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## Research Paper

# Overexpression of *Arabidopsis thaliana* gibberellic acid 20 oxidase (AtGA20ox) gene enhance the vegetative growth and fiber quality in kenaf (*Hibiscus cannabinus* L.) plants

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Kenaf (*Hibiscus cannabinus* L.; Family: Malvaceae), is multipurpose crop, one of the potential alternatives of natural fiber for biocomposite materials. Longer fiber and higher cellulose contents are required for good quality biocomposite materials. However, average length of kenaf fiber (2.6 mm in bast and 1.28 mm in whole plant) is below the critical length (4 mm) for biocomposite production. Present study describes whether fiber length and cellulose content of kenaf plants could be enhanced by increasing GA biosynthesis in plants by overexpressing *Arabidopsis thaliana* Gibberellic Acid 20 oxidase (AtGA20ox) gene. AtGA20ox gene with intron was overexpressed in kenaf plants under the control of double CaMV 35S promoter, followed by *in planta* transformation into V36 and G4 varieties of kenaf. The lines with higher levels of bioactive GA (0.3–1.52 ng g<sup>-1</sup> fresh weight) were further characterized for their morphological and biochemical traits including vegetative and reproductive growth, fiber dimension and chemical composition. Positive impact of increased gibberellins on biochemical composition, fiber dimension and their derivative values were demonstrated in some lines of transgenic kenaf including increased cellulose content (91%), fiber length and quality but it still requires further study to confirm the critical level of this particular bioactive GA in transgenic plants.

**Key Words:** biocomposite, fiber quality, GA20ox gene, *Hibiscus cannabinus*, overexpression, transgenic kenaf.

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## Introduction

There are increasingly growing demands for the natural fibers which are predominantly obtained from the woody plants of the forests as the main commodity for most of the fiber-based industries. Along with other biocomposite materials, global consumption of paper was estimated to have increased from 300 million tons in 1998 to 425 million tons in 2010 (Labidi *et al.* 2008, Rowell and Cook 1998). However, woody fiber resources are diminishing rapidly through extensive felling of trees for natural fibers. Kenaf (*Hibiscus cannabinus* L.), which belongs to the Malvaceae family, is a non-woody multipurpose annual plant that is very close to cotton and okra (Ayadi *et al.* 2011) and it possesses excel-

lent potential to fulfil this demand without cutting and depleting forest resources. It has also been recognised as the third largest fibrous crop following cotton and jute (Ahmed *et al.* 1998, Pande and Roy 1996), an excellent source of cellulosic fiber for manufacturing large range of paper products (Ayadi *et al.* 2011). Pulping from kenaf requires less energy and chemical inputs for processing compared to other standard wood sources (Bhardwaj *et al.* 2005, Villar *et al.* 2009). It grows very fast even under minimum amount of requirements and provides a wide range of commercial values for the fiber industry. The stalk of this plant consists of outer fiber “bast”, while its inner fiber “core” comprises roughly 40% and 60% of the stalk’s dry weight, respectively. These refined fibers are compatible for engineering wood, insulator and clothing-grade clothes, security notes, bullet proof jackets, helmets, packing materials, etc. Meanwhile, cut bast fibers are commonly blended with resins for plastic composites that are used in making car interiors

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known as bolsters for more sustainable vehicles. Since the fibers of this plant are mainly used in various manufacturing industries, the enhanced quality of fibers, as well as species biomass, is a lucrative target of many researchers, manufacturers and investors alike for overall production system. However, the average length of kenaf fibers is 2.6 mm in bast and 1.28 mm in whole plant (Jamaludin 2008), with some exceptions of 3–4 mm in bast (Farshes *et al.* 2011), which is below the critical fiber length for biocomposite (biopolymer and paper industries) manufacturing (Jamaludin 2008). The larger the fiber length means greater mechanical strength than the shorter ones for flexural and impact properties. Studies have shown that flexural strength in specimens is considerably higher by 67% in longer fiber while these decreases significantly with reduced fiber length including the ones below the critical length (Shibata *et al.* 2006) since the end use of fibers primarily depend upon their physical, chemical and structural properties.

Elongation of kenaf plant and fiber is believed to be obtained through environmental manipulation or genetic modification. Plant hormones play critical roles in growth and development of plants. Gibberellin (GA) is a plant hormone of particular interest to researchers due to its broad spectrum of effects on plant growth, elongation, flowering development, seed formation and germination (Davies 2004, Fleet and Sun 2005, Hedden and Thomas 2012, Mutasa-Göttgens and Hedden 2009, Schomburg *et al.* 2003). Exogenous application of gibberellins (GAs) has created a consensus on altering the plant growth and different developmental features. Although a large number of different GA species (136 GA structures in nature) have been identified (Giacomelli *et al.* 2013), only few have been proven to possess biological active GAs. GA1 and GA4 are the most common active forms in higher plants (Giacomelli *et al.* 2013). Since most of the biosynthesis genes in gibberellin biosynthesis pathway have been identified and extensively studied in many species (Hedden and Thomas 2012, Yamaguchi 2008), genetic modification of GA biosynthesis has become an alternative approach targeting the crop breeding (Barboza *et al.* 2013) to alter plant growth. There are a number of genes encoding enzymes in GA metabolism pathway that have been identified and characterized in *Arabidopsis* (Hisamatsu *et al.* 2005, Plackett *et al.* 2011, 2012, Rieu *et al.* 2008), as well as in cucurbita (Lange *et al.* 2012), tomato (Serrani *et al.* 2007), apple (Kusaba *et al.* 2001), tobacco (Biemelt *et al.* 2004), grape (Giacomelli *et al.* 2013), chrysanthemum (Miao *et al.* 2010), sago palm (Jamel *et al.* 2011) and burley (Jia *et al.* 2009, 2011).

In gibberellin biosynthesis pathway, the last steps are catalyzed by soluble 2-oxo-glutarate-dependent dioxygenases, referred to as GA-oxidase (Lange *et al.* 2012, Yamaguchi 2008) which are involved in the production of bioactive GA1 and GA4. GA20 oxidase has been identified as one of the key enzymes for the production of bioactive GAs that controls various aspects of plant development, stem elongation, flower and fruit development and seed germination

(Barboza *et al.* 2013, Carrera *et al.* 2000, Hisamatsu *et al.* 2005, Jia *et al.* 2009, 2011, Plackett *et al.* 2012, Rieu *et al.* 2008). Initial studies on *Arabidopsis* showed that regulating GA20 oxidase could successfully modify the gibberellins levels. The enhanced level of GA through their biosynthetic gene expression is known to elongate shoots in many plants while decreasing the level of GA will lead to plant dwarfness. Overexpression of GA20 oxidase gene has been reported to increase the indigenous GAs in plants and elongate the stem in *Arabidopsis* (Hisamatsu *et al.* 2005, Huang 1998, Rieu *et al.* 2008), potato (Carrera *et al.* 2000) and tobacco (Biemelt *et al.* 2004, Vidal *et al.* 2001) that are regulated spatially and temporally during the development. Another study has shown that the antisense expression of GA20 oxidase gene caused earlier tuberization in potato (Carrera *et al.* 2000). Therefore, GA20 oxidase genes are currently being used in plant improvement programmes in a wide range of species, particularly in crop plants. Thus, genetic manipulation of these genes could be the useful tool to generate new crop varieties of desired trait.

Although kenaf is considered as one of the important multipurpose fiber crop plants to have excellent potentials for manufacturing versatile value added products, effort has not been made to enhance the fiber quality and biomass production of the plant till date. There is no report describing the molecular aspect of the plants except for the characterization of ferulate 5-hydroxylase (Kim *et al.* 2013a) and caffeic acid O-methyltransferase (*COMT*) (Kim *et al.* 2013b) gene from kenaf. Therefore, the present research work was the first to specifically investigate whether overexpression of GA20 oxidase can accelerate the stem elongation and fiber quality through enhancing bioactive GA synthesis in transgenic kenaf plants aimed for an overall increment of growth of the species. *Arabidopsis thaliana* GA20 oxidase (*AtGA20ox1*) gene was overexpressed in two kenaf varieties; G4 and V36. The gateway recombination cloning system was adapted to develop the plant transformation vector. Meanwhile, pMDC32 plasmid vectors were used for the overexpression of *AtGA20ox* gene under duplicated CaMV 35S promoter in the kenaf plant through *Agrobacterium tumefaciens* mediated transformation system *in planta*.

## Materials and Methods

### Amplification and molecular cloning of *Arabidopsis AtGA20 Oxidase 1* gene

Total genomic DNA was extracted through CTAB method from the young leaves and floral buds of 4–6 weeks old soil grown plants in growth room under standard growth condition. The *Arabidopsis thaliana* GA20 oxidase genomic (*AtGA20ox*) clone was amplified by PCR with genomic DNA as a template. The gene specific primers for the amplification of the *AtGA20ox* gene (Forward) 5'-AGGATCCATGGCCGTAAGTTTCGTAAC-3' and *AtGA20ox* (Reverse) 5'TGGATCCTTAGATGGGTTTGGTGAGCC3' were designed from the published sequence (Accession no: