of infected bulbs (minimum of five nuclei per replicate) were expressed as a percentage of average NA from comparable locations of non-infected bulbs.

TABLE 1.—Mean nuclear area[a] in relation to location[b] in the outermost live bulb scale (inner epidermis) of onion.

	Nuclear Area (10^{-6} cm^2)	Location	Nuclear Area (10^{-6} cm^2)
1	3.42	6	8.65
2	6.68	7	7.24
3	7.08	8	7.69
4	9.14	9	6.32
5	8.41	10	3.59

a. Six replicates, minimum of 10 nuclei per location per replicate.
b. Locations 1 through 10 are 10 equal distances from base to apex of the bulb scale.

RESULTS

The location means (Table 1) indicate that nuclear areas in different locations of the bulb scale are not uniform but are distributed according to a pattern. The mean nuclear area was least in location 1, generally increased to maximum in location 4, gradually declined to locations 7 and 8, and fell sharply from location 8 to 10. The nuclear areas of location 1 and 10 were nearly equal, and the greatest changes in nuclear areas were found between locations 1 and 2 and between 9 and 10.

An analysis of variance was performed which yielded F values of 4.54 and 16.28 for differences among replicate means and location means, respectively. Since the corresponding tabular values are 3.48 and 2.85 at the 1% level of probability (LeClerg, Leonard and Clark, 1962), then differences among both components are judged to be significant. Adjacent location means are compared using the Duncan mul-

TABLE 2.—Duncan multiple range analysis of differences between adjusted location means. Differences greater than 16.60 are significant at the 5% level and those greater than 22.19 are significant at the 1% level of probability. The line separates the significant differences above from the non-significant below at the 1% level. Those marked * are significant at the 5% level.

Location	Location								
	4	6	5	8	7	3	2	9	10
1	66.67	63.30	60.40	51.57	48.97	49.29	39.40	35.07	1.84
10	64.83	61.46	58.56	49.73	47.13	42.45	37.66	33.23	
9	31.60	28.23	25.33	16.50	13.90	9.22	4.33		
2	27.27	23.90	21.00*	12.17	9.57	4.89			
3	22.38	19.01*	16.11	7.28	4.68				
7	17.70*	14.33	11.43	2.60					
8	15.10	11.73	8.83						
5	6.27	2.90							
6	3.37								
4									

tiple range test (Table 2). The differences between locations 1 and 2, 3 and 4, and 9 and 10 are significant, whereas, the differences between all other adjacent locations are non-significant at the 1% level of probability. Although other comparisons can be made using Table 2, we would like to generalize as follows: no significant difference exists in the average nuclear sizes of locations 1 and 10; little difference exists among the location means in the equatorial region; and nuclear sizes at the polar regions are significantly smaller than those of the equatorial region.

In infected bulbs, the average NA in locations with mycelium and those adjacent to mycelium were found to be less than the NA averages in the same locations in non-infected bulbs (Table 3). However, for cells more distant from the infection, average NA increased. In the case of mycelium present in the location, the greatest reduction in NA occurred in location 7 and least in 10. These also were the locations initially largest and least in average NA (for the three locations studied in which the mycelial front was observed). The reduction in NA in locations adjacent to those with

TABLE 3.—Mean nuclear area (NA) of inner epidermal nuclei adjacent to and distant from the mycelial front in neck-rotted white onion bulbs. Percentages are comparisons of the mean NA of infected with the mean NA of non-infected bulbs. Locations 1 through 10 are equal distances from base to apex of the outermost turgid bulb scale. Infections were in apical locations.

Location	Number of Replications	NA x 10^{-6} cm^2 Infected	Percent of Non-Infected
Infected in location 10.			
10	4	2.9	81
9	4	6.1	97
Infected in locations 10 through 9.			
9	2	3.2	51
8	1	5.5	71
Infected in locations 10 through 7.			
7	2	3.4	47
6	2	5.6	64
Infected in 10, 10 through 9, and 10 through 7, above.			
6	8	9.7	111
1	8	3.6	106

mycelium was similar to that described above; the amount of reduction was related to the initial size. In all of the infected bulbs, the NA averages for locations 6 and 1 were greater than those in these locations in the non-infected bulbs.

Discussion

In the observed patterns of nuclear sizes in non-infected bulbs, two biological interpretations are possible: nuclear size increases with cell enlargement during development; and nuclear size increases with distance from the basal meristematic zone and then declines with senescence and death (at the apex) after bulb maturation and harvest.

In the first interpretation, it is possible that nuclear area and other nuclear properties vary with physiological condition of cells based on their location, *per se*, rather than on distance from the meristematic zone at the base. If a cell-nuclear size relationship exists (Giese, 1962), then significant differences between adjacent location means would be anticipated near the poles and only slight differences near the equator.

In the second interpretation, the youngest cells in the onion bulbs are the basal ones (Hoffman, 1933). Nuclear areas increase by approximately 2.7x from location 1 through 4. Some of the largest cells occur near the equator of the bulb, but it is not known if the increase in nuclear area is concommittant with cell enlargement. The bulb scales are leaf bases which either subtend or at one time subtended leaf blades. The drying and dying of blades at the top of a bulb begins in the field and ultimately results in dead bulb scale apices (Hoffman, 1933). It could be interpreted that the decrease in mean nuclear area from location 4 through 10 may represent a progressive deterioration during senescence in these locations after harvesting. Although the sharp difference in nuclear area between locations 9 and 10 may represent developmental differences, in all likelihood it represents progressive senescence and cell death since segment 10 borders the apical dead zone.

The nuclear sizes in Table 1, the analysis of nuclear size means in Table 2, and our observation of largest cells in the equatorial region and smallest at the poles support both interpretations. Studies of the cell-nuclear size relationship during bulb development, pre-harvest, and post-harvest phases of growth are required to resolve this question.

Although the NA is known to increase or decrease in pathological conditions (Bessis, 1964; Cameron, 1951; Eckert and Cooper, 1937; Goodman, *et al.*, 1967; Kasten, 1958; Kulfinski and Pappelis, 1969; Ogilvie, 1962), the pattern of NA observed in onion epidermal cells appears to be more related to normal growth and development processes. In this and other studies, we observed that NA of onion host cells decreased near the fungi and increased at some distance from the fungi (Kulfinski and Pappelis, 1969a, 1969b). In this study, the initial host NA varied due to location. We are assuming that the small nuclei in location 10 are less able to respond because they are in senescing cells. An additional test of this hypothesis would be to determine whether small non-senescing nuclei at the base of the bulb would be reduced in NA following infection in their vicinity.

From the study of infected bulbs, we conclude that neckrot fungi liberate chemical factors (enzymes and/or toxins) at the site of the infection, which diffuse through the tissue away from the infection site

and cause the nuclei of the host cells to decrease in size as a result of a combination of degradation of nuclear materials and changes in nuclear membrane character. The increase in NA at greater distances from the infection may be a response to metabolic changes in living host cells or to products from dead host cells nearer the infection. It appears that dying of host cells takes place very quickly in those cells in contact with the fungus and more slowly in cells more distant from the mycelium. It is also concluded, since host cell death in fungal parasitism and cellular autolysis (Kasten, 1958) have similar symptoms, that these degenerative changes and others as well may have common pathways, differing only in the specific form of initiation of the changes.

We conclude that the onion bulb epidermis represents a model in which to study nuclear control of cell development, senescence, death, and pathological changes. The fact that quantitative interferometric studies can be made on these living cells (Kulfinski and Pappelis, 1969a) without the obvious disruptions of morphology and composition which occur as a result of histological procedures contributes an important method to the study of the above-mentioned processes. Since NA correlates with nuclear dry mass (Courtis, 1964), and since there is a pattern of NA in the bulb scale, it may be that quantities of nuclear components vary with location and NA. Until the DNA content has been determined in these nuclei, an explanation of nuclear mass and NA changes can not preclude the possibility of endoploidy (Lorz, 1947). The determination of DNA, RNA, chromosomal histone, and total nuclear protein would be useful in the studies of development, senescence, cell death, and cellular changes in response to pathogens.

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