The Cytohistology of Foliar and Floral Initiation in *Andropogon gerardi* Vitman

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The present study was conducted to determine the time of inflorescence initiation and the sequence and derivation of foliar and floral organs in *Andropogon gerardi* (Big bluestem). It has become increasingly evident that such detailed information is essential for the precise interpretation of the results of physiological and agronomic research. Recent researches (Barnard, 1955; Holt, 1954) have demonstrated a need for broader coverage of anatomical knowledge in the genera of the family *Gramineae,* with emphasis on the cytohistology of floral initiation.

The literature on this subject has been reviewed in a previous paper (Holt, 1954). In a more recent study (Barnard, 1957) the following species were examined: *Bambusa arundinacea, Triticum aestivum, Lolium multiflorum, Bromus unioloides* Kunth (*Bromus catharticus* Vahl in "Stand-

ardized Plant Names"), *Danthonia setacea, Ehrharta erecta* and *Stipa hyalina.* Barnard found very little difference in the histology of floral organogenesis in these species. He stated that they represent a reasonable cross section of the gramineous types. The present paper deals with differences found in the histology of big bluestem.

MATERIALS AND METHODS

Plants were collected from a railroad right-of-way and on open prairie near Ames, Iowa, as early as 1951. Subsequent collections of similar clones were made from an open prairie near Stillwater, Oklahoma, in 1954 and 1956. Chromosome counts on the types studied were made to identfy similar clones. The 70 chromosome types were selected for this particular study.

Both fresh and preserved materials were used in the diagnosis of the conditions of the shoot apex. In sampling winter and early spring material, portions of the sods were removed to the laboratory, washed free of soil and the stems (rhizomes) traced back to the crown to insure the proper identification of each shoot (Bennett, 1950; Hitchcock, 1950). This precaution was necessary because the sods were contaminated with other perennial and annual grasses.

The growing points of each sample were excised by splitting them down the center and removing the portion above the node of the largest expanded leaf. The excised pieces were immediately evacuated in an Allen-Bouin killing solution (Formula II) and stored pending further processing. A second preservative, a modified FAA-glycerin formula, was used to maintain a stock of material for dissection under the stereoscopic binocular microscope. Materials preserved in Allen-Bouin's fluid were processed and embedded in paraffin. All materials were sectioned at ten microns and stained in safranin O-fast green for diagnostic and histological interpretations. Both median longitudinal sections and transverse sections were used for the interpretations of stage of development from vegetative through the flowering phases.

OBSERVATIONS

The Dormant Vegetative Apex

Big bluestem is a perennial grass which produces culms from either rhizomes or old crowns. The culm-producing buds are formed the previous year in the axils of the oldest foliage leaves. A membranous prophyllum is the only protective covering during dormancy. The outer three leaves of the bud are firm and lightly enclose the shoot apex. The shoot apex may contain from five to six foliage leaves and one or two leaf primordia. The number of foliage leaves present in buds ranges from 10 to 12 in material collected in early April.

Histogens

The shoot apex is very short and slightly bent to one side in the plane of leaf initiation, and alternates in a rhythmic pattern of bending as foliage leaves are initiated in acropetal order (Fig. 1). The dome is round to elliptical when viewed in a transverse plane above the last produced leaf (Fig. 2). The apex consists of a one-layered tunica which covers a mass of elongate, polygonal cells which make up the corpus, the central mass of the apex. The peripheral layer of the corpus appears at times to retain continuity by normal anticlinal cell division, but serial sections to either side of this longitudinal level show several periclinal as well as random planes of cell division. The cells of this layer may be columnar or isodiametric in shape.

The apex has only two leaf primordia at any one time during the vegetative phase. After a leaf has grown above the level of the shoot apex, it elongates very rapidly and the provascular tissues differentiate first.

Provascular elements are evident in the youngest leaf primordia which have a well-developed marginal meristem (Fig. 1). From one to three protoxylem and protophloem elements are evident in the third and fourth leaves near the midrib section. Successive leaf primordia are produced in acropetal, distichous order, throughout the entire growing season. The growing season extends from early spring until mid-summer.

Leaf initiation is preceded by many anticlinal cell divisions in the tunica and in the first layer of the corpus (Fig. 2). The first evidence of leaf histogenesis occurs in the peripheral layer of the corpus where periclinal cell divisions arise, approximately at the level of the axil of the last-produced leaf. This activity is followed by periclinal divisions in the tunica adjacent and continguous to those of the corpus. Many periclinal divisions may occur in the tunica layer. As a result of this mitotic activity a definite bulge is formed on the side of the apex opposite the last-produced leaf (Fig. 2). The activity of histogens continues around the stem and forms a meristematic ring. The leaf margin is first formed by rapid anticlinal cell divisions on the upper edge of the annual meristem, followed by periclinal divisions and cell enlargement. The marginal meristem of the young leaf primordium is produced laterally by the same pattern of meristematic activity, beginning at the point of leaf initiation. As a result of this developmental activity, the young leaf soon arches over and remains very closely appressed to the apex for a brief period of time. A vegetative apex may range from 56 to 84 microns in width above the last-produced leaf, from 126 to 196 microns in width at the base of the first leaf, and from 126 to 195 microns in length. The central mass of the leaf is derived from corpus (especially provascular strands), however, marginal cell divisions of the tunica also contribute a portion of the leaf mass.

Initiation of the Inflorescence

All shoots remain vegetative through spring and early summer. The first evidence of inflorescence initiation occurred in material collected during the latter days of June. At the time of initiation, histogen activities in the apex change in relation to organogeny. The shoot apex becomes broader and longer and somewhat flattened at the distal end (Fig. 3). Cell divisions in the tunica occur rapidly in anticlinal planes, and the increase in mass is produced by cell division in the corpus in many random planes. The initiation of a leaf primordium may or may not occur at a point above the level of the axil of the last-produced leaf, but histogenesis becomes retarded in the zone of leaf initiation and increased histogen activity is detected above the leaf primordium. This zone of activity in the histogen gives rise to a branch of the first-order. Anticlinal divisions in both tunica and the peripheral layer of the corpus give rise to stratified layers of cells within the apex (Fig. 4). A dome-like apex arises in the axil of a rudimentary leaf primordium (Fig. 3). Continued periclinal and anticlinal cell divisions in the corpus produce radial rows of elongate cells, which are oriented with their lengths to parallel to the main axis of the branch primordium. This phase of histogenesis marks the end of foliage leaf formation and represents the transition to the flowering phase. The apex is asymmetrical after the second first-order branch is initiated. The second first-order branch may be initiated at a point less than 180° from the position of the first-produced branch (Fig. 4). From 16 to 20 first-order branches are produced acropetally in an inflorescence. Branches of the second-order range from 13 to 20 in number and are produced in acropetal order (Fig. 5). Branch primordia of all orders

are initiated by the process described for the branches of the first-order. Externally, the branches of the inflorescence have been described as racemes subtended by a peduncle, but from the standpoint of histological derivation, they are branches of successively higher orders. The branches become bifacial after the branches of all orders have been initiated, and the branches tend to face one another and form a bowl-shaped cluster, with the floral primordia on the inside (Fig. 6).

Development of floral organs

The initiation of spikelets is first detected on the lower side of the second- and third-order branches at the distal end of the leaf-cluster. Activity in the tunica is the first evidence of glume initiation. Periclinal cell divisions give rise to a ridge or bulge, which is propagated laterally around the apex. A shelf-like ring meristem below the apex dome is the final result (Fig. 8).

Marginal activity of the first glume is detectable in the pedicellate spikelet very early in spikelet ontogeny. Glume initiation is basipetal in every branch-order of the inflorescence. The second glume is initiated in rapid succession followed by a sterile lemma in acropetal distichous order. The sterile lemma is initiated in the peripheral layer of the corpus and the tunica. The fertile lemma is organized in the same layer and elongates rapidly from the point of initiation before the annular meristem encircles the young floret primordium. Stamen primordia are evident before the palea primordium is initiated, thus, the distichous, acropetal order of floral ontogeny is interrupted. The pistil primordium becomes evident with the initiation of the carpel primordium in the same plane as that of the fertile lemma. The initiation of the carpel wall is leaf-like, and encloses the remaining mass of dome-shaped meristem which gives rise to the ovule (Fig. 8). The two lodicule primordia arise from the tunica and peripheral layer of the corpus, and become flattened on the upper surface. The order of floral organogenesis is sterile lemma, fertile lemma, stamens, palea, pistil, and lodicules in both sessile and pedicellate spikelets. The terminal floret is fertile. The pedicellate spikelet was used for demonstration (Fig. 8) because of its favorable position and orientation . The pistil is abortive in the pedicellate spikelet following the initiation of the stigmas. Archesporia of abortive pistils were not observed in the material studied. A physiological mechanism is suggested as the possible control of archesporial development in the two types of spikelets.

Discussion

The time of floral initiation has been established for several grasses of the spring-blooming types. Very little knowledge of floral development in mid- and late-summer varieties is known. This study of a summer type shows that a definite vegetative phase exists until late June when the shoot apex undergoes functional and structural changes to produce an inflorescence. There is no over-wintering of inflorescences in any of the grasses, including big bluestem, studied by Holt (1954) or of Brome (Sass and Skogman, 1951).

The shoot apex in *A. gerardi* is very similar to that of the annuals studied by Barnard (1957), Abbe and Phinney (1951), Bonnett (1935, 1936, 1937, 1940), Chen (1938), Kliem (1937), Sass and Skogman (1951), and Sharman (1945). The shoot apex is short and tapered with two leaf primordia. A leaf primordium is pedagogically defined as a young leaf with no differentiated cells, e.g. protoxylem or protophloem.

The histogens of big bluestem differ from other grasses studied, in having a one-layered tunica, like that of the small grains, which becomes two-layered during branch initiation, then becomes uniseriate again during

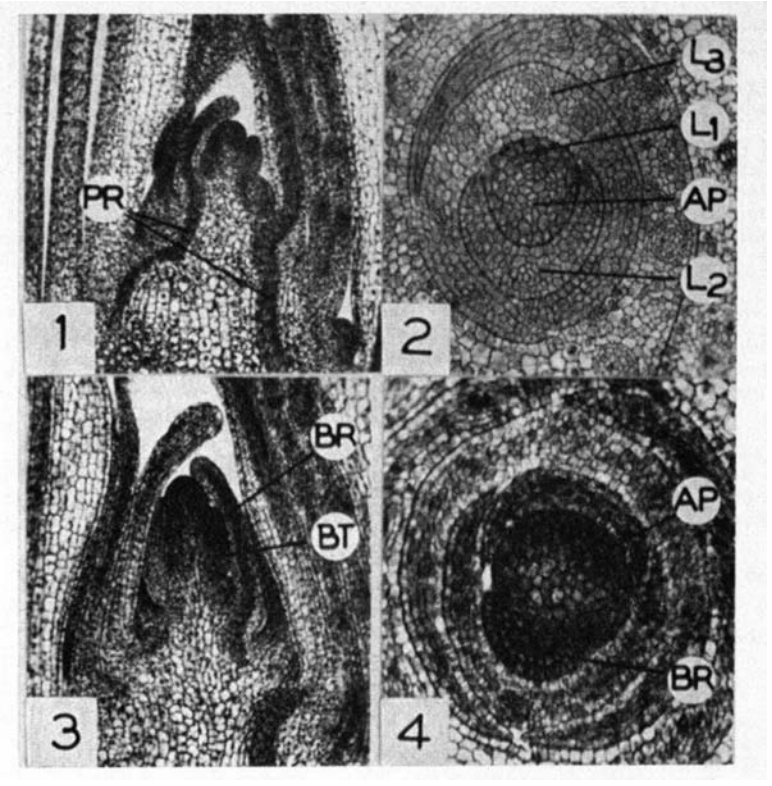

Plate I

Figure 1. Longitudinal section of the vegetative apex. Two leaf primordia are evident. Well developed provascular strands (PR) occur in the older leaf primordia. Note the axillary bud in the axil of an older leaf. (73X).

Figure 2. Transverse section of the vegetative apex. Cell lineage in the corpus is indicative of periclinal cell divisions in the peripheral layer of the corpus and subsequent divisions in the tunica. Apex (AP); Successively older leaves are numbered by subscript (L_1), (L_2), (L_3). Note the provascular strands in the midrib area of L_2 and L_3. (200X).

Figure 3. Longitudinal section of a transitional apex of *Andropogon*. Branch (BR); Bract (BT). (73X).

Figure 4. Transverse section of a transitional apex at a level, through the distal part of the apex of the first-order branch primordium. Note the stratified layer of cells at the junction of the branch to the main axis. (260X).

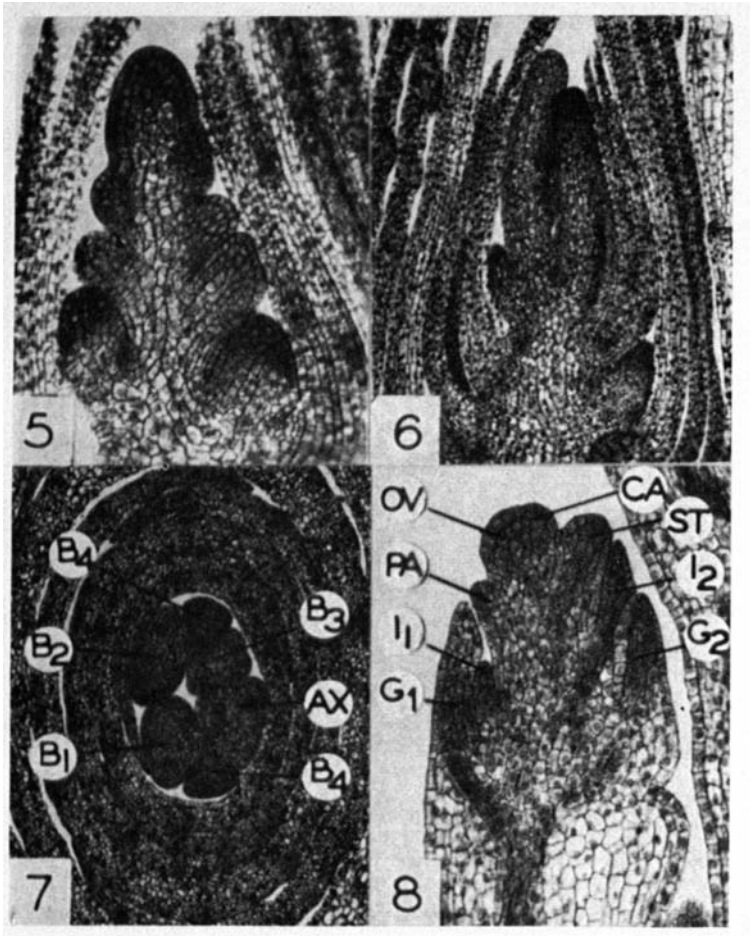

Plate II

Figure 5. Longitudinal section of the main axis of the inflorescence. No leaves or bracts subtend the branch primordia. (164X).

Figure 6. Longitudinal section of the inflorescence which shows two first-order branches, the main axis cannot be seen in the same level. Note the initiation of second-order branches on the interfacial walls of the two branches. (73X).

Figure 7. Transverse section of an inflorescence in its leaf envelope. The arrangement of the main axis (AX) with respect to branches of higher orders (B_1), (B_2), (B_3) and (B_4). (73X).

Figure 8. Longitudinal section of a young pedicellate spikelet. First glume (G_1); second glume (G_2); sterile lemma (L_1); fertile lemma (L_2); stamen (ST); ovary (OV); palea (PA); carpel (CA). (164X).

floral organogeny. The corpus contributes to the mass of the branch, leaf and some of the floral organs.

The change in histogen activity during the transition from the vegetative to the flowering phase in big bluestem is similar to previous studies on perennial grasses (Holt, 1954; Barnard, 1957). The precocity of bifaciality of the racemes in early branch initiation (Figures 6 and 7) is different from that in other materials studied. The basipetal initiation of spikelets in *A. gerardi* is less obvious than that in the indeterminate floral types. This grass demonstrates a determinate type of development which is associated with the less obvious basipetal development of spikelets.

SUMMARY

A study was made of the initiation and development of the inflorescence in *Andropogon gerardi* Vitman. The approximate date of floral initiation has been established and the cytohistology of organogeny during the vegetative and flowering phases is described.

The vegetative shoot apex has a uniseriate tunica and two leaf-primordia. The transition from the vegetative to the flowering phase is described. The leaves, glumes, lemmas, stamens and pistils are all derived from both tunica and corpus. The palea and lodicules have their origin in the tunica only.

The carpel is foliar in its ontogeny and the ovule is derived from the residual dome of meristem.

Archesporia were never visually evident in the ovules of the pedicellate spikelet.

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