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GROWTH AND DIFFERENTIATION IN THE SHOOT APICAL MERISTEM OF THE SAFFRON PLANT (*CROCUS SATIVUS* L.)[1]

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ABSTRACT

Qualitative and quantitative changes taking place in the shoot apical meristem in corms of saffron plant (*Crocus sativus* L.) during the period between middle of May to the middle of August are described. No appreciable growth occurs in the shoot apex between middle of May and the middle of July; this period is the phase of corm dormancy. The cells in the shoot apex during this phase show low mitotic activity. From the middle of July onwards, the shoot apex begins to form leaf primordia which continue to increase in number till the beginning of August. In the period between the beginning of August and middle of August, rapid changes occur in the topography of the shoot apex leading to flower initiation and differentiation, the latter being of protandrous type. The onset or break of dormancy in the corms does not appear to be triggered by a specific temperature experience, since no sharp transition in temperature occurs during initiation of growth and differentiation in the shoot apex.

INTRODUCTION

Saffron, the costliest of all spices is an important cash crop of the state of Jammu and Kashmir. Saffron crocus (*Crocus sativus* L.) is a member of the family Iridaceae and bears a condensed underground shoot in the form of a corm. The plant is sexually sterile and is propagated vegetatively through corms.

Development and differentiation in the shoot apex of certain bulbous plants has been reviewed by Wardlaw (1965) and by Rees (1972). Sass (1944) described the development of foliar and floral structures in tulip, whereas Riviere (1963) described such details for *Lilium candidum*. Floral development in *Iris* has been investigated by Rodrigues Pereira (1962). Information relating to seasonal periodicity in developmental events, particularly differentiation in the shoot apical meristem, is lacking in the saffron plant. A study has therefore been made of the changes occurring in the shoot apex in corms of saffron crocus especially with a view to study flower initiation and differentiation.

MATERIAL AND METHODS

Buds were excised from corms together with a portion of surrounding tissue and were immidiately fixed in alcohol red -acetic acid (3:1). After fixation, the buds were washed with distilled water and transferred to 70% alcohol. Infiltration and embedding in paraffin wax was carried out by passing the material

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through the usual alcohol-xylene series. The dehydrated material was gradually infiltrated with, and finally embedded in, paraffin wax. Longitudinal sections, 10μm thick, were cut. The sections, after being stained with safranin and light green, were mounted in canada balsam.

Axillary buds in notches towards the apical part of the corms were excised at fortnightly intervals between middle of May and middle of July, at weekly intervals from middle of July to the last week of July, and later on at intervals of three days till the second week of August. Buds were excised on May 15 and 31 ; June 15; July 1, 16, 23, 27 and 30 and August 2, 5, 8, 11 and 15. At each stage buds were excised from corms of nearly uniform diameter (3-4 cm).

Observations were made on median longitudinal sections. At each stage quantitative observations were made on five buds taken from diffrerent corms and considered as replicates. Besides studying the morphological changes taking place with progressive intervals, the length and the breadth of shoot apex were also measured. Length and breadth of the shoot apex was measured directly by an ocular micrometer; generally the youngest leaf primordium was taken as the base line. The ratio length/ breadth was computed at each stage.

Cells showing mitotic divisions in the meristematic regions of the apex were marked out on camera lucida drawings and the number of such cells per section was counted to obtain the frequency of mitotic figures.

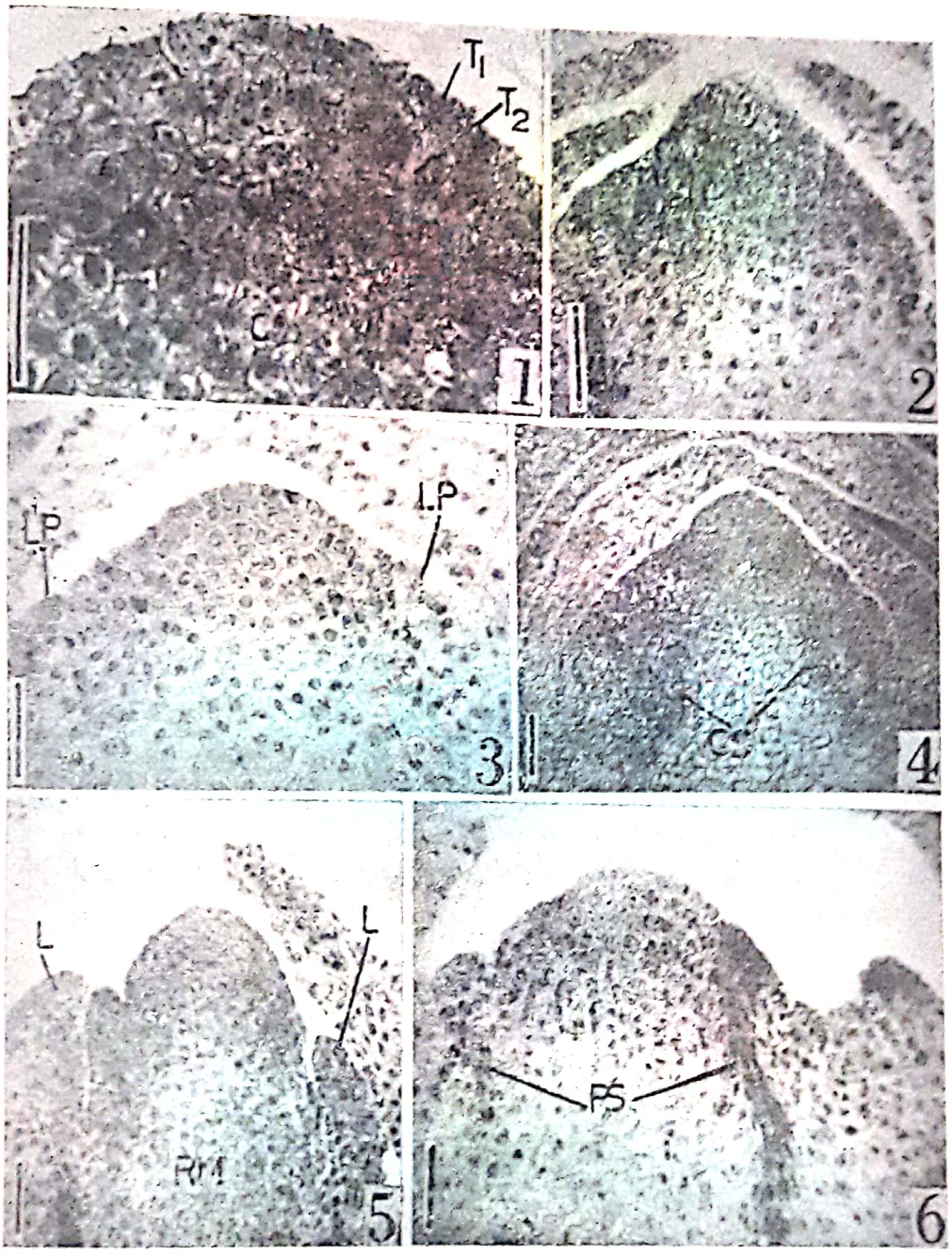
The work done, first during the year 1978, was repeated in 1979 with a fair degree of reproducibility. The results presented here pertain to work carried out during 1979.

RESULTS

Transition from vegetative to floral apex

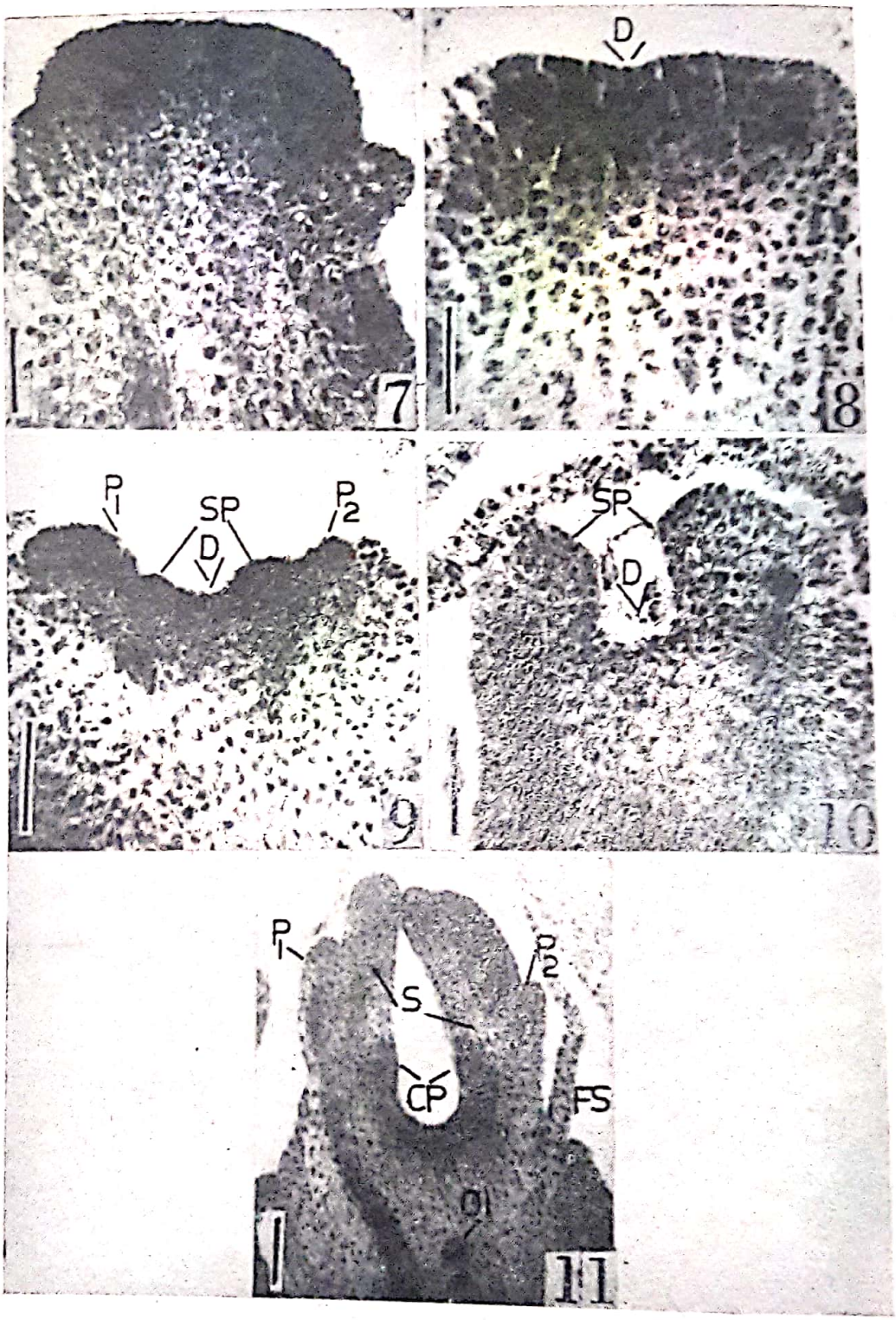
The shoot apex has a typical tunica-corpus configuration the two layered tunica covering a core of unlayered cells, the corpus (Fig. 1). The shoot apical meristem as present during middle of May continued to persist upto the first week of July (Fig. 2).

The first signs of growth in the shoot are manifest sometime between the first and second week of July (Fig. 3). The shoot apex typically resembles the vegetative apex in form and structure. The apex then shapes like a dome and some incompeletly differentiated leaf primordia are observed laying well below the tunica layers on either side, lateral to the axis. The leaf primordia arise as lateral protuberances initiated from the tunica of the apical meristem.The initiation is marked by anticlinal divisions along the lower flank of the apical meristem. Subsequently, one to several periclinal divisions appear in tunica layers. A lateral protuberance is thus formed and continued anticlinal and periclinal divisions in the tunica layers contribute to gradual enlargement of the primordium. The peripheral regions of the corpus do not seem to contribute to the formation of the internal tissue. In such a manner, from the middle of July upto the beginning of August, the apex continues to produce a series of leaf primordia which undergo transition from primordial to young leaf stages of development (Fig. 4 and 5). Apex is well differentiated into an axial zone of rib meristem comprising parallel rows of cells and peripheral pro-vascular meristem. The shoot is broadly domed and bilaterally symmetrical, structurally simple with two-layered tunica and a homogenous corpus composed of large nu-



Figs. 1-6. : Fig. 1. L.S. of vegetative apex showing a two-layered tunica (T_1, T_2) and a multi-layered corpus (C). Fig. 2. L.S. of a cone shaped quilcent apex. Fig. 3. L.S. of a dome shaped vegetative apex showing initiation and differentiation of leaf primordia (LP). Fig. 4. L.S. of apex showing pro-cambial strand developing towards the apex. Fig. 5. High dome stage of the apex with well differentiated young leaves (L) and rib meristem (RM). Fig. 6. The apex prior to flattening with a pro-vascular strand (PS) developing towards the terminal floral apex.

Note : Vertical scales in each figure represent 50 μm.



Figs. 7-11. Fig. 7. Fully flattened terminal floral apex. Fig. 8. Terminal flattened floral apex in L.S. showing a central depression (D) for the initiation of perianth primordia. Fig. 9. L.S. apex showing primordia of the outer perianth (P_1) and the inner perianth (P_2) and also a central depression (D) with staminal primordia (SP). Fig. 10. L.S. floral apex showing well differentiated staminal primordia (SP) on either side of the central depression (D). Fig. 11. L.S. of a well differentiated terminal flower showing floral sheath (FS), outer perianth (P_1), inner perianth (P_2), stamens (S), carpel primordia (CP) and ovular initials (OI).

Note : Vertical scales in each figure represent 50 μm.

cleated cells with dense cytoplasm devoid of vacuoles. The tuncia cells are elongated at right angles to the surface of the apex (Fig. 5). After the high dome stage is reached the apex completely flattens out (Fig. 6, 7 and 8)., and differentiates into the terminal flower during the second week of August. The floral differentiation is of the protandrous red type.

Perianth initiation: Perianth initiation is expressed when the flattened floral apex depresses into a concavity (Fig. 8). The lobes are initiated by periclinal divisions in the tunica layers. Both outer and inner perianth primordia are formed in succession. Outer perianth differentiates first and then the inner perianth. The perianth primordium swells into protuberances as a result of contribution from cells underlying tunica. Pro-vascular cambium extends acropetally along the boundary between peripheral zone and the rib meristem (Fig. 9).

Stamen initiation: Stamen initiation is accompanied by further depression of the floral apex (Fig. 9 and 10). Stamens are initiated by anticlinal divisions in the surface layer followed by similar divisions in the cells lying below epidermal layers. Each staminal primordium is initiated below the primordium of inner perianth and opposite the outer perianth primordium.

Carpel initiation: The concavity gets fully depressed forming a notch enclosed between well differentiated perianth and stamens. The uniseriate tunica and subepidermal layers which displayed localized meristematic activity now undergo active cell division at the base of the notch indicating the initiation of gynoecium by forming small protuberances on both sides (Fig. 11). A deeply stained mass of cells in the centre, below the staminal notch, expressess the differentiation of ovular initials.

Frequency of mitotic figures

From the middle of May till late in June, the frequency of mitotic divisions as observed in the longitudinal sections is relatively low both in the tunica and the corpus regions. Thereafter, it continues to increase progressively with time till early August. In the second week of August, however, a decline in the trend is observed. The highest frequency of mitotic figures is observed during the first week of August (Text fig. 1).

Length and breadth of the shoot apex

A small but gradual increase in the length of the apical meristem occurs from middle of May till early August after which the length of the mristematic region shows a marked decline (Text fig. 2).

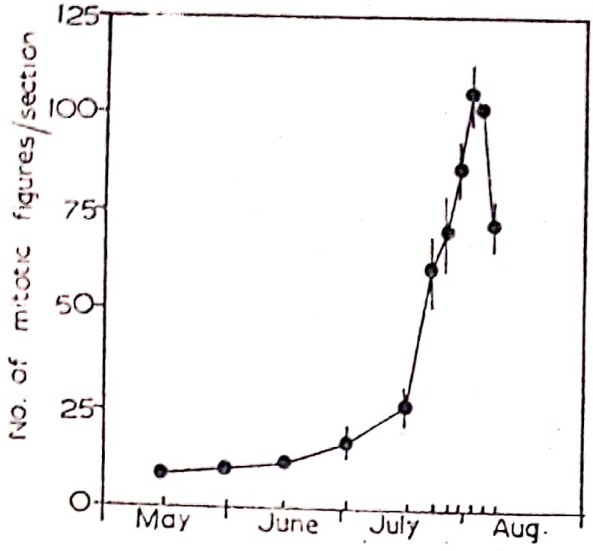
A continued increase in the breadth of the apical meristem is observed from middle of May upto middle of July, after which it shows a sudden decrease. Subsequently, the breadth again continues to increase till early August (Text fig. 3).

Length to breadth ratio

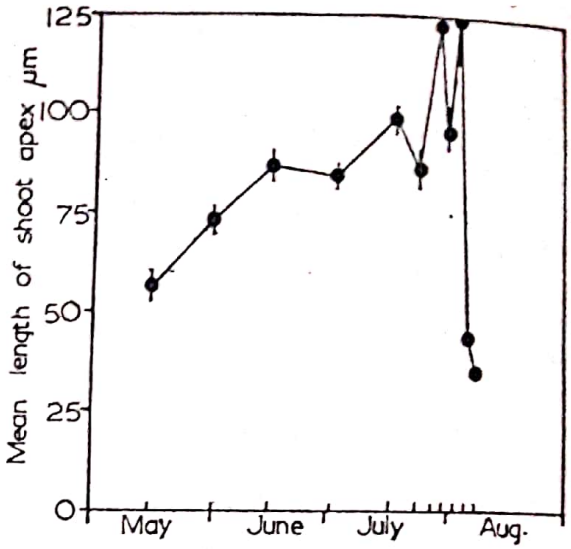
There is generally a progressive increase in the ratio of length to breadth in the apical meristem from second week of May till late July after which the ratio begins to decrease, the decrease being more pronounced during the first week of August (Text fig. 4).

DISCUSSION

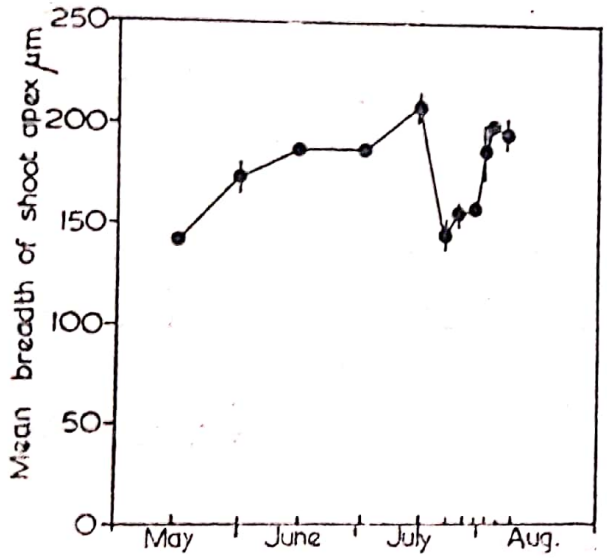
The structure of vegetative shoot apex in the saffron plant compares well with that of *Iris* as described by Rodrigues Pereira (1962), both being members of the same family Iridaceae. In each



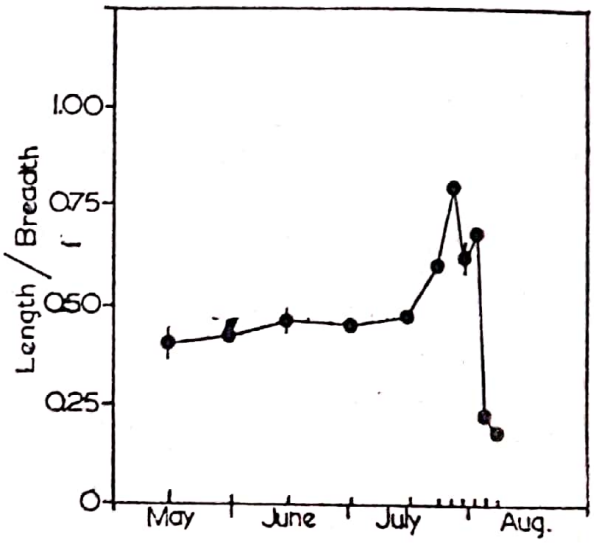
Text fig. 1



Text fig. 2



Text fig. 3



Text fig. 4

Text fig. 1. Changes in frequency of mitotic figures in the apical meristem of saffron plant with progressing dates between May and August.

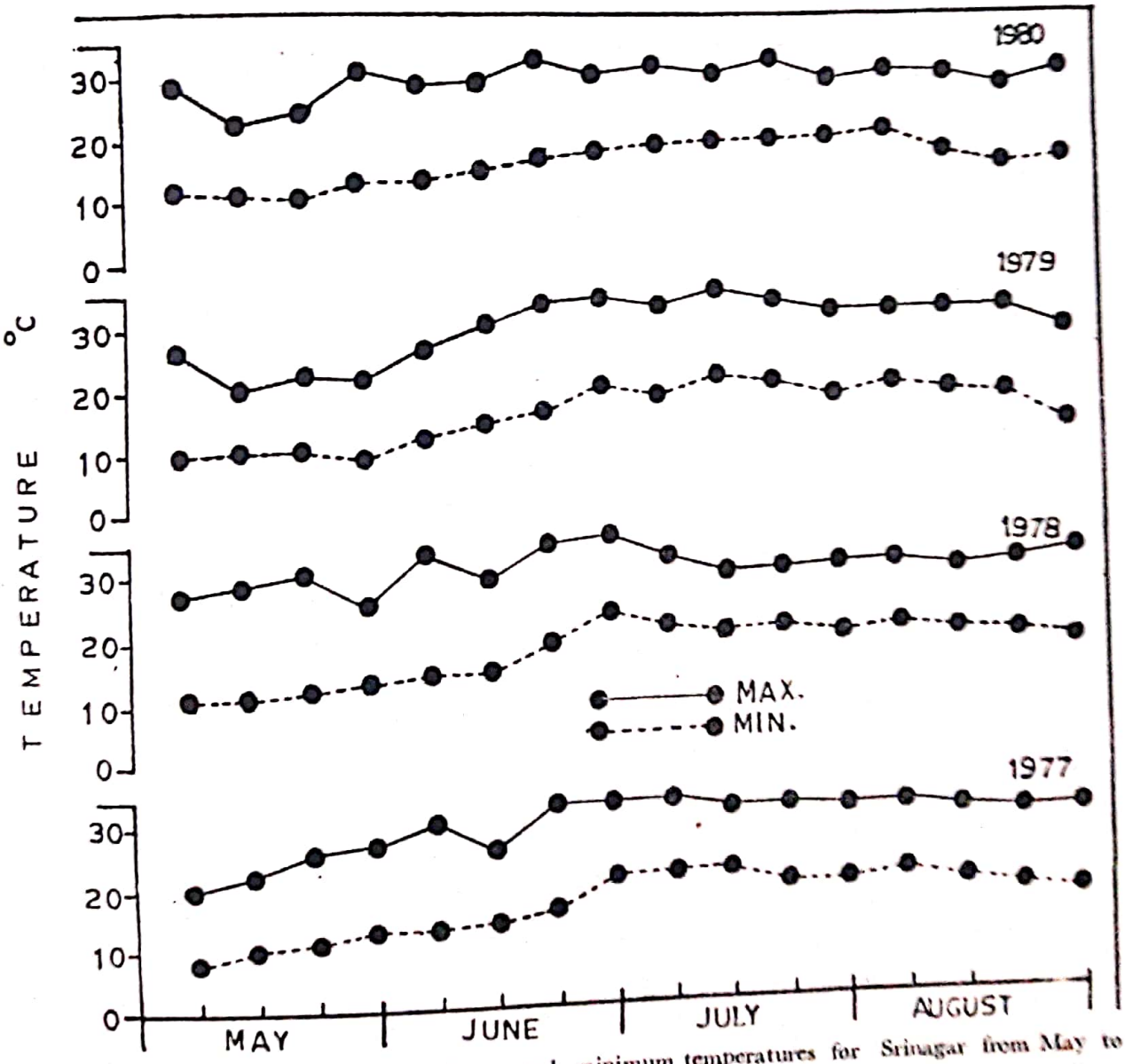
Text figs. 2 to 4. Changes in length, breadth and length/breadth ratio in the shoot apical meristem of saffron plant with progressing dates between May and August.

case there is a two layered tunica enclosing a corpus of larger unlayered cells, and the rate of cell division is initially very slow. In *Iris* the first sign of transition from vegetative to flowering phase is an increase in cell division in the rib meristem. This, howerver, is not true of the saffron plant, and the difference may be due to the fact that flowers in *Iris* are borne on an elongated scape. The changes taking place in the shoot apex of the saffron plant during transition from vegetative to reproductive phase are in conformity with those described for *Iris*. Certain major differences, however, exist in the structure of

the shoot apex in saffron plant and that described for tulip (Sass, 1944) and *Lilium candidum* (Riviere, 1963). Tunica in case of tulip is only one layered while in *Lilium candidum* it is three layered.

The present observations on growth and differentiation in buds of saffron plant during the period between May to middle of July suggest that this period is a phase of arrested growth and developmental activity or dormancy in the corms. With the resumption of growth, a steady increase in cell division and elongation occurs. However, marked fluctuations are observed in the quantitative parameters *viz* length, breadth and the length/breadth ratio during early August. These fluctuations correspond with the rapid changes in the topography of shoot apex at the time of floral differentiation.

The role played by a specific temperature experience in the control of dormancy is well known for a wide range of bulbous plants (Rodrigues Pereira 1962; Rees, 1972). However, tempera-



Text fig. 5. Mean weekly maximum and minimum temperatures for Srinagar from May to August for four consecutive years (1977-80).

ture, high or low, apparently plays no role in the onset or release of dormancy in corms of saffron plant, since, the period between May to August comprises the summer season in Kashmir, as also in other regions where the plant is is cultivated. During this period the mean weekly temperatures in Kashmir valley do not show wide fluctuations and range between 20°C to 30°C maximum and 10°C to 18°C minimum (Text fig. 5). This together with the fact that storage of corms for four to eight weeks at a wide range of high and low temperatures could not break their dormancy leads to the conclusion that the dormancy in corms of saffron plant is innate in character.

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