A Morphological Study on the Time of Reproductive Differentiation of the Apical Meristem of *Brassica oleracea* L. var. *italica,* Plenck, cv. 'Coastal'[1]

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Abstract. Various histological criteria were employed in studying the chronological and morphological development of the apex of field-grown plants of *Brassica oleracea* var. *italica,* cv. 'Coastal'. Time of differentiation was based on changes in the size and configuration of the apex. The earliest evidence of reproductive differentiation was 5 weeks after sowing or at the time of macroscopic appearance and unfolding of the eighth true leaf. First order floral stalks began to appear at 7 weeks or at the time of macroscopic appearance of the 14th true leaf. Thus, the time interval for the transition from a vegetative to a reproductive apex appears to be approximately 2 weeks, under field conditions. By 9 weeks after sowing, or after the macroscopic appearance of the 22nd true leaf, second order floral stalk initiation and hence inflorescent head formation predominated.

Introduction

ALTHOUGH there has been no histological study on flower differentiation in green sprouting broccoli, considerable work has been done with such Brassicas as cabbage (2, 10, 18), cauliflower (13, 14), Brussels sprouts (16), collards (11), white mustard (1), and field mustard (3). Thompson (18) found that elongation of the terminal bud was the first indication of flower differentiation in cabbage and was accompanied by a change in the configuration of the apex from a flattened to a conical form. Boswell (2) determined this period of differentiation (or change of configuration) to extend over a 2 week period, which was marked by a rapid increase in the width and depth of the meristem. The transition in Brussels sprouts was similar to that of cabbage (16).

The purpose of this study was to determine the chronological time and the morphological stage of reproductive differentiation of green sprouting broccoli, thus providing some background for future work aimed at increasing the uniformity of the harvest date through chemical or cultural means.

Materials and Methods

On July 15, 1966, seeds of the cultivar 'Coastal' were direct-seeded in the field in 30-ft rows with a 4-ft spacing between rows. Each plot of 8 such rows was replicated 4 times. Within each replication each row was randomly designated as to time of sampling, e.g., 3 weeks, 4 weeks. By 2 weeks after sowing, seedlings had been thinned to 1 plant every 12 inches.

Terminal bud samples were taken at weekly intervals from August 4 (3 weeks from sowing) to September 16 (9 weeks from sowing), a time when most of the plants had macroscopically visible inflorescent heads or "buttons". On each sampling date 15 samples were randomly taken from each replication and the leaf number and size of the last expanding leaf subtending the apex was recorded. In all, 60 samples were taken on each sampling date.

For the period between sowing and the taking of the 5 week sample the average minimum and maximum temperatures were 61.8° and 86.1°F, respectively. The average minimum and maximum temperatures for the 2 week period just prior to the 5 week sampling were 64.1° and 86.1° respectively.

At the time of sampling each sample was immediately killed and fixed in a formalin-acetic acid-alcohol (FAA) mixture consisting of 50% ethyl alcohol, glacial acetic acid, and 40% formaldehyde (18:1:1). Prior to sectioning, the samples were passed through a tertiary butyl alcohol (TBA) dehydration series and embedded in Tissue Mat (M.P. 52.5°C). Serial longitudinal sections of the embedded tissue were made with a Spencer rotary microtome at a thickness of 10μ. The iron alum hematoxylin (Heidenhain's)-Safranin staining procedure of Sass (15) was followed as modified by Gauss (6). This procedure was followed for all of the samples except for 12% and 33% of the samples taken at 8 and 9 weeks, respectively. These samples had already formed macroscopically visible inflorescent heads which were too large to section via the above procedure. After staining, the samples were microscopically examined, data recorded, and photomicrographs taken of representative samples for each sampling date with a Leitz Ortholux research microscope supplemented with a Leica ⅓× camera. Only photomicrographs of critical stages of development are presented here.

In addition to recording the width and depth of the apical meristem, the number of apical tunica layers was recorded. As illustrated in Fig. 1, the

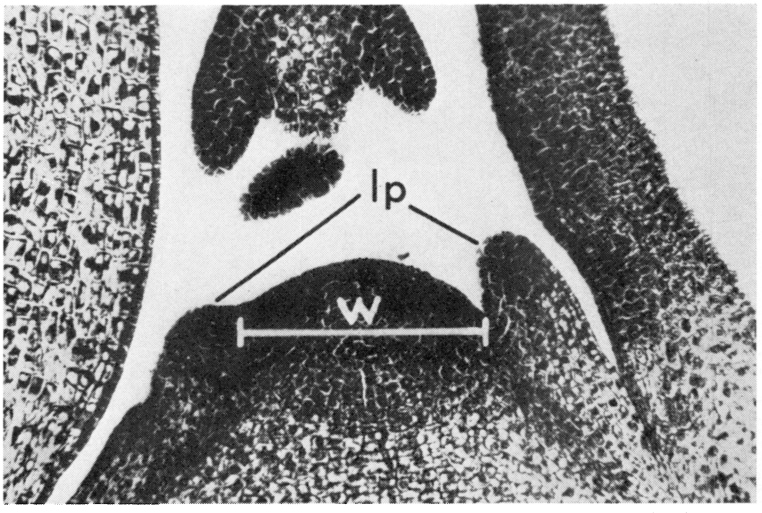

Fig. 1. A representative apex of green sprouting broccoli. The horizontal line represents the width (W) of the apical meristem between the 2 uppermost leaf primordia (1 p). 150×.

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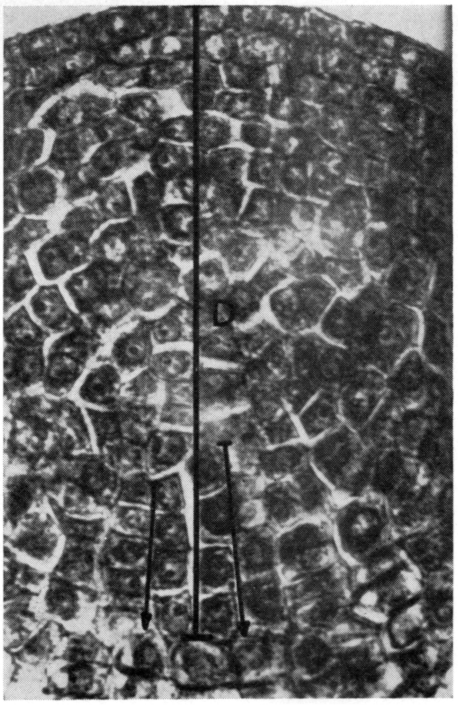

Fig. 2. A section of Fig. 1 enlarged showing the depth (D) of the apical meristem as indicated by the vertical line. Note the groups of cells, each having 4–5 cells in a vertical file (arrows). These groups represent the pith rib meristem region. 1225×.

width of the meristem was considered to be the horizontal distance between the 2 uppermost leaf or flower stalk primordia. Since the entire meristem is important in the differentiation process, the depth was considered to be from the top of the apex to the end of the linear files of cells which represent the pith rib meristem (Fig. 2). In recording this data only median longitudinal sections of the apex were used. Due to tissue tearing or improper sample orientation in the microtome block some samples could not be measured. However, for each sampling date at least 67% of the samples were measured in the above manner, and all of the samples taken were observed microscopically to determine their stage of apical development.

Results and Discussion

In general, under field conditions, with an increase in the chronological and morphological age of the green sprouting broccoli plant there was an increase in the width and depth of the apex (Fig. 3, 4). This was in contrast to Boswell's (2) findings with cabbage, where he observed that there were no significant differences in apical size or shape in plants of different size. In fact, in Boswell's work many apices remained small and vegetative even though plant size increased greatly.

Based on histological examination,

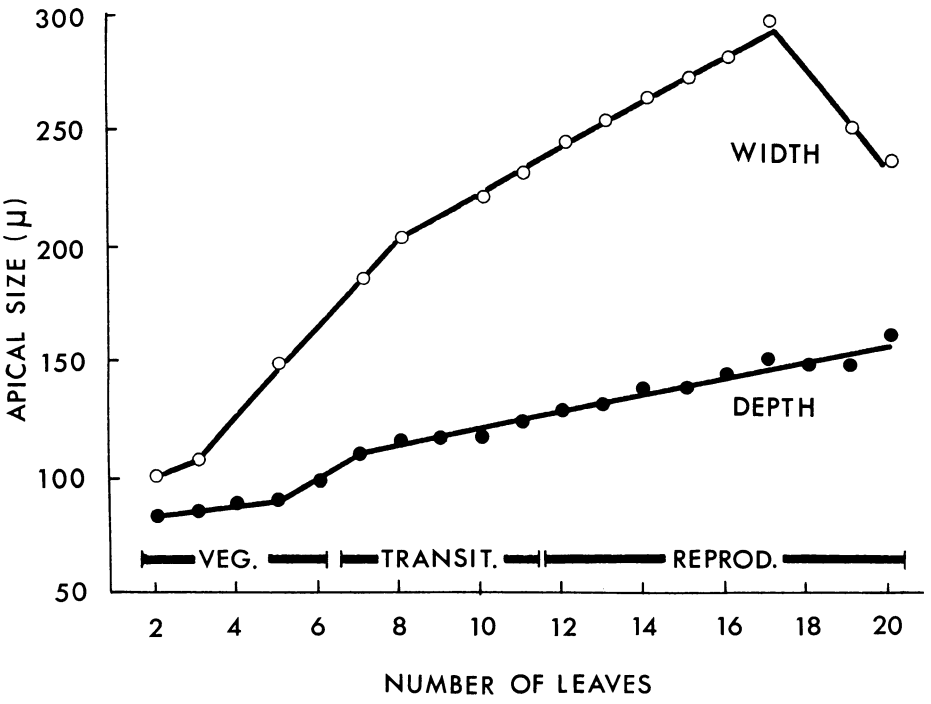

Fig. 3. The rate of change in apical size as influenced by the chronological age of the plant.

the vegetative phase of apical growth continued for 4 weeks following seeding (Fig. 5). By 4 weeks the fifth macroscopically expanding leaf had appeared. At this time the apex was small (147μ wide, 90μ deep) and dome-shaped as in the vegetative apices of other Brassicas (11, 12, 16, 18). The width of the apical meristem was increasing rapidly between 3 and 4 weeks while the depth of the meristem remained almost constant (Fig. 3). Increase in apical depth did not occur until 5 weeks after sowing or until the macroscopic appearance of the sixth leaf (Fig. 3, 4, 6). Such a lag period for an increase in apical depth has also been reported by Bernier et al. (1) for white mustard. These authors associated increases in apical width and depth, which occurred after an inductive long day, with the prefloral phase of development. Although Bernier et al. used the depth of the meristem

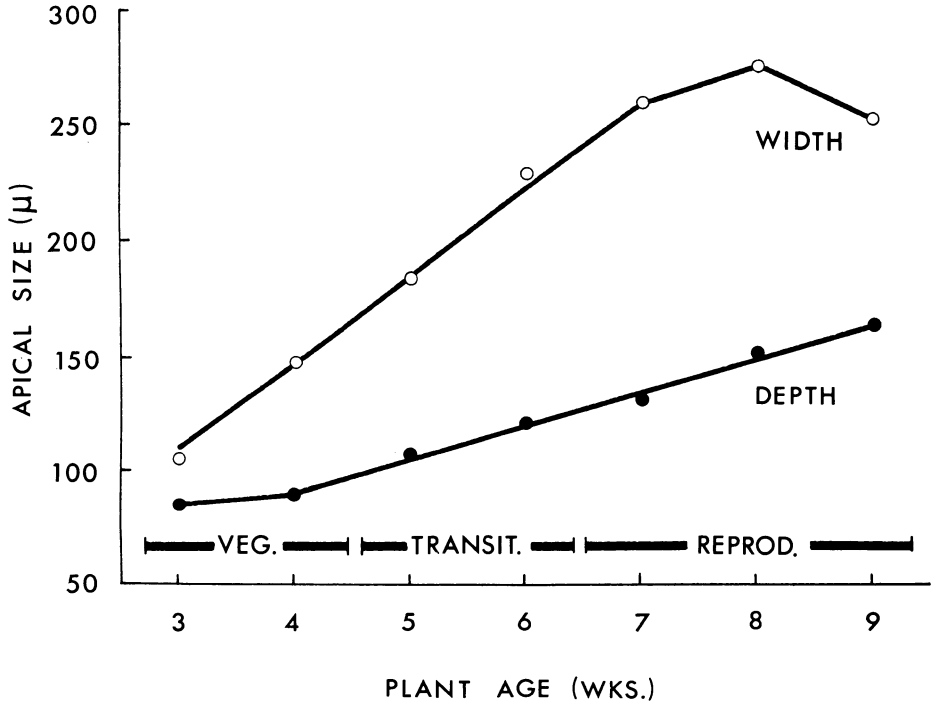

Fig 4. The change in apical size in plants of different morphological age.

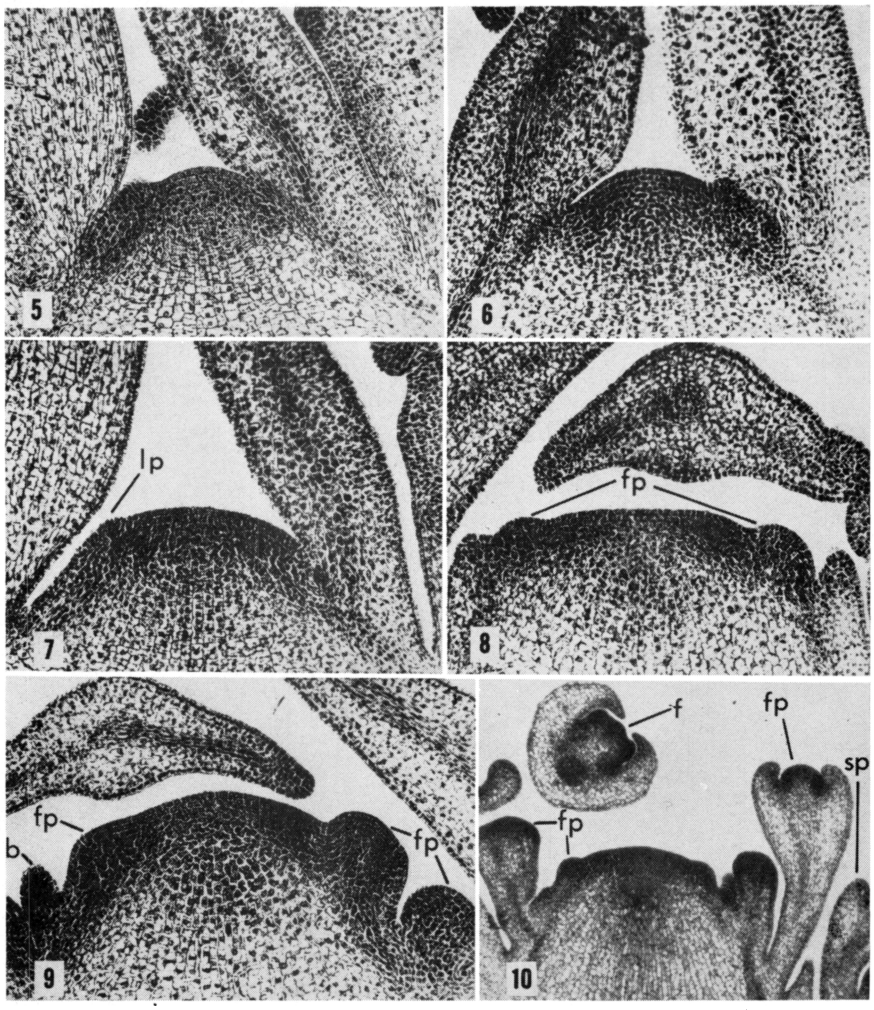

Fig. 5–10. Transition from vegetative to floral apices in broccoli. *Fig. 5.* A large vegetative apex of a 4-week old seedling. *Fig. 6.* A large, domed apex of a 5-week old plant. This apex may represent the first histological evidence of a transition from a vegetative to a reproductive apex. *Fig. 7.* A large, flat, and broad apex of a 6-week old plant. This apex represents the early reproductive stage prior to initiation of first order floral stalks. Note the base of a leaf primordium (1 p). *Fig. 8.* A large, extremely flat apex of a 7-week old plant which is initiating first order floral stalks (fp). Note the phenomenon of leaf cupping. *Fig. 9.* A large, somewhat cone-shaped apex of a 9-week old plant which is initiating many first order floral stalks (fp) and bracts (b). *Fig. 10.* An apex of a 9-week old plant which is in an advanced reproductive stage of elongation of first (fp) and second order floral stalks (sp), and producing florets (f). *Fig. 5–9,* 150×; *Fig. 10,* 70×.

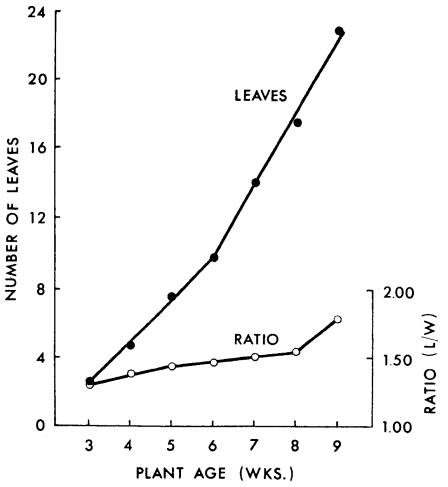

Fig. 11. The rate of leaf formation and the change in the ratio of length to width (L/W) of the last expanding leaf subtending the apex as a function of chronological plant age.

above the leaf primordia as a criterion of prefloral development it should be noted that in green sprouting broccoli the dome of the apex extended ca. 5 cells above the uppermost leaf primordia at 5 weeks as compared to ca. 2 cells at 4 weeks (Fig. 5, 6). On this basis then, as well as on the basis of entire meristematic depth, there was a marked increase in meristem depth between 4 and 5 weeks after sowing, or after the macroscopic appearance of the sixth leaf.

From the above changes in the apical meristem it appears that between 4 and 5 weeks, or after the appearance of the sixth leaf, there was a marked change in the histological activity of the meristem. Perhaps such a change was indicative of an active meristem which was initiating many leaf primordia prior to becoming a reproductive apex. Such a stimulation in the initiation of leaf primordia prior to flowering has been reported by Stokes and Verkerk (16) in Brussels sprouts and by Thomas (17) in panicle-forming *Chenopodium amaranticolor.* Although there was no direct measurement taken of the number of new leaf primordia initiated during this study, it is of some importance to note that the number of new leaves appearing markedly increased between 6 and 7 weeks after sowing (Fig. 11). Depending upon the interval between initiation of a leaf primordium and the macroscopic appearance of the resulting leaf, it is possible that this increase in the number of leaves between 6 and 7 weeks was suggestive of an increase in the number of leaf primordia initiated between 4 and 5 weeks.

Waterkeyn et al. (19) working with tobacco which produces indeterminate panicles or racemes, observed that the irreversible prefloral stage occurred when the plants possessed a wide, dome-shaped apex. Since the Brassicas produce inflorescences of the same type as tobacco, it is possible that the earliest histological evidence of reproductive initiation in green sprouting broccoli was at 5 weeks from sowing. At this time the apex was broad (183μ wide) and high-domed as in the prefloral stage of tobacco (Fig. 6). Similar prefloral configurations have also been reported for collards (11) and Brussels sprouts (16).

At 6 weeks, when the plants had an average of 10 leaves, the apex was broad (227μ wide) and practically flat (Fig. 7). This indicates its early reproductive nature as reported for many plants (4) and particularly for cabbage (18). Occurring also at this time was a phenomenon in which the uppermost leaves became "cupped" over the apex in 25% of the samples, i.e., the youngest leaves were folded over the apex in a horizontal fashion rather than in a vertical fashion as in vegetative apices (Fig. 8). This phenomenon has not been previously associated with any stage of reproductive development in Cruciferous crops. However, it does appear in drawings and photomicrographs of the early reproductive stage of many Brassicas (2, 11, 13, 16, 18), as well as in lettuce which has a panicle-type inflorescence (8). This phenomenon may not be directly associated with reproductive initiation or differentiation, but may be a result of the physical stress put on the upper-

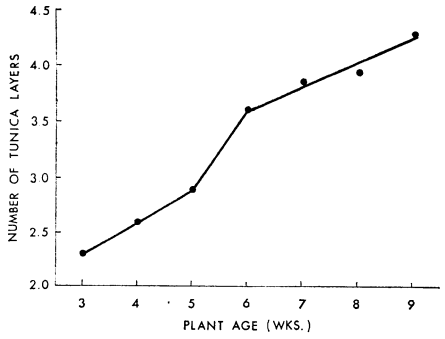

Fig. 12. The increase in the number of tunica layers in plants of increasing chronological age.

most leaves by the rapidly expanding apex, thereby "causing" the leaves to curve in over the apex. Between 5 and 6 weeks from sowing a marked increase in the number of tunica layers occurred (Fig. 12). Such an increase in the number of tunica layers in the transition from a vegetative to a reproductive apex has been reported for other Cruciferae (3, 9) and for *Chenopodium album,* which forms an indeterminant panicle (7).

Since reproductive differentiation appeared to be taking place between 5 and 6 weeks after sowing, it is noteworthy that the average minimum and maximum temperatures for the 2 week period prior to reproductive initiation at 5 weeks were 64.1° and 86.1°F, respectively. On the basis of this field observation it would appear that this cultivar of green sprouting broccoli does not have a qualitative cold requirement for floral induction.

Seven weeks from sowing representative plants exhibited large (259μ wide, 131μ deep), extremely flat, but somewhat raised apices, 25% of which were initiating first order floral stalk primordia (Fig. 8). Of all the samples 83% exhibited the phenomenon of the youngest leaves cupping over the apex. Between 7 and 8 weeks the width of the apex increased very little, possibly due to the increased activity of the peripheral zone of the apex in floral stalk initiation and formation which, consequently, prevented the apical meristem from attaining a maximum width. During this same period the depth of the meristem as well as the number of tunica layers continued to increase at a constant linear rate (Fig. 3, 12).

At 8 weeks the apex reached its maximum width (275μ) and 83% of the apices were initiating first order floral stalks (67% of these were also initiating second order floral stalks). Of the samples forming second order floral stalks 13% represented reproductive buttons greater than 5.0 mm in diameter.

Between 8 and 9 weeks the width of the apex decreased (from 275μ to 259μ), while the depth of the apex continued to increase (Fig. 3). This period was marked by rapid formation and elongation of first order floral stalks and initiation of second order floral stalks (Fig. 9, 10). This decrease in apical width after initiation of first order floral stalks has also been reported by Stokes and Verkerk (16) for Brussels sprouts and by Bernier et al. (1) for white mustard. As suggested by Bernier et al. this decrease in apical width is probably due to a reduction in the size of the peripheral zone of the apex with the initiation of floral stalk primordia.

At 9 weeks, when the plants had an average of 17 leaves, all apices were in some stage of flowering, and 52% of the plants sampled had inflorescent heads or buttons greater than 5.0 mm in diameter. Although the apex was in an advanced reproductive stage, apical zonation persisted. This is typical of plants producing indeterminant inflorescences (5). After the appearance of the 17th leaf the width of the apex decreased rapidly with an increase in morphological age, possibly denoting that a progressively greater number of plants were forming reproductive buttons (Fig. 4). During this chronological time (8 or 9 weeks) in which the apex was decreasing in size, the last expanding leaf subtending the growing point became markedly elongated and strap-shaped in form. Stokes and Verkerk (16) observed a similar phenomenon accompanying flowering in Brussels sprouts and referred to these leaves as "generative" leaves.

This study was not definitive in nature, but it does provide some information as to the chronological and morphological sequence of events in the transition from vegetative to reproductive growth in this cultivar of green sprouting broccoli. It should also serve as an impetus for future related studies with this and other green sprouting broccoli cultivars.

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