African Journal of Biotechnology Vol. 12(19), pp. 2581-2586, 8 May, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2547
ISSN 1684-5315 ©2013 Academic Journals

Full Length Research Paper

Variations in *in vitro* and *in vivo* indices of photoperiod sensitivity among kenaf (*Hibiscus cannabinus*) accessions in Nigeria

M. O. Balogun[1]*, A. O. Olabisi[1] and S. R. Akande[2]

[1]Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria
[2]Institute of Agricultural Research and Training, Obafemi Awolowo University, P.M.B. 5029, Ibadan, Nigeria.

Accepted 13 March, 2013

In equatorial climates, fibre yield is higher in photo-insensitive kenaf cultivars. To develop a rapid screening method, *in vivo* and *in vitro* indices were evaluated. Seven genotypes were grown at natural photoperiod and growth rates before and after flowering, days to flowering and fibre yield were recorded. *In vitro,* stem and leaf explants of genotypes Tainung and V400 which showed contrasting photoperiodic responses *in vivo*, were tested for callus induction at 0 and 12 h photoperiod. Calli were transferred to differentiation medium at 12 and 9 h photoperiod and numbers of green spots and embryogenic callus clusters were recorded. Flowering was delayed by at least 27 days in V400 relative to other genotypes. Growth rate reduced by 30% after flowering in all genotypes except V400 where it increased by 60%. Highest yield of 76 g / plant was recorded in V400. Eighty-four percent degree of callus formation by stem was higher than 51% by leaf explants of V400 irrespective of light regime. In Tainung, callus formation varied with explants and photoperiod. Green spots and embryogenic clusters were three times more in Tainung than V400. Both *in vivo* and *in vitro* results showed Tainung as photosensitive and V400 as photoinsenstive. Incubating kenaf callus in differentiation medium in 12 h light and evaluating for greenness was useful in screening for photoperiod sensitivity.

Key words: Kenaf, *Hibiscus cannabinus*, photosensitivity, *in vitro* screening, somatic embryogenesis.

INTRODUCTION

In Nigeria, kenaf has been accepted as an industrial crop, and will soon find considerable use in making absorbents for cleaning oil spills in the Niger Delta. However, supply of kenaf raw materials is scarce, with Africa producing only 2.91% of the global production (FAO, 2003) compared to China (30.5%) and India (47.4%). This is due in part to cultivation of varieties with photoperiod sensitivities that do not match equatorial climates. It is the latitude, north or south of the equator, which determines the daily photoperiod of a location yearly. Kenaf is a short day plant, and most varieties are photosensitive. They initiate flowering when day length reduces to 12.5 h, and are suited for countries at latitudes 10 to 27° north or south of the equator (Scott, 1982; Webber et al*.,* 2002). However, planting these varieties in the tropics (latitude 0 to 10° N or S) where day length is more uniform from June to September induces early flowering which causes a reduction in vegetative growth and low fibre yields. Photo-insensitive (day-neutral) cultivars are therefore preferred in that, they flower late (Stricker et al., 2001), or when they flower early, their vegetative growth is not significantly reduced. Identification of photoinsensitive genotypes is therefore a priority in kenaf yield improvement programmes.

To date, serial field planting at natural photoperiod (Balogun et al., 2007) or controlled illumination *in vivo* has been used to screen for photosensitivity using days to maturity and relative growth rates before and after flo-

*Corresponding author. E-mail: kemtoy2003@yahoo.com. Tel: +2348037038766.

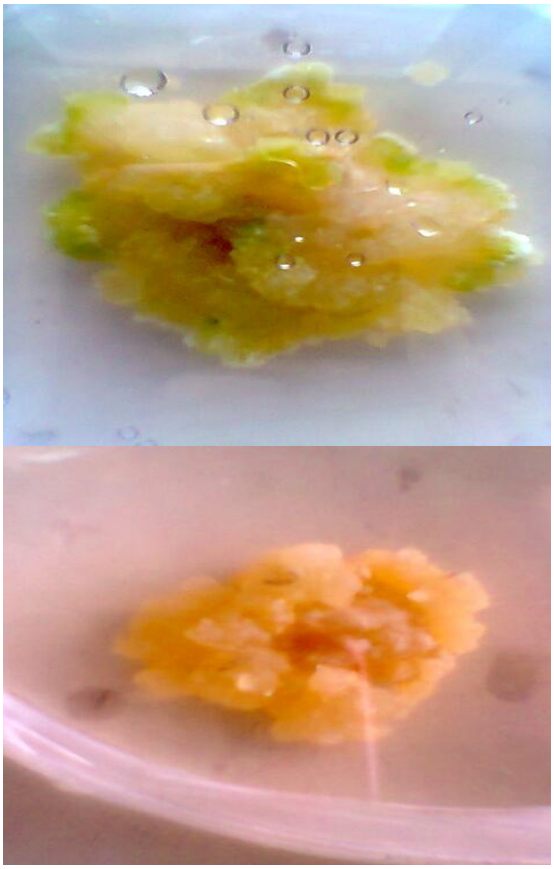

Plate 1. Differences in greenness of callus of Kenaf genotypes Tainung (above) and V400 (below).

wering as indices. However, these methods require considerable space, time and labour. Thus, an *in vitro* approach may be more efficient. Long photoperiods were reported to enhance high chlorophyll contents in cells, manifested by high greening level (Uozumi et al*.,* 1993). We therefore hypothesize that quantifying greenness of callus cultures can show the threshold photoperiod sensitivity among kenaf genotypes. We investigated and compared genotypic variations in growth rate before and after flowering, days to flowering and fibre yield (*in vivo*) on one hand; and callus induction, formation of green spots and embryogenic clusters (*in vitro*) on the other.

MATERIALS AND METHODS

In vivo determination of photoperiod response

The study was conducted in Ibadan (07°22'N, 03°58'E), South western Nigeria. Seven kenaf genotypes (Tainung, 20C, 8B, Local 35, V400, S72 and Cuba108) obtained from the gene bank of the Institute of Agricultural Research and Training, Ibadan were planted in pots in the screen house in January in a completely randomized design with four replicates. Fertilizer (N-P-K 15-15-15) was applied four weeks after planting. Every 10 days before and after flowering, plant height was measured. Growth rate was determined by increase in plant height per 10 days and means were calculated for pre- and post-flowering phases. Numbers of days to flowering and fibre yield (g) per plant were recorded. During the period of experimentation, mean natural photoperiod was 11.8 h. Analysis of variance was done using the generalized linear model procedure of SAS (SAS, 2000) and means were separated at p=0.05.

Controlled environment studies: *In vitro*

The study was conducted at the National Biotechnology Development Agency (NABDA) South west Zonal Laboratory hosted in the department of Agronomy, University of Ibadan, Nigeria.

Effect of photoperiod on callus induction

The genotypes Tainung 1 and V400, which expressed contrasting photoperiodic responses in the screenhouse studies, were used. Seeds were cultured in Murashige and Skoog (Murashige and Skoog (MS), 1962) medium containing (per litre) 30 g sucrose, 0.1 g myo-inositol and 7 g agar set at a pH of 5.7. When the embryos had germinated (four days after culturing), the hypocotyls and cotyledons were excised, cut into approximately 1 cm^2 sizes and cultured in modified MS medium containing 30 g sucrose, 0.1 g myo-inositol, 0.1 mg 2, 4-dichlorophenoxy acetic acid (2, 4- D), 0.5 mg kinetin and 8 g agar per litre of medium set at pH 5.7 for callus induction. Petri plates containing explants were divided into two and one half was incubated in a closed cupboard (photoperiod=0 h) while the other half was incubated at 12 h photoperiod (Plate 1). The experiment was laid out in a completely randomized design with three replicates. Four weeks later, the degree of callus induction was recorded as the fraction of the cultured explant on which callus had been induced on a scale of: 0: no callus, 1: low (up to 35%), 2: medium (40 to 75%) and 3: high (80 to 100%). Analysis of variance was performed and means were separated at p=0.05.

Effect of photoperiod on greenness of callus and formation embryogenic clusters

Calli were transferred into differentiation medium (Balogun et al., 2006) containing (per litre) 4.43 g Murashige and Skoog basal medium 30 g Sucrose, 0.1 g myo-inositol, 0.3 mg naphthalene acetic acid (NAA), 0.3 mg 6-benzyl amino-purine (BAP) and 8 g agar set at pH 5.7 and incubated at 12 h photoperiod for two weeks. Thereafter, the calli cultures were divided into two and each half was incubated at either 12 or 9 h photoperiod for two weeks and the numbers of green spots were recorded. The calli were washed with sterile distilled water, agitated to separate embryogenic callus clusters (Uzuomi et al*.,* 1993) and viewed under the dissecting microscope. Numbers of embryogenic callus clusters per culture were recorded. Analysis of variance was done using the Statistical Analysis System (SAS, 2002) and the means were separated at p = 0.05. Correlation analysis was done among numbers of days to flowering, green spots and embryogenic callus clusters.

RESULTS

Screenhouse studies

A mean growth rate of 15 cm /10 days before flowering in genotype V400 was significantly lower than 24 to 29 cm/10 days in other genotypes (Table 1). The growth rate however increased to 25 cm per 10 days after flowering in V400 but reduced significantly in other genotypes, with as low as 7 cm per 10 days in S72. Percentage increase in growth rate after flowering in V400 was 60% but reduced

Table 1. Pre- and post- flowering growth rates of seven kenaf genotypes grown in Ibadan, South-western Nigeria.

Genotype	Growth rate before flowering (A)	Growth rate after flowering (B)	B-A	Standard error
20c	24.56[a]	13.21[b]	-11.35	2.92
8[b]	27.28[a]	17.48[b]	-9.80	1.44
Cu[b]a	26.89[a]	15.97[b]	-10.92	1.29
Local35	27.71[a]	15.16[b]	-12.55	2.18
S72	29.09[a]	7.82[b]	-21.27	1.21
Tainung1	28.8[a]	16.46[b]	-12.34	2.46
V400	15.06[a]	24.95[a]	+9.89	8.80

For parameters A and B, means in each row followed by the same letters are not significantly different at p=0.05.

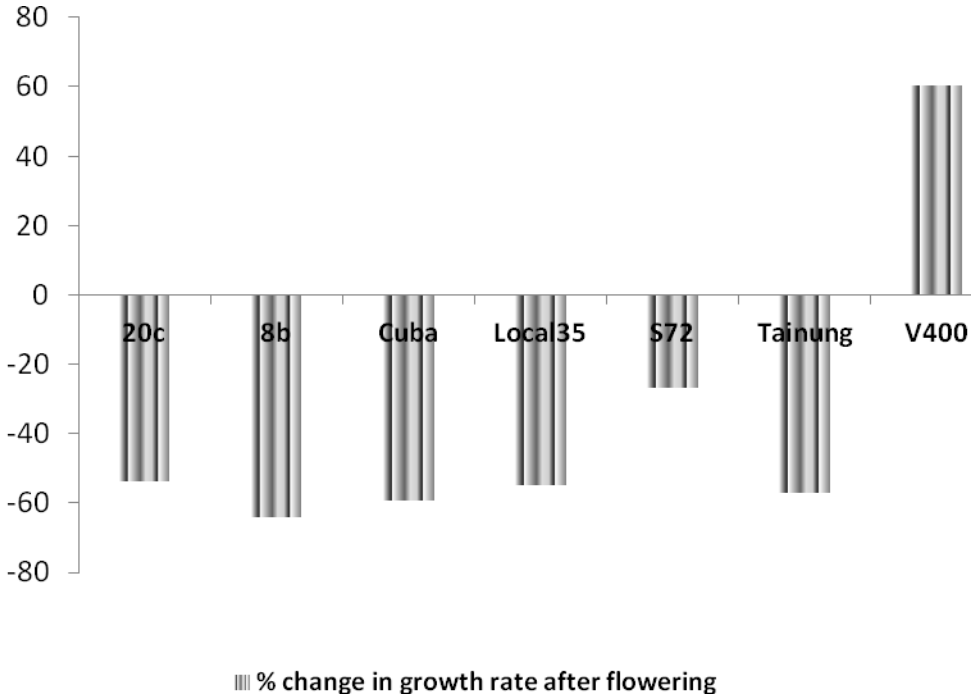

Figure 1. Percentage change in growth rate after flowering in seven kenaf genotypes.

by approximately the same amount in other genotypes (Figure 1). Dempsey (1975) reported that vegetative growth did reduce significantly after flowering in photo insensitive kenaf varieties, even when they flower early. Monitoring growth rate indicates rate of progress towards flowering, and is a better indicator of the degree of photo-sensitivity than time to flowering (Summerfield et al., 1991).

Number of days to flowering ranged from 45 to 51 in other genotypes (Figure 2); significantly lower than 79 in V400. Most kenaf varieties are photosensitive, and flowers when daylength reduces below 12.5 h (Dempsey, 1975; Carberry et al*.,* 1992). The natural photoperiod during the experimentation period was short, ranging from 11.6 to 11.9 h (Balogun et al*.,* 2007). This low photoperiod therefore induced early flowering in the photosensitive genotypes compared to late flowering in the photoinsensitive VI400. It is therefore important to measure the response of genotypes to photoperiod at equatorial climates both in quantitative (degree) and qualitative (presence or absence) terms (Dempsey, 1975; Muchow and Wood, 1983; Balogun et al., 2007), since genotypes have different thresholds of photoperiod sensitivity.

On the average among genotypes, highest yield was recorded in V400, suggesting its compatibility with equatorial climates for optimum fibre yields. However, genotypes may also respond differently to an interaction between photoperiod and other factors like temperature and humidity in the environment (Webber and Bledsoe, 1993; Balogun et al., 2007; Summerfield et al., 1991) which may affect the incidence and severity of pest infestation and disease infection. A multi-locational trial is therefore necessary to reveal specific adaptation of genotypes to agroecologies for optimum yield.

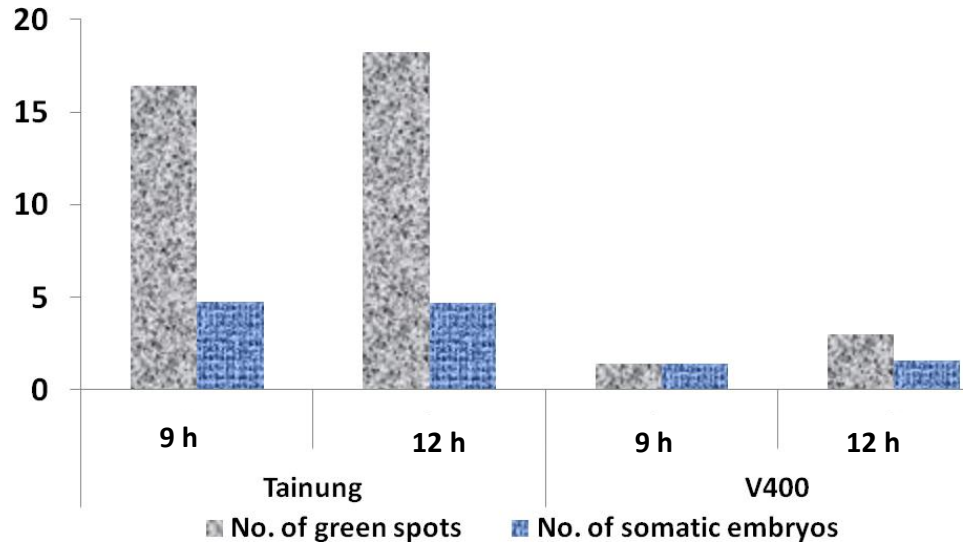

Figure 2. Number of green spots and embryogenic clusters in callus of 2 kenaf genotypes.

Table 2. Percentage callus induction in two kenaf genotypes cultured at 0 and 12 h photoperiods.

Genotype	Photoperiod	Explant	Percentage (%) Callus induction
Tainung1	Dark	Leaf	100
		Stem	53.55
	12 h	Leaf	40.95
		Stem	71.4
V400	Dark	Leaf	51.45
		Stem	75.25
	12 h	Leaf	50.75
		Stem	93.45

In vitro results

Degree of callus formation

The main effects of variety explant and photoperiod regimes were not significant for degree of callus formation but the interactions were significant. In Tainung1 cultured in darkness, 100% degree of callus formation by leaf explants was higher than 53.55% by stem explants while in light, stem explants had a higher degree of callus formation (71.4) than leaf explants (40.95). In genotype V400 however, stem explants had a higher degree of callus formation (75.25 and 93.45) than leaf explants (51.45 and 50.75) in both light and darkness, respectively (Table 2). Thus, a particular plant part (leaf or stem) of V400 responded similarly to different light regimes, while the response varied with light regime in Tainung1. This also suggests that Tainung1 is photosensitive while V400 is not. Mclean et al. (1992) reported the induction of callus tissue and plant regeneration in kenaf using genotype Tainung1 but did not evaluate the effect of photoperiod regimes.

Formation of green spots and embryogenic callus clusters in response to light

The main effect of photoperiod on number of green spots per callus of about 5 cm^2 in area was not significant. However, the genotypic effects were significant, and the genotypes differed significantly for this trait. A mean of 2.2 spots in V400 was significantly lower than 17.34 in Tainung1. Genotype by photoperiod interaction was also not significant, showing the high resolution capacity of this screening technique since results will not be distorted or complicated by interactions among prevailing cultural conditions.

Table 3. Days to flowering and fibre yield (g/plant) in seven kenaf genotypes grown in Ibadan, South-western Nigeria.

Genotype	Days to flowering	Fibre yield (g/plant)
20c	51.6[b]	47.3[b]
8b	51.6[b]	25.2c
Cuba	52.0[b]	45.7[b]
Local35	48.0[b]	28.2[c]
S72	45.8[b]	32.8[c]
Tainung	49.8[b]	54.0[b]
V400	79.3[a]	76.7[a]
Standard error	4.30	6.75

Means in each column followed by the same letters are not significantly different at p=0.05.

T**able 4.** Correlation coefficients of *in vitro* and *in vivo* indices of photoperiod sensitivity averaged over two kenaf genotypes.

Parameter	DAYSFFL	NGREEN	NEMBRYO	PRERATE	POSTRATE
DAYSFFL	1				
NGREEN	-0.75*	1			
NEMBRYO	-0.16	0.40	1		
PRERATE	-0.39	0.81*	0.48	1	
POSTRATE	0.31	-0.09	-0.36	-0.16	1

DAYSFL, Number of days to first flowering; NGREEN, number of green spots per callus; NEMBRYO, number of embryogenic clusters per callus; PRERATE, pre-flowering growth rate; POSTRATE, post-flowering growth rate. *Correlation is significant at 0.05 level of probability.

Only genotype main effect was significant for number of embryogenic callus clusters per callus. Tainung1 had a mean of 4.73, significantly higher (three-fold) than 1.50 in V400. Number of days to flowering was negatively correlated with number of green spots (r=-0.76) per callus (Table 3). Also, there was significant positive correlation between number of green spots *in vitro* and growth rate before flowering (Table 4). These show that late maturing genotypes had fewer green spots on their calli while vigorously growing plants prior to flowering had more green spots on their calli.

DISCUSSION

Greenness in cell or tissue cultures is a light mediated process which reflects chlorophyll synthesis (Uozumi et al., 1993). The insignificant difference in number of green spots per callus between 12 and 9 h light exposure per 24 h cycle suggests the existence of light-independent chlorophyll synthesis in kenaf. Chlorophyll synthesis in angiosperms was reported to involve at least two types of enzymes, one (NADPH-protochlorophyllide oxidoreductase) requiring light and the other (*chl*L, N, B-type enzyme) which does not (Uozumi et al., 1993; Adamson et al., 1997). Also, using root greening as an indicator, Kobayashi et al. (2012) showed that in addition to light, auxin/cytokinin signaling pathways are responsible for coordinated expression of the key genes in chloroplast biogenesis of *Arabidopsis thaliana*.

Significant genotypic effects emphasize the genetic attributes of the light-dependent and light-independent steps. In kenaf, light insensitivity and light sensitivity were reported to be controlled by a pair of gene, photoperiod insensitivity being the recessive gene (Shilin et al., 1996). This mutant gene of kenaf light-insensitivity was reported to be located in genome DNA, and has no relationship to mitochondria and chloroplast genome DNA.

In transformation of kenaf with the B-glucuronidase gene, Reichert et al. (1999) reported significant differences among kenaf cultivars for the prevalence of GUS-positive sectors. Balogun et al. (2006) also posited that, in as much it is desirable that there is a general procedure for kenaf regeneration; it may be difficult to remove the influence of genotypes. It will be worthwhile to investigate whether or not photo-sensitivity or -insensitivity in kenaf is related to ability to regenerate *in vitro*, since formation of embryogenic callus precedes plantlet regeneration (Balogun et al., 2006; Reichert, 1999). This will be facilitated by the recent construction of genetic linkage map of kenaf (Zhang et al., 2011).

The significant negative correlation between number of green spots and days to flowering showed that fewer

green spots were observed on the calli of late maturing genotypes and this is suggestive of photo-insensitivity. Similarly, significant positive correlation between number of green spots and growth rate before flowering showed that genotypes with high vigour before flowering relative to vigour after flowering had more green spots on their calli in response to light and this is suggestive of photo-sensitivity. The authors have not come across reports of *in vitro* flowering in kenaf wherein time to flowering of *in vitro* plantlets can be monitored in response to photo-period. It may be worthwhile to also quantify amount of chlorophyll in leaves and stems *in vivo*. These will allow direct comparison of the same traits at *in vitro* and *in vivo* conditions

In determining photoperiodic response of kenaf genotypes, the results obtained *in vivo* which utilized growth rate and maturity period as indices matched in *vitro* results which utilized green spot formation by callus cultures. In both cases, Tainung1 was photosensitive while V400 was not. Incubating callus in differentiation medium at 12 h light and evaluating for greenness by counting number of green spots showed photoperiod response in genotypes. This *in vitro* screening method is faster and cost effective in space and labour requirements. Quantification of callus chlorophyll content in addition to greenness will better show the degrees of photoperiod sensitivity in genotypes. More genotypes should be screened *in vitro* to further establish the efficiency of this procedure and the relationship between ability to regenerate plantlets and sensitivity to photoperiod.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Akin Akisanya of the Institute of Agricultural Research and Training for the technical assistance on the evaluation for embryogenic clusters and Prof. V. O. Adetimirin of the Department of Agronomy for facilitating the use of the NABDA laboratory.

REFERENCES

Adamson, HY, Hiller RG, Walmsley J (1997). Protochlorophyllide reduction and greening in angiosperms: an evolutionary perspective. Journal of Photochemistry and Photobiology 41(3): 201-221.

Balogun MO, Akande SR, Ogunbodede BA (2006). Effects of plant growth regulators on indirect somatic embryogenesis in Kenaf (*Hibiscus cannabinus*). Nig. J. Genet. 20: 41-48.

Balogun MO, Raji JA, Akande SR, Ogunbodede BA (2007). Variations in Photo- and thermal- sensitivities among local, improved and exotic kenaf accessions in Nigeria. J. Food Agric. Environ. 5(1): 385-388.

Carberry PS, Muchow RC, Williams R, Sturtz JD, McCown RL. (1992). A simulation model of kenaf for assisting fibre industry planning in northern Australia: 1. General introduction and phenological model. Aust. J. Agric. Res. 43: 1501-1513.

Dempsey JM (1975). Fibre Crops. University of Florida Press, Gainesville, FL, pp. 203-304.

Energy Information Administration (EIA), 2003. Country Analysis Briefs. Statistical Agency of the United States Department of Energy. On the Internet: www.eia.doe.gov/cabs/nigenv.html.

FAO. 2003. The production and consumption of kenaf in China. ESC-Fibres Consultation no. 03/6.

Kobayashi KS, Baba T, Obayashi M, Sato K, Toyooka M, Keränen E, Aro H, Fukaki H, Ohta K, Sugimoto, Masuda T. (2012). Regulation of Root Greening by Light and Auxin/Cytokinin Signaling in *Arabidopsis*. The Plant Cell. 24 (3) 1081-1095.

McLean KS, Lawrence GW and Reichert NA. (1992). Callus induction and adventitious organogenesis of kenaf (*Hibiscus cannabinus* L.) Plant Cell Reports Volume 11, Number 10 Pp. 532-534

Muchow RC, Wood LM. (1983). Effect of sowing date on the growth and yield of kenaf (*Hibiscus cannabinus*.) grown under irrigation in Tropical Australia. I. Phenology and seed production. Field Crops Res. 7: 81-90.

Murashige T and F. Skoog. (1962). A revised medium for rapid growth and bioassays with tobbacco tissue cultures. *Physiologia Plantarium* 15: 473-497.

Reichert NA, Lim T and Young MM (1999). Method for transformation of cotton and kenaf and organogenic regeneration. United States Patent 5,998207. On the internet.

Rymsza TA. (1999). Utilization of Kenaf raw materials. Paper presented to the Forest Products Society. Boise, Idaho.

Scott AW. Jr., Taylor CS. (1988). Economics of Kenaf Production in the lower Rio Grande Valley of Texas. Paper presented at National Symposium on New Crops, Oct. 23-26, 1988, Indianapolis, Indiana, pp. 292-297.

Shilin T. (1996). Exploitation and Investigation of Insensitive Materials to Short Daylength in Kenaf. Acta Agronomica Sinica. 1996: 001-007. Statistical Analysis Systems (SAS). Copyright 1999-2000.SAS (r) Proprietary Software Release 8.2 (TS2M0), SAS Institute Inc., Cary, NC, USA.

Stricker JA, Prine GM, Riddle TC. (2001). Kenaf – A possible New Crop for Central Florida. University of Florida Cooperative Extension Service. Institute of Food and Agricultural Sciences. Gainesville, Florida. On the Internet: http://www.edis.ifas.ufl.edu/BODY_AA220.

Summerfield RJ,Roberts EH, Ellis RH, Lawn RJ (1991). Towards the reliable prediction of time to flowering in six annual crops I. The development of simple models for fluctuating field environments. Expt. Agric. 27: 11-31

Uozumi N, Kohketsu K, Okamoto A, Kobayashi T. (1993). Light dependency in celery somatic embryogenesis and plantlet development in suspension culture. Plant Tiss. Cult. Lett. 10 (1):25-32.

Webber CL III, Bledsoe VK (1993). Kenaf: Production, Harvesting, Processing and Products. In: Janick, J. and Simon, J.E. (Eds.), New Crops. Wiley, New York, pp. 416-421.

Webber CL III, Bhardwaj HL, Bledsoe VK. (2002). Kenaf Production: Fibre, Feed and Seed. In: Janick, J., Whipkey, A. (Eds.), Trends in new crops and new uses. ASHS Press, Alexandria, VA, pp. 327-339.

Zhang G, Qi J, Zhang X, Fnag P, Su J, Tao A, Lan T, Wu W, Liu A (2011). A Genetic linkage map of Kenaf (*Hibiscus cannabinus* L) Based on SRAP, ISSR and RAPD Markers. Agric. Sci. China 10 (9): 1346-1353.