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Effect of Plant Growth Regulators on the Yield and Quality of Bast Fibres in *Hibiscus sabdariffa* L. var. *altissima* Wester

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Abstract: The present investigation highlights the effect of plant growth regulators like gibberelic acid (GA) and Naphthalene acetic acid (NAA) on the yield and quality of bast fibres in *Hibiscus sabdariffa*. The fibre quality and yield was best in GA 100 + NAA 50 μg mL^{-1} treatment. Fibre macerate studies showed increase in fibre length and the slenderness ratio was also high. The Runkel's ratio for the above treated fibre was between 1 and 2 rendering them suitable for textile industry. Proximate analysis of retted fibres revealed lower moisture and ash content and an increase in wax content. Fourier transform infra-red (FTIR) spectroscopy analysis registered a high crystallinity index. The physico-mechanical properties showed considerable improvement of fibre quality. Considering the above criteria, GA100+ NAA 50 μg mL^{-1} treatment brought about advantageous changes for improving the quality of fibres. This study is significant in promoting the exploitation of a non-conventional bast fibre source for use in textile industry.

Key words: Bast fibers, gibberellic acid, *Hibiscus sabdariffa*, naphthalene acetic acid, plant growth regulators, quality

INTRODUCTION

Hibiscus sabdariffa L. var. *altissima* Wester commonly known as Roselle belongs to the family Malvaceae. The fibre obtained from this plant is a useful substitute to jute and can be mixed with jute and spun on jute machinery. It is used in the manufacture of coarse sackings, cordage and ropes and is silky, soft, lustrous white to pale yellowish to brown in color with chemical and physico mechanical properties similar to those of jute. The fibre is also suitable for the manufacture of paper and cardboard (Anonymous, 1985). This plant has been used for the manufacture of sacking cloth but is not found suitable for use in textiles. In the modern world where there is a demand for natural textile fibres other than cotton and other synthetic fibres, attempts were made to improve the yield and quality of these fibres using Plant Growth Regulators (PGRs) such as Gibberellic acid (GA) and naphthalene acetic acid (NAA) in various combinations. The earlier studies on bast fibres were directed towards yield and quality but studies on improvement of bast fibres for textiles using plant growth regulators are fragmentary (Atal, 1961; Aloni *et al*., 1990). The present study was directed towards improvement of fibre quality for textile industry using plant growth regulators like NAA and GA in *H. sabdariffa*.

MATERIALS AND METHODS

Plant material: Seeds of *Hibiscus sabdariffa* were procured from National Seeds Corporation, Ambattur, Chennai, India and used to raise plants for the experiments. Seedlings were raised in wide pots of 60 cm diameter and transplanted to pots of uniform size of 30 cm diameter. The pots were filled with sand, red soil and farmyard manure in the ratio of 1:1:1 and maintained under garden land conditions. Five to six plants were grown in each pot and ten pots were maintained for each treatment including controls. Plants were irrigated uniformly throughout the period of experiment and treatments were given when plants were 30 days old.

Various combinations of different concentrations of Plant Growth Regulators (PGRs) like NAA and GA were applied as foliar sprays (Table 1). The spraying was done at the end of each week for seven consecutive weeks. The experiment was repeated thrice and plants were harvested after 120 days. Ten samples were observed and analyzed in each of the studies undertaken. The parameters taken for study included stem height, length of internodes, diameter, fibre yield, fibre dimensions, analytical characters like moisture content, ash and wax content and crystallinity index of fibres and physico-mechanical properties of fibres.

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Table 1: Combinations and concentrations of Plant Growth Regulators (PGRs) applied as foliar sprays

Treatment	PGRs used	Concentration
Control	-	-
GA 10	GA	10 ($\mu g\ mL^{-1}$)
GA 100	GA	100 ($\mu g\ mL^{-1}$)
GN1	GA+NAA	100 + (50 $\mu g\ mL^{-1}$)
GN2	GA+NAA	100 + (100 $\mu g\ mL^{-1}$)
GN3	GA+NAA	100 + (200 $\mu g\ mL^{-1}$)

Fibre dimensions: Fibre macerates were prepared from stripped bark samples, which were cut into 10×5 mm segments. They were treated in Jeffrey's Maceration fluid (Johansen, 1940) for duration of 10 h at 50°C in an oven. Samples were washed thoroughly and stored in 70% alcohol for studying the microscopic features and for collecting the morphometric data. Macerated fibres from base, middle and tip regions were pooled from ten samples for each treatment and 100 fibres were chosen at random for measurement and the mean was calculated. Length, diameter, Lumen width and Wall thickness were measured using micrometer. The following derived values were calculated from the data on dimensions of fibres following Tamolong *et al.* (1980).

$$\text{Slendernessratio} = \frac{\text{Lenght of fiber}}{\text{Diameter of fiber}} \quad (1)$$

$$\text{Flexibilityratio} = \frac{\text{Lumen width of fiber}}{\text{Diameter of fiber}} \times 100 \quad (2)$$

$$\text{Runkel ratio} = 2 \times \frac{\text{Wall thickness}}{\text{Lumen width}} \quad (3)$$

Studies on wall characteristics: Unstained fibre macerates from both control and treated samples were viewed under polarized light. Photomicrographs were taken under a Nikon microscope equipped with a polariser and analyser in cross-position. A first order red plate was placed over the polariser at an angle of 45° to create a red background (Bennet, 1950; Dayanandan and Pon Samuel, 1979). Documentation was done using 100 ASA Konica colour negative film, processed and printed at local commercial laboratories.

Analytical studies: The retted fibres were sun-dried and powdered using a homogeniser. The crude powder samples from each of the control and experimental species were passed through 40 mesh and by repeated sieving, a fine powder was obtained. Proximate chemical analysis including estimation of percentage moisture, estimation of ash and alcohol-benzene solubility percentage were carried out for both treated and control samples employing the protocol mentioned in Tappi test methods.

The crystallinity index of the control and treated samples were calculated by Fourier Transform Infrared Spectroscopy (FTIR) following the protocol of Silverstein (1980) and Willard *et al.* (1986). The retted fibres were homogenized, sieved and ground with potassium bromide in the ratio of 1:80 using a mortar and pestle. From the ground sample, pellets of 0.2 mm thickness were made according the protocol developed by Kemp (1991). The pellets were used in Bruker IFS66V spectrophotometer and the spectrum was recorded as percentage transmittance over a wave number range of 4000-400 cm^{-1}.

Crystallinity index was calculated following Nelson and O'Connor (1964) formula: A1372 / A2900 where the band appearing at 1372 cm^{-1} was taken as the crystalline band and the band at 2900 cm^{-1} as amorphous band. In the present study, the bands around 1378-1374 cm^{-1} and 2923-2916 cm^{-1} were taken for arriving at the crystallinity index.

Formula

A 1372 = Absorbance for band at a frequency of 1372 cm^{-1}

A 2900 = Absorbance for band at a frequency of 2900 cm^{-1}

Studies on physico - mechanical properties: The different physico-mechanical properties of fibres studied were tensile property, elongation percentage and fibre fineness. The tensile properties were measured using Instron Model 1121 Tensile tester (Booth, 1967). A gauge length of 15 mm with a rate of extension of 5 mm min^{-1} pretension of 1 kg d tex^{-1} was used. Cross head speed of 50 mm min^{-1} was used while testing all the fibres. The maximum load supported by the fibre was reported as breaking load and the corresponding elongation as the breaking elongation. Tenacity and elongation were determined from the average of 20 tests. Elongation was expressed as elongation percentage.

$$\text{Tenacity} = \frac{\text{Breaking strength of fiber}}{\text{tex of fiber}} \text{ expressed as g/tex}$$

The gravimetric fineness of fibre expressed as mass per unit length was determined by cutting and weighing method developed by Duraiswamy (1991) and was referred to as the tex of the fibre. The mean fibre weight

per unit length was calculated and expressed in terms of tex values.

$$\text{Tex of fiber} = \frac{\text{Weight of fiber bundle}}{\text{Length of fiber bundle}}$$

Statistical analysis of data: Data on fibre dimensions, analytical studies and physico-mechanical parameters were subjected to statistical analyses. Arithmetic mean and standard deviation were calculated for all the data of treated and control samples. Tests of significance for comparison of fibre dimensions, morphological and analytical data in the different treated and control samples were done taking the respective sample means at 0.05 level of significance (Schefler, 1969) Analysis of variance (ANOVA) was conducted for comparison of physico-mechanical properties between the different treated and control samples (Schefler, 1969). Studentised range, the least significant difference at 0.05 level between any two means was calculated for each parameter according to Snedecor and William (1967).

RESULTS AND DISCUSSION

Exo-morphological characters: The exo-morphological characters like stem height, length of internodes and diameter in control and treated plants is given in Table 2. The mean height at zero hour was 9 cm. The plant height was maximum (115.6 cm) in GA100 treated plants followed by plants treated with GA(100 μg mL^{-1}) in combination with NAA (50 μg mL^{-1}). Plants treated with GA in combination with NAA at a concentration of 200 μg mL^{-1} showed least increment in height. A similar trend was observed for length of internodes where in a maximum increase of 5.13 cm was observed at the end of seven weeks of spray in plants treated with GA at a concentration of 100 μg mL^{-1}. The difference in internodal diameter between the treated and control plants was not significant though all the treated plants showed a higher value than that of the control (Table 2).

Fibre yield: The fresh weight was maximum (131.9 g) in plants treated with GN1 followed by GA10 and GA100. The fresh weight of all the treated plants was higher than that of the control except in the case of plants treated with GN3 (Table 3). Dry weight also showed a similar trend in the control and treated plants.

Fibre dimensions: The quality of fibres and their use in textiles is determined by criteria such as (i) microscopic characters (ii) fibre dimensions and their derived values (iii) chemical and (iv) physico-mechanical properties.

Length of fibres was maximum in plants treated with GA100 μg mL^{-1} (5122 μm) followed by plants treated with GN1. Breadth of fibres was maximum (24.68 μm) in plants treated with GN3 and minimum (14.59 μm) in GN1 treated plants. Wall thickness was maximum (7.99 μm) in plants treated with a combination of GA100 and NAA200 μg mL^{-1}, followed by plants treated with GN2 and GA100. Wall thickness was minimum (3.63 μm) in control plants. Lumen width was minimum (6.53 μm) in GN1 treated plants and maximum (15.64 μm) in control plants (Table 4).

Wall characteristics: The characteristics of fibre as observed under polarisation microscope show variations in lamellation and composition of cell wall. The lamellae, which appear pink, are rich in cellulose and the green and bluish green are indicative of lignin. The blue color, under plane polarized light indicates broader lamellae in which the orientation of microfibrils are parallel to the long axis of the fibre, whereas the narrow lamellae with the orientation of fibrils at right angles to the long axis of the fibre take yellow coloration. The number of lamella or layers were more in the treated plants and is maximum in

Table 2: Comparison of exo-morphological characters in control and treated plants of *H. sabdariffa*

			Treatments					
Parameters	F-value	p-value	Control	GA 10	GA 100	GN 1	GN 2	GN 3
Height (cm)	48.25	0.000**	53.6±11.90a	78.3±10.81b	115.6±11.37d	112.1±10.68d	94.1±11.06c	76.2±8.43b
Length (cm)	126.13	0.000**	1.71±0.21a	3.32±0.49b	5.13±0.61d	4.26±0.18c	2.96±0.26b	2.05±0.21a
Diameter (mm)	11.04	0.000**	5.52±0.50a	6.77±0.50b	7.17±0.92bc	7.52±01.07bc	6.96±0.27b	7.83±0.96c

**Significance at 0.01 level, Different alphabets between treatments indicate significance at 0.05 level

Table 3: Comparison of fibre yield in control and treated plants of *H. sabdariffa*

			Treatments					
Parameters	F-value	p-value	Control	GA 10	GA 100	GN 1	GN 2	GN 3
Fresh weight (g)	253.15	0.000**	66.86±2.31b	116.47±2.63c	105.4±1.66c	131.92±4.8e	101.62±3.29c	55.9±3.48a
Dry weight (m)	26.05	0.000**	1.12±0.208a	2.213±0.296b	1.95±0.098b	2.388±0.231b	1.859±0.172b	0.961±0.097a

**Significance at 0.01 level, Different alphabets between treatments indicate significance at 0.05 level

Table 4: Effect of PGRs on fibre dimensions of *H. sabdariffa*

Dimensions	Treatments	Mean± SD	t-value	p-value
Length	Control	3412±342.69		
	GA 10	4108±439.09	1.75	0.031*
	GA 100	5122±346.13	2.71	0.000**
	GN 1	4782±497.89	2.32	0.025*
	GN 2	3847±410.04	2.52	0.086
	GN 3	3756±302.83	2.54	0.093
Breadth	Control	22.90±5.00		
	GA 10	20.39±5.06	1.76	0.084
	GA 100	19.47±4.52	2.55	0.014*
	GN 1	14.59±5.15	5.8	0.000**
	GN 2	23.69±4.69	0.58	0.566
	GN 3	24.68±6.54	1.08	0.285
Lumen width	Control	15.64±4.70		
	GA 10	7.59±3.99	6.54	0.000**
	GA 100	7.06±3.28	7.48	0.000**
	GN 1	6.53±3.21	8.00	0.000**
	GN 2	9.17±3.02	5.79	0.000**
	GN 3	8.71±3.42	5.96	0.000**
Wall thickness	Control	3.63±0.93		
	GA 10	6.40±2.30	5.58	0.000**
	GA 100	6.20±2.25	5.29	0.000**
	GN 1	4.03±1.72	1.01	0.317
	GN 2	7.26±2.86	6.04	0.000**
	GN 3	7.99±2.72	7.57	0.000**

**Significance at 0.01 level, *Significance at 0.05 level.

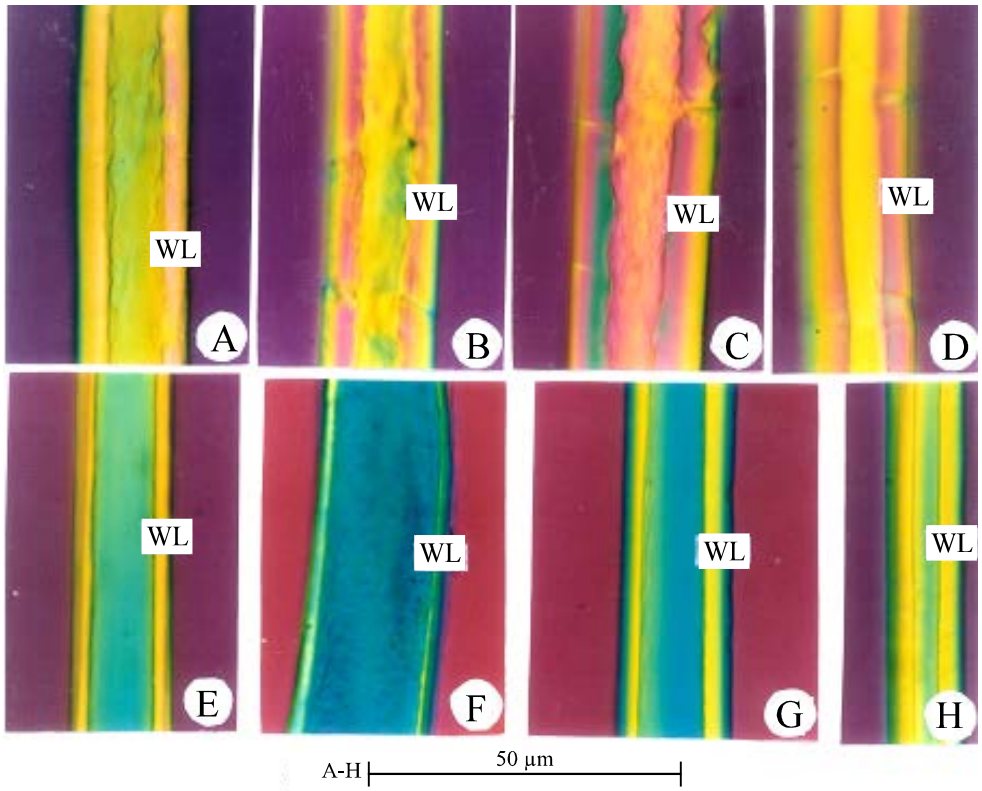

Fig. 1: Fibre macerates of *H. sabdariffa* as seen under polarization microscope
A: Control, B: GA 10 µg mL, C: GA 100 µg mL^{-1} + NAA 50 µg mL, D: GA 100 µg mL^{-1} + NAA 50 µg mL,
E: GA 100 µg mL^{-1} + NAA 100 µg mL^{-1}, F: GA 100µg mL^{-1} + NAA 200 µg mL,
G: GA 100 µg mL^{-1} + NAA 200 µg mL, H: GA 100 µg mL, WL: Wall Layer,
The color green and blue are indicative of lignin while yellow and pink indicate presence of cellulose

Table 5: Effect of PGRs on derived values of fibre dimensions in *H. sabdariffa*

Derived values	Treatments	Mean± SD	t-value	p-value
Slenderness Ratio	Control	149±27.15	-	-
	GA 10	201.5±36.91	0.48	0.004**
	GA 100	263.1±51.79	3.01	0.000**
	GN 1	375.4±87.52	4.98	0.000**
	GN 2	162.4±22.39	2.27	0.636
	GN 3	152.2±25.26	2.03	0.814
Flexibility Ratio	Control	67.13±9.90	-	-
	GA 10	36.99±16.19	7.94	0.000**
	GA 100	36.71±15.67	8.20	0.000**
	GN 1	44.74±13.66	6.63	0.000**
	GN 2	39.99±14.69	7.66	0.000**
	GN 3	35.94±11.77	10.14	0.000**
Runkel's Ratio	Control	0.53±0.28	-	-
	GA 10	2.21±1.28	6.42	0.000**
	GA 100	2.37±1.75	5.20	0.000**
	GN 1	1.44±.73	5.80	0.000**
	GN 2	1.92±1.27	5.35	0.000**
	GN 3	2.06±.95	7.73	0.000**

**Significance at 0.01 level, *Significance at 0.05 level.

Table 6: Effect of PGRs on proximate chemical analysis of retted fibres *H. sabdariffa*

Analytical Parameters	Treatments	Mean± SD	t-value	p-value
Percentage of Moisture	Control	9.7±0.20	-	-
	GA 10	8.87±0.20	5.51	0.005**
	GA 100	8.6±0.23	6.61	0.003**
	GN 1	8.3±0.20	6.12	0.004**
	GN 2	8.7±0.20	8.57	0.001**
	GN 3	8.9±0.20	4.89	0.008**
Percentage of Ash	Control	1.3±0.01	-	-
	GA 10	1.36±0.10	0.73	0.50
	GA 100	0.69±0.20	4.64	0.01**
	GN 1	0.98±0.05	1.25	0.28
	GN 2	1.15±0.10	3.91	0.017*
	GN 3	1.6±0.20	2.32	0.081
Alcohol-benzene	Control	0.9±0.02	-	-
solubility percentage	GA 10	3.43±0.20	21.5	0.000**
	GA 100	2.6±0.20	14.6	0.000**
	GN 1	1.93±0.26	6.5	0.003**
	GN 2	2.89±0.17	18.8	0.000**
	GN 3	3.64±0.34	13.4	0.000**

**Significance at 0.01 level, *Significance at 0.05 level.

Table 7: Effect of PGRs on Crystallinity Index of cellulose in *H. sabdariffa*

Treatments	Crystallinity Index
Control	0.3780
GA 10	0.4254
GA 100	0.4524
GN 1	0.4805
GN 2	0.4592
GN 3	0.3399

plants treated with GA100 + NAA50. The pitting on the fibres is oblique and multi seriate. The wall shows deposition of lignin in plants treated with GA10 and GA100 while cellulose deposition is high in plants treated with a combination of GA and NAA (Fig.1 A-H).

These observations on fibre dimensions and yield were in accordance with earlier observations of Atal (1961) on hemp where gibberellin treatment at 100 μg mL^{-1} led to an increase in fibre length. The observations were also in corroboration with the findings of Aloni (1987), where combinations of PGRs such as GA and NAA brought about increased fibre length in a wide variety of commercially important plants. The increase in length of fibres could be correlated with the plant height and increase in internodal length. The observations on lumen width and wall thickness were in conformity with earlier observations where Aloni *et al.* (1990) reported that IAA alone or low GA and high IAA combination induced short fibres with thick lignified walls. Lumen width of fibres showed a strong correlation with mechanical properties. Lesser lumen width imparted favorable mechanical properties to the fibres. Maiti (1970) reported that higher strength is shown by long fibre cells because short fibre cells have many weak points at the cementing region of fibre cells forming fibre strands, but in fibre filaments having long fibre cells, there are few weak points at the time of tension caused by the breaking load. Thus, longer fibres showed higher tenacity. It was also reported that increase in number of wall layers increased the fibre

tenacity (Maiti, 1980). Hence, in the present investigation, plants treated with a combination of 100 μg mL^{-1} GA and 50 μg mL^{-1} NAA showed favorable fibre dimension and wall characteristics related to better physico-mechanical properties in terms of length and width.

Derived values of fibre dimensions: The slenderness ratio was maximum in GN1 followed by GA100 while the flexibility ratio was low in GN1 when compared to control samples. Runkel's ratio was ideal in GN1 treatment with a value of 1.44 (Table 5).

Slenderness ratio is related to the dimension of fibres such as length and breadth. It may also be referred to as L/B ratio. The preferred L/B ratio for use in textile industries is between 200-300 (Maiti, 1980). Observation on the L/B ratio on the control plants in *H. sabdariffa* was in accordance with Maiti (1980). But in GA100 and GN1 treated plants, a high L/B ratio of 263 and 375, respectively was noticed.

Runkel's ratio is related to lumen width and wall thickness of fibres. Runkel's ratio between 1 and 2 rendered the fibres suitable for use in textiles (Manimegalal, 1999) while Runkel's ratio of 1 or less than 1 was considered to be favorable for paper making in bamboos (Tamolong *et al.*, 1980). In this study GN1 treatment gave a value of 1.44 that is preferable for textile industry.

Aloni (1979) reported that auxin and gibberelic acid control the differentiation of primary phloem fibres in *Coleus blumei* Benth. The effect of gibberelic acid on fibre cell length, width, number and yield in *Cannabis sativa*, *Corchorus olitorius*, *C. capsularis* and *Hibiscus cannabinus* was studied by Atal (1961) and Sircar and Chakravarthy (1960). Aloni (1987) reported that spraying of leaves with NAA and GA in plants used as fibres sources in industry increases the fibre yield. Aloni *et al.* (1990) have reported that auxin and gibberellin are involved in controlling lignin formation. The present study corroborates the above results revealing the increase in fibre length, width and yield on treatment with PGRs.

Analytical studies: The moisture level and ash content was found to be low in GN1 treatment when compared to control. The alcohol-benzene solubility percentage indicative of wax content increased on GN1 treatment and the maximum value was observed in GN3 treatment (Table 6).

The crystallinity index was estimated by FTIR analysis of the samples based on the protocol of Nelson and O'Connor (1964). Crystallinity index was maximum (0.4805) in GN1 treated plants followed by GN2 (0.4592) and GA100 treated plants (0.4524). The crystallinity index was minimum in GN3 treated plants (Table 7).

The moisture content is directly related to strength and extension of fibres besides dye absorption. Low moisture content enhanced drying of fabric (Anonymous, 1960). The results in the case of GA100 and GN treatments are similar to the values obtained for retted pineapple leaf fibre (Duraiswamy and Chellamani, 1993) and those for control plants corroborate the earlier observations of Snehlata and Verma (1995) on *Cannabis sativa*.

In view of the above, GN treated plants showed favorable proportions of ash and percentage moisture. Wax in fibres could bring about reduction in wettability, which in turn increased the duration of dye uptake. An optimum content of wax imparted desirable lubricating properties for spinning. The present observations corroborated the earlier reports of Sekar (2000) on jute as far as control plants were concerned.

The degree of crystallinity decreased with increase in percentage moisture in kenaf and jute while in mercerised cotton the negative effect was observed (Mukherjee and Radhakrishnan, 1972). The degree of crystallinity improved with delignification and partial or complete removal of hemicellulose. In the present study, the crystallinity index was maximum in GN1 treated plants rendering them spinnable.

Studies on physico-mechanical properties: Elongation was maximum in control plants (12.848%) followed by the GA10 treated plants (9.6432%). Elongation was minimum (3.5132%) in GN1 treated plants. Fineness of fibre was minimum in GA100 plants (2.6 tex) and maximum (5.3 tex) in plants treated with 100 μg mL^{-1} GA and 100 μg mL^{-1} NAA (GN2). Tenacity was minimum in GN3 plants (18.56 g tex^{-1}) and maximum in GA10 (53.12 g tex^{-1}) followed by GA100 and GN1 treated plants (Table 8).

Table 8: Comparison of Physico – mechanical properties in *H. sabdariffa* as effected by PGRs

Physico-mechanical Properties	F-value	p-value	Control	GA 10	GA 100	GN 1	GN 2	GN 3
Elongation %	13.2359	0.000**	12.848±4.9392c	9.6432±1.5312bc	5.35±0.6623ab	3.5132±0.8876a	3.8586±0.9625a	5.629±1.3170ab
Fineness tex	46.4054	0.000**	3.23±0.1384b	2.6±0.2041a	4.14±0.1654c	3.6±0.5208bc	5.3±0.1061d	3.9±0.4121c
Tenacity g/tex	33.7395	0.000**	19.72±1.6a	53.12±1.7d	42.38±1.425c	32.28±1.346b	27.63±1.524b	18.56±1.853a

**Significance at 0.01 level, Different alphabets between treatments indicate significance at 0.05 level

Flexibility of fibres has direct correlation with elongation. Maximum elongation percentage was observed in GN1 treatment in *H. sabdariffa*. The tenacity of fibres depends on the breaking load of the fibres and is inversely proportional to fineness or tex of the fibre. In the present investigation, fibres of GN1 treated plants in the investigated species show a high tenacity value. Present results of the control samples were similar to those in cotton, jute and sunn hemp (Istiaque *et al*., 2000; Sinha and Chatterjee, 1977; Navin *et al*., 1986). The fibres in treated plants presently investigated, show higher tenacity value over that of cotton and pineapple leaf fibres, used for apparel (Duraiswamy and Chellamani, 1993). The present findings revealed that the tenacity of fibres could be correlated with the dimensional characters and their derived values, which in turn have a direct impact on the physico-mechanical properties taken that determines the use of fibres in industry.

Clothes are one of the essential requirements of man that may be produced from various natural and synthetic fibres. But the synthetic fibres cause pollution, degrade very slowly and pose a pollution hazard. Hence the need to identify non- conventional fibres sources for use in the textile industry. Commercialization of natural fibres is losing prominence owing to competition caused by synthetic fibres and the difficulty in large-scale production due to the cultivation of cash crops by farmers. The crop plant presently investigated could be used as unconventional fibre source besides being used as a vegetable. This study is significant in not only promoting the exploitation of unconventional bast fibre sources for use in textile industries, but also in the advocation of growing of these plants as dual crops by farmers.

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