| Phyton (Horn, Austria) | **Vol. 48** | Fasc. 1 | 99–115 | 29. 8. 2008 |
|---|---|---|---|---|

# Effect of Exogenous Hormone Application on the Stem of Kenaf (*Hibiscus cannabinus* L.)

By

Preeti MISHRA*), Aarti KAVANE*) and K. S. RAO*)

With 5 Figures

Received November 11, 2007

Accepted June 6, 2008

Key words: *Hibiscus cannabinus*, growth regulators, cambium, vascular differentiation.

Summary

MISHRA P., KAVANE A. & RAO K. S. 2008. Effects of exogenous hormone application on the stem of Kenaf (*Hibiscus cannabinus* L.). – Phyton (Horn, Austria) 48 (1): 99–115, with 5 figures.

Anatomical variations in the stem of Kenaf following application of different concentrations and combinations of indole-3-acetic acid (IAA), gibberellic acid (GA) and 6-benzyl aminopurine (BAP) have been studied. The application of the low concentrations of IAA, IAA+GA and BAP+IAA caused active cambial cell division and more differentiation of cells towards phloem. Earlier lignification of cell walls in both xylem and phloem was induced by the application of higher concentration of BAP alone. BAP in combination with low concentration of IAA induced the formation of multiple vessels. GA alone caused differentiation and enlargement of cells towards phloem. Higher concentration of IAA+GA induced rapid cambial cell division and differentiation towards xylem as well as phloem. Cambial cell division and enlargement were directly proportional to the concentration of GA applied. The combination of IAA and BAP induced elongation of core fibers while GA and IAA at higher concentration promoted elongation of bast fibres.

Seeds soaked in GA showed higher percentage of germination and in the resulting plants elongation of xylem and phloem elements was promoted in the stem. The extent of xylem and phloem was more in the plants raised from IAA treated

*) P. MISHRA, A. KAVANE, K.S. RAO, BRD School of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120, India. Corresponding author: e-mail: kayesrao@yahoo.com

seeds. The application of GA and IAA on seeds together caused core fiber elongation, while IAA, GA and BAP together promoted elongation of vessel elements. Plants raised from BAP treated seeds showed less stem elongation.


## Zusammenfassung

MISHRA P., KAVANE A. & RAO K. S. 2008. Effects of exogenous hormone application on the stem of Kenaf (*Hibiscus cannabinus* L.). [Die Auswirkungen extern applizierter Phytohormone auf den Spross der Kenaf-Pflanze (*Hibiscus cannabinus* L.)]. – Phyton (Horn, Austria) 48 (1): 99–115, mit 5 Abbildungen.

In dieser Arbeit wurden anatomische Veränderungen im Spross von Kenaf-Pflanzen nach Behandlung mit Indol-3-Essigsäure (IAA), Gibberellinsäure (GA) und 6-Bezyl-Aminopurin (BAP) untersucht. Die Anwendung von niedrigen Konzentrationen von IAA, IAA+GA und BAP+IAA führten zu vermehrten Zellteilungen im Kambium und einer verstärkten Phloembildung. Eine frühere Verholzung der Zellwände, sowohl im Xylem, wie auch im Phloem wurde durch die Applikation von höheren Konzentrationen von BAP hervorgerufen. BAP in Kombination mit niedrigen IAA Konzentrationen. erhöhte die Anzahl der Gefäße. GA alleine führte zu einer vermehrten Phloem-Bildung und einer Vergrößerung der Zellen. Höhere Konzentrationen von IAA+GA bewirkten eine Beschleunigung der Zellteilung im Kambium und eine vermehrte Bildung des Xylems und des Phloems. Beide (Zellteilung und Xylem-Vermehrung) waren mit der angewendeten GA Konzentration direkt korreliert. Eine Kombination von IAA und BAP bewirkte eine Verlängerung der Markfasern, während GA und IAA in höheren Konzentrationen eine Verlängerung der Bastfasern hervorrief.

In GA gequollene Samen zeigten eine erhöhte Keimrate und in den sich daraus entwickelten Pflanzen eine Verlängerung der Xylem- und Phloemelemente des Sprosses. Der Anteil des Xylems und des Phloems war in IAA gezogenen Pflanzen größer. GA und IAA gemeinsam führte zu einer Verlängerung der Faserzellen des Markes, während IAA, GA und BAP gemeinsam eine Verlängerung der Gefäße bewirkten. Pflanzen, entwickelt aus mit BAP behandelten Samen, zeigten eine verringerte Sprosslänge.


## Introduction

Understanding the biology of vascular differentiation is important from an applied and biotechnological perspective, because biomaterial such as cellulose and lignin in xylem represent the prominent part of the terrestrial biomass and therefore play an important role in carbon cycle (BOUDET & al. 1995). Anatomical and physiological studies have provided important clues, for understanding the process of xylem differentiation in response to hormone supply in plants.

It is clear from experimental manipulation that the patterns of cell divisions in cambial zone are not genetically programmed (SAVIDGE 1996, 2001, CHAFFEY 1999). Rather, it appears that cells perceive their local environments, including hormonal signals, physical factors and intercellular messenger molecules, and act accordingly. A key organizer as cambial growth and development is an auxin, indole-3 acetic acid (IAA). It is a very

important morphogen as it has potential to induce differentiation of vascular strands in callus and explants. In intact plants, polar flow of IAA is essential for the initiation of spatially organized patterns of vascular tissues as well as for maintaining of the vascular cambium (UGGALA & al. 1996). Much evidences indicated that this hormone is transported in the cambial region at the rate of about 1 $cmh^{-1}$. Regeneration experiments indicate that low concentrations of auxin stimulate phloem differentiation, whereas a higher level induces xylem differentiation (ALONI 1987, 1995, 2001).

It is not yet clear if GA play a role in the control of cambial growth. It likely participates in regulation of treachery element cell elongation to build up the well ordered bundle architecture of vascular tissues in plants (KURIYAMA & FUKUDA 2000).

There is yet no definite conclusion to be drawn concerning the involvement of cytokinin in vascular development of plants. It remains undetermined. However, whether cytokinin induces differentiation of vascular cells or simply promotes cell proliferation and thus provides room for the formation of larger and increased vascular bundle. The enlargement of vascular system by increased cell division is also observed in other cases (STEINDLER & al. 1999).

Therefore, the present work was aimed to understand the pattern of vascular differentiation process i) in response to exogenously applied growth regulators directly on the growing main stem and ii) in response to exogenously applied growth regulators directly on the growing main stem following histological and histochemical methods.

The experiments were carried out on Kenaf (*Hibiscus cannabinus*) a multipurpose annual fiber yielding plant.

## Material and Methods

### Selection of Plant Materials

Kenaf is a woody to herbaceous annual plant, mostly unbranched, fast growing, with prickly stem and up to 4.2 m tall. It is a fibre plant belonging to family Malvaceae. Kenaf is native to east central Africa. It was selected in the present study as it grows rapidly with a straight stem having distinct and sufficient amount of secondary vascular tissues. Six month old plants having 4–5 feet height growing in the botanical garden of the Sardar Patel University were used All experiments on Kenaf were carried out from August to October 2006.

### Treatments

Hormonal application was carried out at the eleventh to twelfth internode of the main stem where the secondary growth was prominent. To the cut end of stem microtip was fixed by using strips of parafilm. Then it was filled with the hormonal solution through the pointed tip using a syringe. The leaves and axillary buds below the point of application were removed. For control, microtip was filled with distilled

water. At an interval of every 4 days, microtip was replaced after removing a 1–2 mm tissue from the cut surface and the hormone solution was applied. Segments of the stem one cm below the site of application were collected and fixed in FAA solution after 10, 20 and 30 days of hormonal treatment.

Preparation of Hormone solution: IAA (Qualigen, Bombay) and GA (Himedia, Mumbai) were dissolved in 3ml of 0.1N NaOH (Qualigen, Bombay) whereas BAP (Himedia, Mumbai) was dissolved in 3ml of 0.1 N HCl (Qualigen, Bombay) and diluted with distilled water. The stock solutions were stored in refrigerator. Desired concentrations of different hormones were prepared from the stock solution and pH was maintained at 5.5–5.6.

### Seeds Treatment

Seeds of Kenaf were soaked in different hormone concentrations and combination solutions and allowed to germinate in petriplates. 20 seeds were soaked for each treatment. The length of radicals after 32 h and 72 h of seed germination was measured. Distilled water treated seeds were considered as control. Seedlings were sown in the pots and irrigated with hormone solutions at an interval of one week for a month. The height of the plants was measured after two weeks and two months of growth. Stem samples were collected at $12^{th}$ internode of two months old plants for histological studies.

### Sectioning and Staining for Histology

The samples were subjected to both hand and microtome sectioning. For microtomy the stem pieces were dehydrated in Tertiary Butyl Alcohol series and finally embedded in paraffin with $58–60^0$ melting temperature. 15–20 µm thick sections were taken with a rotary microtome. The sections were subjected to different staining methods mentioned below.

### Toluidine Blue 'O' Method (William 1973)

Sections along with paraffin were stained in 0.05% Toluidine Blue 'O' (Sigma, Germany) prepared in benzoate buffer (pH 6.5) for 15 min and washed several times in water. After air drying the slides were deparaffinized in xylene (Qualigen, Bombay) and mounted in DPX (Qualigen, Bombay). Lignified walls stains in greenish blue colour and unlignified walls stains in purple colour.

### Histochemical Staining Methods

Hand cut transverse sections taken from either fixed or freshly collected samples were subjected to Phloroglucinol (Sigma, Germany)/HCl method for lignin Localization (Gahan 1984), Ruthenium red (Himedia, Mumbai) staining for pectic polysaccharides (Johansen 1940) and Toluidine Blue 'O' staining to differentiate lignified and unlignified cell walls (Mccully 1966).

### Maceration

Stem samples collected after 10, 20 and 30 days of hormone treated and seeds treated plants were macerated to measure the length and width of bast fibers, core fibers and vessel elements. Small matchstick size stem pieces were macerated by in-

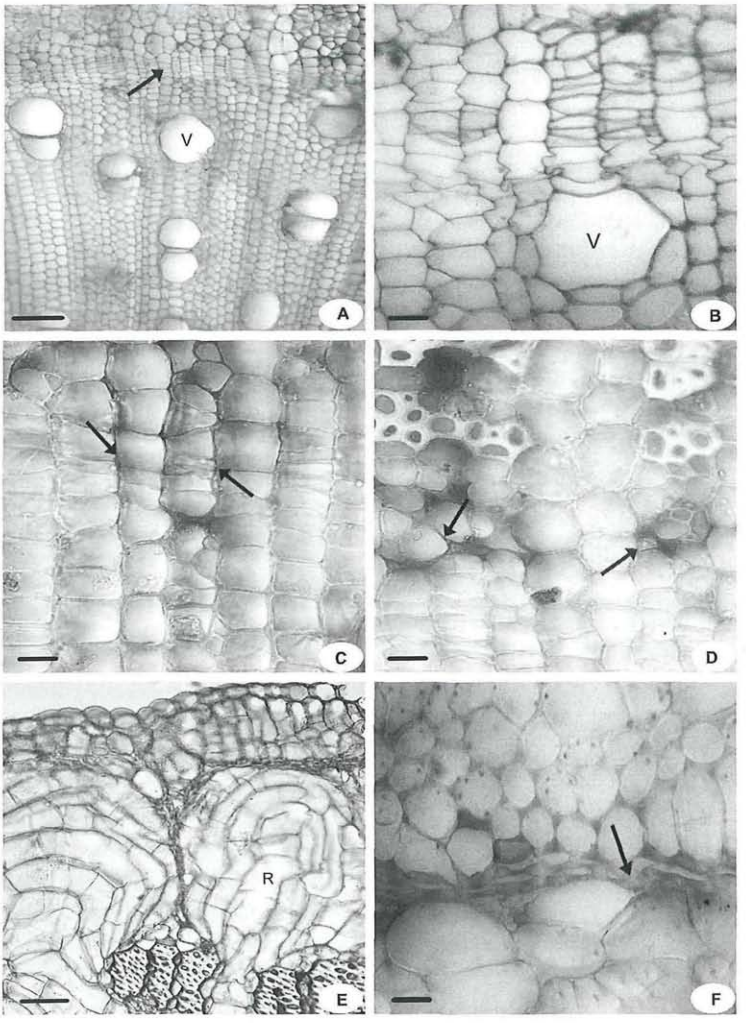


Fig. 1. (A-F) Transverse sections of Kenaf stem. A. Stem of control plant showing a distinct cambial zone (arrow) surrounded by secondary xylem and phloem (Ruthenium red). Bar = 50 μm; B. Active cambial zone with differentiating xylem and phloem after 20 days of treatment with low concentration of IAA (Ruthenium red). Note the differentiating vessel (V) near the cambial zone. Bar = 10 μm; C. Lignification of cambial radial cell walls (arrows) after 30 days of treatment with high concentration of BAP (Phloroglucinol). Bar = 10 μm; D. Lignification of recently formed bast fibers with no secondary walls (arrows) after 30 days treatment with high concentration of BAP (Phloroglucinol). Bar = 10 μm; E. Dilated ray (R) in the cortical region following 20 days of treatment with high concentration of IAA+GA (Ruthenium red). Bar = 50 μm; F. Obliterated and lignified cortex after 30 days of treatment with high concentration of BAP (Ruthenium red). Bar = 10 μm.

cubating in Jeffrey's fluid (BERLYN & MIKSCHE 1976). After thorough washing in water the macerated elements were stained with safranin (Himedia, Mumbai) before mounting in 50% glycerol (Merck, Mumbai).

### Measurements

The length and width of bast fibers, core fibers and vessel elements were measured with an ocular micrometer scale mounted in a research microscope. For each parameter 100 readings were taken from randomly selected elements and they were statistically analyzed to determine the mean and standard deviation.

Stained sections were observed and photographed using a Zeiss microscope with Carl Zeiss (KS 300) Image Analyzer.

## Results

### Hormone Application on Stem

Histological and histochemical observations were made on stem tissues after treating with different concentrations and combinations of IAA, GA and BAP for a period of ten, twenty and thirty days.

### Effect of IAA

Ten days of treatment with low concentrations (0.5, 1, 1.5 and 2 mg/l) did not bring any apparent changes in the structure of stem vascular tissues. Following 20 days of treatment cambium became active showing periclinal and anticlinal divisions. Periclinal divisions lead to differentiation of xylem and phloem. Phloem ray cells underwent dilation resulting obliteration of cortical cells. The differentiating xylem and phloem continued following 30 days of treatment (Fig. 1B). Ray dilation in phloem was more prominent.

Application of low concentrations of IAA induced relatively more phloem differentiation as compared to that of control (Fig. 1A). However higher concentrations (4, 8 and 12 mg/l) maintained the distinct cambial zone and caused differentiation of more xylem elements.

### Effect of GA

GA caused swelling of stem 2.5 mm below the point of application. Stem diameter increased with the rise in concentration of hormones and measured 7 mm in 3 mg/l, 10 mm in 10 mg/l and 12 mm in 100 mg/l of hormone.

After 10 days, phloem rays dilated and formed balloon shaped structure among cortical cells. Bast fibers appear as individual fibers instead of fiber bundles. Phloem ray parenchyma closer to cambium were more enlarged and irregularly arranged. Epidermal cells above the dilated rays underwent tangential enlargement followed by anticlinal divisions. Hypodermal cells enlarge radially resulting obliteration of adjacent cortical

cells. The observations made after 20 and 30 days of hormone application were similar to those of 10 days treatment.

### Effect of BAP

BAP (10 mg/l) following 10 days of application did not bring any histological changes in the stem. However after 20 days there was differentiation of xylem with multiple vessels, fibers and parenchyma cells. Initiation of lignin deposition was noted in the cell walls of pith, cortex, cambium, hypodermis, epidermis and bast fibers.

After 30 days, multiple vessels appeared with more lignified walls. Bast fibers produced after the treatment was lignified without deposition of secondary walls (Fig. 1D). Irregular lignin deposition has been observed in the walls of cambial cells (Fig. 1C), cortex (Fig. 1F), hypodermis, pith, and phloem ray cells.

### Effect of IAA + GA

After 10 days of treatment with low concentration of IAA (0.5, 1, 1.5 and 2 mg/l) and GA (1, 2 and 3 mg/l) cambium was found active with ray cells showing dilation. Swelling of stem was noticed below the point of hormone application.

After 20 days of treatment, cambial derivatives centripetally differentiated into vessels, fibers and parenchyma cells. Xylem ray parenchyma cells had more radial and tangential diameter compared to those produced before the treatment. After 30 days cambium was more active with differentiating xylem and phloem elements. Phloem ray underwent extensive dilation.

With the higher concentration of IAA (4, 8 and 12 mg/l) + GA (10 and 100 mg/l) the stem showed swelling below the point of hormone application. Phloem rays dilated following cell enlargement. Xylem derivatives differentiated into radially elongated thin walled cells with lignified walls. By $20^{th}$ day dilated phloem rays appeared balloon like below the epidermis (Fig. 1E). The epidermal cells just above the dilated rays underwent anticlinal divisions. Phloem fibers appeared as isolated cells with poorly developed cell walls. Xylem derivatives formed after the application were radially enlarged with poorly lignified thin walls. The changes resulted in the stem after 30 days of treatment were similar to those recorded earlier. The xylem derivatives formed in the beginning of treatment had more radial diameter than those formed later (Fig. 2A).

### Effect of IAA + BAP

Low concentration of IAA (0.5, 1, 1.5 and 2 mg/l) + BAP (1, 1.5 and 2 mg/l) after 10 days of treatment showed a distinct cambial zone with cell differentiation.

Multiple vessel formation (Fig. 2B) and ray cell dilation closer to cambial zone were significant changes found in the stem after 20 days of treatment.

Following 30 days of treatment, active periclinal and anticlinal divisions were noticed in the cambial zone. Phloem differentiation appeared to be relatively more in the stems compared to other hormone combinations.

The histological changes obtained with high concentration of IAA (4, 8 and 12 mg/l) + BAP (10 mg/l) application were very much similar to those with high concentration of BAP alone. However the lignin deposition appears irregular on the cell walls, particularly at cell corners and radial walls.

### Effect of Hormones on Length of Vascular Elements

It can be seen from Fig. 3 that core fiber length increased greatly by the low concentration of IAA+BAP. Higher concentration of IAA+BAP also showed elongation of core fibers. IAA when applied alone at low concentration also caused increase in length of core fibers. The length of core fiber also increased by application of GA alone. Combination of low concentration of IAA+GA also promoted the elongation of core fibers. None of the growth hormones when applied alone or in combination showed any affect on elongation of vessel elements in the stem. All the hormones when applied alone or in combination caused elongation of bast fibers. Highest fiber elongation was caused by the application of higher concentration of IAA+GA i.e. 3553 μm.

### Effect of Hormones on Width of Vascular Elements

IAA alone in high concentration caused increase in width of the bast fibers while the width of vessel elements did not change much with other hormones concentration and combinations. Following GA application the width of core fibers increased while that of bast fibers decreased. Higher concentration of GA+IAA also caused increase in the width of the core fibers upto 33 μm. The width of core fibers and bast fibers did not change when higher concentration of BAP alone and BAP+IAA applied to the stem.

### Hormonal Effect on Seeds

Seeds soaked in different concentrations and combinations of hormone solutions of IAA, GA and BAP were allowed to germinate on Petriplates. Number of seeds germinated after 36 hours and 72 hours of germination were noted. In GA treated seeds the average length of radical was highest measuring 18 mm and 43 mm after 36 and 72 hours respectively. The elongation of radical in BAP treated seeds was less than other hormone concentrations and combinations i.e. 5 mm and 10 mm after 36 and

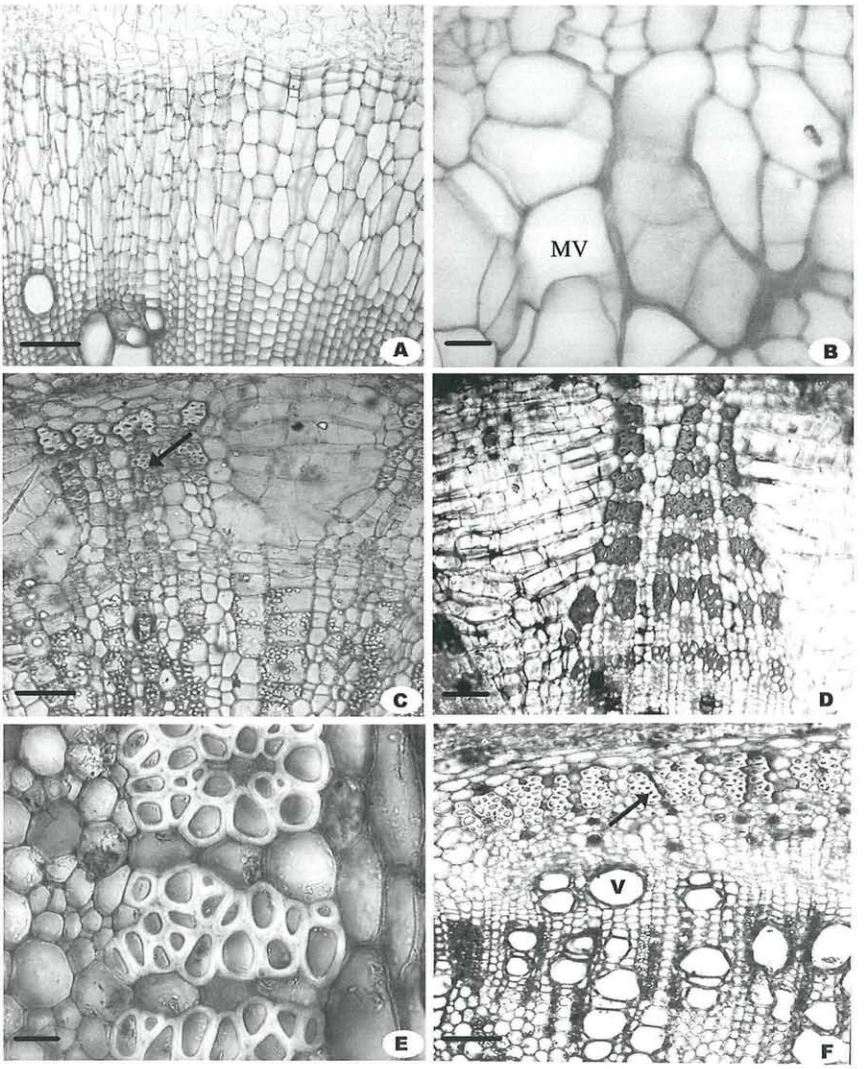


Fig. 2. (A-F) Transverse sections of Kenaf stem. A. Xylem showing radially elongated cells following 10 days treatment of high concentration of IAA+GA. (Phloroglucinol). Bar = 50 μm; B. Formation of multiple vessels (MV) with irregular deposition of lignin after 20 days of treatment with low concentration of IAA+BAP (Ruthenium red). Bar = 10 μm; C. Vascular tissues from the stem of control plant. Arrow indicates the bast fiber bundles (Ruthenium red). Bar = 50 μm; D. Radial rows of bast fiber bundles from the plant irrigated with IAA (12mg/l) (Ruthenium red). Bar = 50 μm; E. Bast fiber bundles showing thinner walls and larger lumen from the stem of plant Irrigated with GA (100 mg/l) (Ruthenium red). Bar = 10 μm; F. Large diameter vessels from the stem of plants irrigated with GA (100 mg/l) (Ruthenium red). Bar = 50 μm.

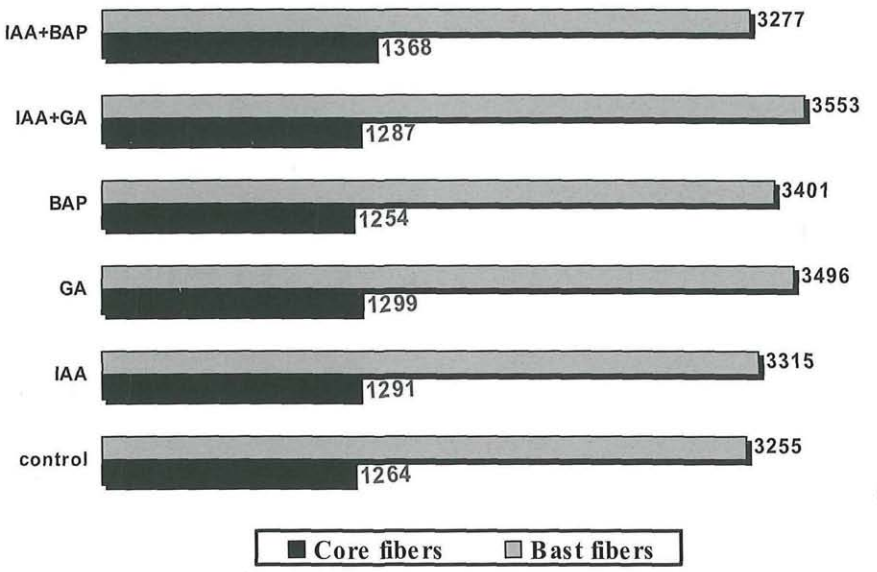


Fig. 3. Effect of hormones on the length (μm) of core and bast fibers.

72 hours respectively (Table 1). While in other hormone concentration and combination the radicle length was more or less equal to that of control. Stem samples from plants raised from hormone treated seeds were collected after two months of growth and observed.

Effect of IAA (12 mg/l)

Transverse sections of stem at $12^{th}$ internodes revealed 3–4 layered cambium. The xylem cylinder was 977 μm (Table 2) in its radial extent. The pith was found to be reduced compare to that of control. Secondary Phloem was also well developed with 5 to 8 bundles of thick walled bast fibers (Fig. 2D). Rays in the phloem showed dilation and cortex was reduced to 2–3 layers.

Effect of GA

GA treated plants were found to be tallest among all other plants. Stem sections showed 4–5 layered active cambial zone and large pith. Xylem cylinder was narrow with less radial extent (600 μm) compared to that of other hormone treated plants except BAP. The lumen diameter of vessels was more compared to that of control (Fig. 2F). Secondary phloem measured 234 μm in its radial extent with closely arranged bast fiber bundles. The fiber lumen was wider with less thickened walls (Fig. 2E) as compared to that of control (Fig. 2C). Cortex was 4-5 layered.

Table 1. Kenaf seeds germinated on different hormone concentrations and combinations after 36 hours and 72 hours.

| Treatment | Number of seeds germinated after 32 hours | Number of seeds germinated after 72 hours | Length of seedling (mm) after 32 hours | Length of seedling (mm) after 72 hours |
|---|---|---|---|---|
| CONTROL | 9 | 13 | 6 | 15 |
| IAA(12mg/l) | 14 | 15 | 8 | 30 |
| GA(100mg/l) | 9 | 11 | 18 | 43 |
| BAP (10mg/l) | 9 | 10 | 5 | 10 |
| IAA(12mg/l)+GA(100mg/l) | 14 | 14 | 16 | 40 |
| IAA(12mg/l)+BAP(10mg/l) | 13 | 13 | 6 | 15 |
| GA(100mg/l)+BAP(10mg/l) | 15 | 15 | 12 | 35 |
| IAA(12mg/l)+BAP(10mg/l)+GA(100mg/l) | 11 | 11 | 12 | 35 |

## Effect of BAP (10 mg/l)

BAP caused less elongation of stem compared to all other hormone treatments. The radial extent of xylem and phloem was 231 μm and 126 μm respectively (Table 2). The dilation of ray cells was less compared to those other hormone treatments. Phloem was composed of 2 to 3 fiber bundles and the cortex was 2 to 3 layered.

Table 2. Dimensional details of seed treated and irrigated plants.

| Treatment | Height (cm) of plants | | Radial extent (μm) | |
|---|---|---|---|---|
| | After 2 weeks | After 2 months | Xylem | Phloem |
| CONTROL | 9 | 28.5 | 792 | 455 |
| IAA(12mg/l) | 9 | 35 | 977 | 425 |
| GA(100mg/l) | 13.5 | 58 | 600 | 234 |
| BAP (10mg/l) | 8.5 | 20 | 231 | 126 |
| IAA(12mg/l)+GA(100mg/l) | 12.5 | 40 | 587 | 261 |
| IAA(12mg/l)+BAP(10mg/l) | 8.5 | 28 | 496 | 326 |
| GA(100mg/l)+BAP(10mg/l) | 8.5 | 36 | 713 | 301 |
| IAA(12mg/l)+BAP(10mg/l)+GA(100mg/l) | 9.5 | 53 | 768 | 495 |

## Effect of IAA + BAP

In this treatment cambium was active with 4 to 5 layers of cells. The radial extent of xylem and phloem measured 496 μm and 326 μm respectively and it was less than that of control. Phloem was composed of 2–3 fiber bundles and dilated ray cells. Epidermal cells lying above the dilated rays underwent tangential elongation.

### Effect of GA + BAP

The height of the plant was found to be more than that of control. Cambium was 2 to 3 layered. The radial extent of xylem and phloem was less compared to that of control. Phloem with 3–4 fiber bundles showing dilated rays. The cortex was 3–4 layered.

### Effect of IAA + GA

In this hormone combination the radial extent of xylem and phloem was 587 μm and 261 μm respectively (Table 2) which was less than that of control. Phloem was composed of 2 to 3 fiber bundles. Cortex was reduced to two layers.

### Effect of IAA+GA+BAP

In this combination the radial extent of phloem increased compared to that of control where as radial width of xylem was less than that of control. Phloem composed of 2 to 3 fiber bundles.

### Effect of Hormones on the Length of Vascular Elements

As shown in the Fig. 4 the elongation of bast fiber was maximal in GA treatment i.e. 3587 μm. With GA (100 mg/l) + BAP (10 mg/l) treatment the fiber elongation was minimum than any other hormone concentration. The

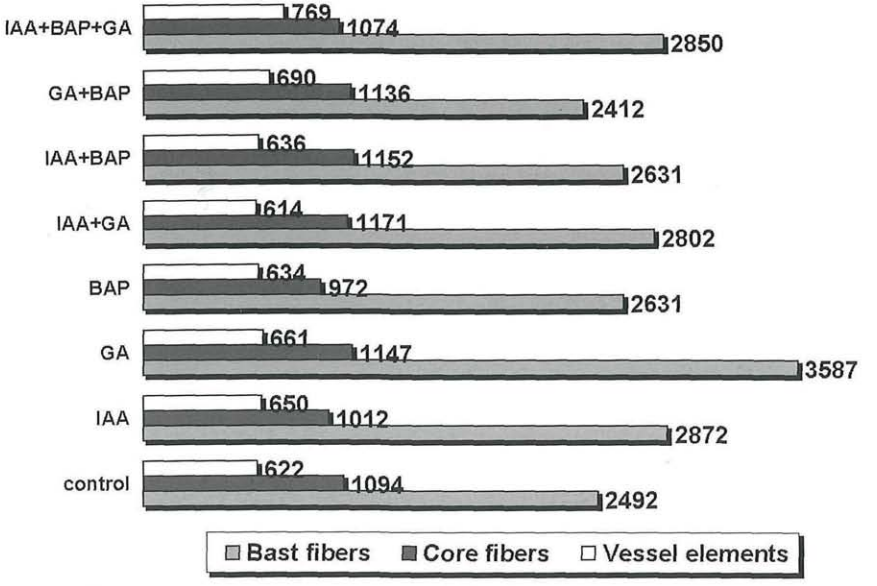


Fig. 4. Effect of hormones on the length (μm) of xylem and phloem elements following two months of hormone irrigation.

length of core fibers was recorded highest in IAA (12 mg/l) + GA (100 mg/l) combination i.e. 1171 µm. However the length was shorter measuring 972 µm in BAP (10 mg/l). The length of vessel elements was highest in the combination of IAA+GA+BAP i.e. 769 µm. However the length decreases measuring 690 µm in GA + BAP.

### Effect of Hormones on Width of Vascular Elements

As shown in Fig. 5 the fiber lumen diameter was more i.e. 22 µm in GA (100 mg/l), IAA+GA, IAA+BAP and least i.e. 19 µm in BAP (10 mg/l). The width of core fibers was found higher i.e. 24 µm in IAA+GA and IAA. Vessel element width reached maximum i.e. 59 µm in GA.

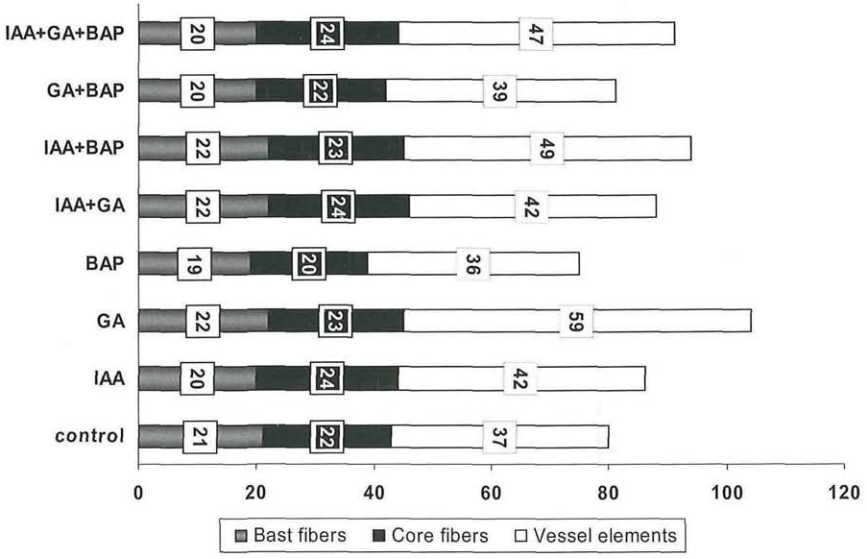


Fig. 5. Average width (µm) of the xylem and phloem elements after two months of hormone irrigation.

## Discussion

In the present study an effort has been made to demonstrate the role of exogenous application of IAA, GA and BAP alone and their combinations on the vascular and ground tissues of Kenaf stem, using histological and histochemical approaches.

### Effect of Growth Hormones on Xylem and Phloem

IAA at low concentration results the activation of cambium. As a result of frequent anticlinal divisions the circumference of the cambial zone

increases. Cambial cell divisions are frequent on the phloem side give rise to larger cells differentiating into sieve tube members and smaller ones into companion cells. Earlier reports indicate that exogenous application of IAA causes activation of the cambium (LEITCH & SAVIDGE 1995, ORIBE & KUBO 1997). Dilation of ray cells is commonly observed in the stem following auxin treatment. The ray cell dilation may be related to the auxin induced cell wall acidification and expansion (TAIZ & ZEIGER 2003). IAA when applied at high concentration causes cambial cell differentiation into xylem elements. It has been reported in *Pinus contorta* that the appearance of cambial zone is maintained when IAA was included in the medium (SAVIDGE 1983). These observations confirm that the IAA induction of secondary xylem and phloem is dose dependent (LITTLE & SUNDBERG 1991, LEITCH & SAVIDGE 1995). GA treated stems show enormous dilation of the phloem ray cells resulting in ballooning of rays below epidermis as compared to the control. Such ray dilation is also promoted by an external application of ethylene (LEV-YADUN 1996). GA caused more differentiation of cambial cells towards phloem but very few towards xylem. The stem below the place of hormone application showed swelling. The stem swelling is caused largely by radial enlargement of phloem cells and dilated rays. Swelling was less at low concentration of GA and more at higher concentration. These observations indicate that concentration of GA is important factor in division and enlargement of the cells. So the division, differentiation and enlargement of the cells are directly proportional to the concentration of GA. There is an evidence that enzyme Xyloglucan Endotrans Glycosylate (XET) is involved in GA promoted wall extension. XET facilitates the penetration of expansin into cell walls inducing cell elongation (TAIZ & ZEIGER 2003).

Application of high concentration of IAA+GA caused more swelling of stem as compared to that of GA alone. This is because of rapid division and differentiation of both xylem and phloem element. Abnormal dilation of rays has also been observed in high concentration of IAA+GA too. Similar to Kenaf, GA promotes diameter growth of *Pinus strobus* cuttings when applied in association with IAA (SAVIDGE 1990). Formation of enlarged thin walled and less lignified cells is unique feature observed following application of IAA+GA at high concentration. Radially elongated thin walled sheets of cells were also observed in the stem of Kenaf after flowering. These cells appear to be produced in the stem due to the increase in levels of GA during flowering. GA and auxin may act together to promote cell wall loosening. Auxin induced proton extrusion while GA stimulated XET activity, which allows expansin protein to penetrate into the cell wall where they become activated by the acidic pH (TAIZ & ZEIGER 2003). Another possible mechanism by which GA controls elongation is through the regulation of cell- wall lignification. The enzyme phenylalanine ammonia lyase (PAL) controls production of phenylpropanoid precursors of ferulic

acid and lignin. GA reduces PAL activity which results in high rate of elongation (BARNES & JONES 1984). At low concentration IAA+GA more phloem differentiation was observed. In many species like *Acer pseudoplantanus, Populus nigra* and *Fraxinus excelsior* IAA+GA application promoted cambial activity (WAREING 1958). Overall the radial extent of xylem and phloem was found maximum in high concentration of IAA+GA. But only division of cells was noticed in cambium with no cell differentiation. IAA+GA (low concentration) also showed xylem and phloem differentiation. IAA+GA at high concentration produced larger bast fibers. This elongation was maximal among all hormone concentrations and combinations. In Populus also longest phloem fibers were found in GA+IAA combination (DIGBY & WAREING 1966).The application of BAP (10mg/l) caused formation of multiple Vessels and irregular lignification of cell walls of pith, cortex, ray parenchyma and cambium. Towards phloem all the cells types showed irregular lignification whereas towards the xylem only vessel elements show lignification more than the earlier formed cells. Similarly when high concentration of IAA+BAP was used irregular kind of lignification was seen in the walls of different cells but did not produce multiple vessels. Present report demonstrates that high concentration of BAP induces early lignification. In tobacco callus also lignin synthesis was found dependent on BAP (BERGMANN 1964). Interestingly, lignin deposits on the cell walls which are primary in nature. Lignification, in general follows secondary wall formation. But when low concentration of BAP+IAA was applied normal lignification was observed with multiple vessel formation. Low concentration of IAA+BAP enhances cambial cell divisions similar to that of low concentration of IAA alone.

## Effect of Hormone Irrigation on Plants

The findings of the present study support the view that GA helps in seed germination (TAIZ & ZEIGER 2003) as well as elongation of stem (SACHS 1965).

GA treatment causes the elongation of bast fibers. Similarly STANT 1963 reported production of long bast fibers with thin walls on application of GA. ERIKSSON & al. 2000 generated transgenic hybrid aspen plants in which a gene encoding GA 20 oxidase exhibit marked increase in level of GAs shoot length, shoot diameter and the number of cells in fully elongated internodes. The present work indicates the role of IAA in the development of more xylem and phloem. IAA alone has pronounced effect on phloem fiber bundle formation. In Coleus also IAA alone stimulates phloem fiber differentiation (ALONI 1978). Present report demonstrates that BAP brings circumfencial expansion of hypocotyls, but has an inhibitory effect on plant growth. Plant height decreases when BAP was applied as well as extent of xylem and phloem was less in the stem. Our re-

sults supports that the combined effect of GA and IAA brings marked effect in the xylem fiber elongation as noticed in Coleus (ALONI 1978). However in Xanthium GA together with auxin did not effect the differentiation of secondary xylem fibres (SHININGER 1971). Combined effect of IAA+ GA+BAP promotes xylem and phloem formation as well as increase in length of vessel elements.

## Acknowledgements

One of us (PM) is thankful to the University Grants Commission for the award of Research Fellowship in Sciences under Meritorious Student Scheme.

## References

ALONI R. 1978. Role of auxin and gibberellin in differentiation of primary phloem fibers. – Plant Physiol. 65: 609–614.

ALONI R. 1987. Differentiation of vascular tissue. – Annual Review of Plant Physiol. 38: 179–204.

ALONI R. 1995. The induction of vascular tissues by auxin and cytokinin. – In: DAIES P. J. (Ed.), Plant hormones Netherlands. – Kluwer Academic Publishers.

ALONI R. 2001. Foliar and axial aspects of vascular differentiation hypothesis and evidences. – J. Plant Growth Reg. 20: 22–34.

BARNES L. & JONES R. L. 1984. Regulation of phenylalanine lyase activity and growth in lettuce by light and gibberellic acid. – Plant Cell and Environment 4: 89–95.

BERGMANN L. 1964. Der Einfluss von Kinetin auf die Ligninbildung und Differenzierung in Gewebekulturen von *Nicotiana tabacum*. – Planta 62: 221–254.

BERLYN G. P. & MIKSCHE J. P. 1976. Botanical microtechnique and cytochemistry. – Iowa State University Press, Iowa.

BOUDET A. M., LAPPIERRE C. & GRIMAPETTENATI J. 1995. Tansley review No.80 biochemistry and molecular biology of lignification. – New Phytol. 129: 203–36.

CHAFFEY N. 1999. Cambium: old challenges – new opportunities. – Trees 13: 138–151.

DIGBY O. & WAREING P. F. 1966. The effect of applied growth hormones of cambial division and differentiation of the cambial derivatives. – Ann. Bot. 30: 139–148.

ERIKSSON M. E., ISRAELESSON M., OLSSON O. & MORITZ T. 2000. Increased gibberellin biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. – Nature Biotechnol. 18: 784–788.

GAHAN P. B. 1984. Plant Histochemistry and Cytochemistry. An Introduction. – Academic Press, Florida.

JOHANSEN D. A. 1940. Plant microtechenique. – Mc Graw-Hill Book company, Inc New York.

KURIYAMA H. & FUKUDA H. 2000. Regulation of tracheary element differentiation. – J. Plant Growth Reg. 20: 35–51.

LEITCH M. A. & SAVIDGE R. A. 1995. Evidence for auxin regulation of bordered-pit-positioning during tracheid differentiation in *Larix laricina*. – IAWA 16: 289–297.

LEV-YADUN S. 1996. Patterns of dilatation growth in *Ficus pumila* and *Ficus sycomorus*. – Aliso 14: 171–177.

LITTLE C. H. A. & SUNDBERG B. 1991. Trachid production in response to indole-3-acetic acid varies with internode age in *Pinus sylvestris* stems. – Trees 5: 101–106.

MCCULLY M. E. 1966. Histological studies on the genus *Ficus*. I. Light microscopy of the mature vegetative plant. – Protoplasma 62: 287–305.

ORIBE Y. & KUBO T. 1997. Effect of heat on cambial reactivation during winter dormancy in ever green and deciduous conifers. – Tree Physiol. 17: 81–87.

SACHS R. M. 1965. Stem elongation. – Anu. Rev. Plant Physiol. 16: 73–96.

SAVIDGE R. A. 1983. The role of plant hormones in higher plant cellular differentiation II Experiments with the vascular cambium and sclereid and tracheid differentiation in the pine, *Pinus contorta*. – The Histochemical Journal 15: 446–447.

SAVIDGE R. A. 1990. Phytohormonal regulation of cambial growth in trees. – In: WERNER D. & MÜLLER P., CASTAN FISHER V. (Ed.), Fast growing trees and nitrogen fixing trees. – Gustav Fischer Verlag, Stuttgart.

SAVIDGE R. A. 1996. Xylogenesis, genetic and environmental regulation. A review. – IAWA J. 17: 269–301.

SAVIDGE R. A. 2001. Intrinsic regulation of cambial growth. – J. Plant Growth Reg. 20: 52–77.

SHININGER T. L. 1971. The regulation of cambial division and secondary xylem differentiation in xanthium by auxin and gibberellin. – Plant Physiol. 47: 417–422.

STANT M. Y. 1963. The effect of GA on cell width and the cell wall of some phloem fibers. – Ann. Bot. 27: 185–196.

STEINDLER C., MATTEUCCI A., SESSA G., WEIMAR T., OHGISHI M., AOYAMA T., MORELLI G. & RUBERTI I. 1999. Shade avoidance responses are mediated by the ATHB6 – 2HD Zip protein, a native regulator of gene expression. – Development 126: 4235–4245.

TAIZ L. & ZEIGER E. 2003. Plant Physiology. 3rd edition, Chapter 20.

UGGALA C., MORITZ T., SANDERBERG G. & SUNDBERG B. 1996. Auxin as a positional singnal in pattern formation in plants. – Proc. Natl. Acad. Sci. 93: 9282–9286.

WAREING P.F. 1958. Interaction between indole-acetic acid and gibberellic-acid in cambial activity. – Nature 181: 1745–1746.

WILLIAM S. 1973. Simple method for differential staining of paraffin embedded material using Toludine Blue 'O'. – Stain Technol. 48: 247–249.