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Effects of Flooding on Growth, Yield and Aerenchyma Development in Adventitious Roots in Four Cultivars of Kenaf (*Hibiscus cannabinus* L.)

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Abstract: A pot experiment was performed to examine the effects of flooding on growth, yield and aerenchyma development in adventitious roots of four kenaf (*Hibiscus cannabinus* L.) cultivars. Three flooding treatments consisting of early season flooding (30 days after planting), mid-season flooding (60 days after planting) and late season flooding (90 days after planting), as well as non-flooding control were used in the present study. The results show that soil flooding significantly increased plant height by 108 and 107% over control in early flooding and mid-flooding, respectively. Early flooding significant decreased the number of leaves and leaf area of whole plant and core dry weights by 15, 19 and 20% over non-flooding control, respectively. Soil flooding did not show any significant effect on plant height and number of leaf among cultivars, but did for leaf area, leaf dry weight and core dry weight. Early season and mid-season flooding significant decreased root dry weight in soil by 71 and 49% over non-flooded control, respectively. No adventitious roots developed in non-flooded control. Adventitious roots located in water above soil surface had dry weight of 18, 11 and 6 g plant^{-1} in early season, mid season and late season flooding, respectively. No significant difference in root dry weight located in soil and root dry weight located in water above soil surface were observed among cultivars. Aerenchyma formed in adventitious roots when the plant was subjected to flooding and was more developed in roots located in water above the soil surface as compared to roots located in soil. All the cultivars formed aerenchyma in their adventitious roots with variation among cultivars. Soil flooding significantly decreased fiber yield by 13% in non-flooded control in early season flooding treatments. However, mid-season and late season flooding did not show any significant difference on fiber yield in comparison with control. The cultivars was not significantly difference on fiber yield in the present experiment.

Key words: Aerenchyma, cultivar, flooding, kenaf

INTRODUCTION

The production of a pre-rice crop during the dry-wet transition period may increase income of farmers in the northeast area of Thailand. The early rains in the dry-wet transition period will be used to successfully grow upland crops such as kenaf, because rice seedlings can be transplanted later in the wet season (Polthanee, 2004) There are, however, three major problems with reference to production of upland crops early in the rainy season, (1) waterlogging due to intermittent heavy rains on soils with poor surface and internal drainage, (2) drought stress due to erratic rainfall and (3) harmful pests and diseases, particularly for legume crops. Among those problems, waterlogging is the predominant one limiting yield in many, if not most, situations (Zandstra *et al*., 1982).

The existing cropping pattern of kenaf as one pre-rice crops has been practiced by the farmers in Chaiyaphum province of northeast Thailand for a long time, possibly because kenaf is considered to be waterlogging-tolerant due to development of many adventitious roots as an adaptive mechanism (Pratcharoenwanich, 2000). Many plant species have been reported to be tolerant for flooding by formation and development of adventitious roots (Kawase, 1981; Steven and Mitchell, 1990; Lizaso *et al*., 2001; Chen *et al*., 2002; Singh and Singh, 2003).

Aerenchyma formation is an another important adaptive response of crops to soil flooding (Vatapetian and Jackson, 1997; Chen *et al*., 2002; Kawase and Whitmoyer, 1980; Das and Jat, 1977; Burdick, 1989). Aerenchyma development in adventitious roots of kenaf (*H. cannabinus* L.) is an adaptive mechanism for tolerance

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to waterlogging (Changdee *et al*., 2008). Aerenchyma provides a low resistance in the internal pathway for gas exchange between the plant parts above and below the water surface and improves the internal supply of oxygen for submerged tissues (Armstrong, 1979; Stevens *et al*., 2002; Shuwen *et al*., 2006).

In general, kenaf as a pre-rice crop will be subjected to unpredictable flooding which depends on variable rainfall distribution year by year. Information on how flooding occurs in early season, mid-season and late season affects kenaf growth and yield is limited. In previous studies, there is no information on aerenchyma development in adventitious roots both in water above soil surface and roots in soil below soil surface. This study was designed to examine the effects of flooding at early season, mid-season and late season on growth, yield and aerenchyma development in adventitious roots of four kenaf cultivars.

MATERIALS AND METHODS

The experiment was conducted in the greenhouse at department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University in 2006. Four cultivars of kenaf (*H. cannabinus*); Khon Kaen (KK), Khon Kaen 60(KK60), Khon Kaen 977-044 (KK977-044) and Nongtakai (NTK) were grown in pots as reported for other crops (Trought and Drew, 1980; Daugherty and Musgave, 1994; Huang *et al*., 1994; Malik *et al*., 2002; Singh and Singh, 2003; Matsuura *et al*., 2005), because it is much easier to control water conditions compared with field experiments. Seeds of all cultivars were sown in soil in plastic pots (30 cm in inner diameter and 60 cm in height) during rainy season in a greenhouse. Chemical compound fertilizer (15-15-15 for N-P_2O_5-K_2O) was applied at rate of 156 kg ha^{-1} when planting. Seedlings were thinned into 1 plant in each pot at 15 days after seeding. Hand weeding was done at 30 days after planting. No pesticides were used in this study. At harvest (135 days after planting), plant height, number and dry weight of leaf, fiber yield and core dry weight (stem without fiber) were determined.

The study was arranged in a factorial in CRD with four replications. Flooding treatments were the factor A and cultivars were the factor B. Flooding treatments included the early season (30 days after planting), midseason flooding (60 days after planting) and late season flooding (90 days after planting). In the non-flooded (control) treatment, soil moisture was maintained at field capacity throughout the growing season. The flooded treatment was initiated at 30, 60 and 90 days after planting, depending on the treatment and standing water maintained at 10 cm above soil surface until harvest. Prior to flooding, soil moisture was maintained at field capacity for optimal growth.

Adventitious roots were taken from above and below the soil surface separately to be weighed. Aerenchyma was observed in randomly selected samples from these two groups of adventitious roots. Cross sections were made at 5 cm from the root tip based on the standard freehand section method (Ruzin, 1999). The sections were stained with toluidine blue 0 for microscopy (Model, Olympus BX51). Images were recorded using a high sensitivity CCD color camera system (Model, Keyence B.7010).

RESULTS AND DISCUSSION

Flooding effect on shoot growth: Plant height was significantly higher in the early and midseason flooded treatments than in the control whereas plant height in the late season flooded treatments was similar to that in the control. The plant height was 108% of the control in the early season flooding and 107% of the control in the mid-season flooded treatment and similar to control in late season flooded treatment. In contrast, flooding reduced the plant height of *Theobroma cacao* by 6-37%, depending on flooding duration (Sena Gomes and Kozlowski, 1986). Similar reduction of plant height by flooding was reported for wheat (Collaku and Harrison, 2002) and black willow (*Salix nigra*) (Shuwen *et al*., 2006). There were no significant differences in plant height among cultivars. Additionally there were no interactions between flooding treatments and cultivars (Table 1).

Table 1: Effect of flooding, time of flooding and cultivar on plant height, number of leaves, leaf Area, leaf dry weight and core dry weight of kenaf at harvest

Treatments	Height (cm)	No. of leaves (No. $plant^{-1}$)	Leaf area (cm^2 $plant^{-1}$)	Leaf dry weight (g $plant^{-1}$)	Core dry weight
Flooding					
None	264.9	33	628.9	3.6b	25.8
Early-season	286.3	28	449.8	2.8	20.7
Mid-season	285.0	34	495.7	3.3	23.6
Late-season	261.8	36	644.0	4.0	26.2
Cultivar					
KK	274.4	30	516.8	2.9	23.7
KK60	272.5	34	638.4	4.1	22.6
KK977-044	268.5	34	671.4	3.9	27.1
NTK	288.6	32	421.8	2.8	23.0
F-test					
Flooding (F)	**	*	**	**	**
Cultivar (C)	ns	ns	**	**	**
FxC	ns	ns	*	**	ns

** and * show significant levels at $p<0.01$ and $0.01 \le p<0.05$, respectively and ns shows not significant

Kenaf plants subjected to flooding in the early season were significantly lower in leaf number and leaf area of whole plant than those of the control. However, leaf number and leaf area per plant in the midseason and late season flooded treatments were similar to those in the control. There was no significant difference in the leaf number of the plant, but these was in the leaf area among cultivars (Table 1). The cultivar Khon Kaen 977-044 had the largest leaf area of the whole plant. There were no interactions of the number of leafs per plant between flooding treatments and cultivars, but the interactions between flooding treatments and cultivars were observed on the leaf area per plant (Table 1). The Nongtakai-cultivar had the smallest leaf area per plant in early season flooding, while the cultivar KK60 had the smallest leaf area per plant in the mid-season flooded (Table 1).

In the present study, the leaf area of the whole plant was decreased to 28 and 21% of the control in the early and mid-season flooded treatments, respectively. Early season flooding significantly decreased the leaf number per plant and leaf area per plant to 15 and 29% of the control, respectively.

Soil flooding reduced the leaf area to 52% of the control in the tolerant genotype of wheat while that in the sensitive genotype decreased to 63% (Huang *et al*., 1994). The total leaf area was reduced by flooding to 76 and 34% of the control in common and Tartary buckwheat, respectively (Matsuura *et al*., 2005). Similar reduction of leaf area of wheat by flooding was reported by Malik *et al*. (2002) and Musgrave and Ding (1998). That soil flooding significantly decreased leaf number of *Theobroma cacao* was reported by Sena Gomes and Kozlowski (1986).

Leaf dry weight in the early season, mid-season and late season flooded treatments were similar to that in the control. However, leaf dry weight in the late season flooded treatment was significantly higher than that in the early season flooded treatment. There were interactions of leaf dry weight between flooding treatments and cultivars (Table 1). The cultivar Nongtakai had the lowest leaf dry weight in early season flooding, while the cultivar Khon Kaen showed the lowest leaf dry weight in the mid-season flooded treatments. Soil flooding significantly decreased leaf dry weight of *Theobroma cacao* when the plant was subjected to flooding for 30, 45 and 60 days but not for 15 days as reported by Sena Gomes and Kozlowski (1986). Core dry weight in the early season flooding was significantly lower than that of the control. However, core dry weight from the mid-season and late season flooded treatments were similar to the control. Sena Gomes and Kozlowski (1986) reported that soil flooding increased stem dry weight of *Theobroma cacao* when the plant was subject to flooding for 15 and 30 days but decreased stem dry weight when the plant was subjected to flooding for 45 and 60 days. Core dry weight varied significantly with the kenaf cultivar. The cultivar Khon Kaen 977-044 had the maximum core dry weight. There were no interactions of the core dry weight between flooded treatments and cultivars (Table 1).

Flooding effect on root growth: Dry weight of adventitious roots in soil from the early, mid and late seasons flooded treatments was significantly lower than that of the control treatments. The plants subjected to flooding in the early season had the least dry weight of adventitious roots in soil. There was no significant difference in the dry weight of adventitious roots in soil among cultivars. There were no interactions between flooding treatments and cultivars regarding adventitious roots in water above soil surface (Table 2).

In present experiment, dry weight of existing roots in soil was decreased by flooding to 71, 49 and 29% of the control in the early, mid and late season flooding, respectively.

Flooding reduced root growth to less than 50% of the control in soybean (Lee *et al*., 2003) and wheat (Malik *et al*., 2002). Root dry weight was reduced by flooding to 82% and 88% of the control in the tolerant and sensitive genotypes of buckwheat, respectively (Matsuura *et al*., 2005). Flooding reduced root dry weight of *Theobrona cacao* by 9-81% of non-flooded control in greenhouse experiment, depending on flooding duration (Sena Gomes and Kozlowski, 1986).

Dry weight of adventitious roots in water above the soil surface from the early season flooded treatments was similar to that of the mid-season flooded treatments . Dry weight of adventitious roots from the late season flooded treatments, however, was significantly lower than that of the early and mid-seasons flooded treatments. There was no roots development in the non-flooded control. Cultivar did not show any significant differences in the dry weight of adventitious roots in water above soil surface. There were no interactions between flooded treatments and cultivars regarding dry weight of the adventitious roots (Table 2).

Flooding effect on fiber yield: Yields in the mid-and late seasons flooded treatments were almost the same as those in the non-flooded control. However, kenaf subjected to flooding in the early season produced significantly lower fiber yield than that of the control. There were no significant cultivar differences regarding fiber yield. Additionally there were no interactions between flooded treatments and cultivars regarding fiber yield (Table 2).

Table 2: Effect of flooding, time of flooding and cultivar on adventitious root in soil and adventitious root in water above soil surface dry weight and fiber yield of kenaf at harvest

Treatments	Adventitious roots in soil dry weight (g plant^{-1})	Adventitious roots in water (g plant^{-1})	Fiber yield (g plant^{-1})
Flooding			
None	12.3	None	12.1
Early-season	3.6	17.6	10.5
Mid-season	6.3	10.5	12.4
Late-season	8.8	5.5	12.8
Cultivar			
KK	7.6	12.9	10.9
KK60	7.3	13.5	11.2
KK977-044	10.3	12.4	12.6
NTK	6.9	10.0	11.1
F-test			
Flooding (F)	**	*	**
Cultivar (C)	ns	ns	ns
FxC	ns	ns	ns

** and * show significant levels at $p < 0.01$ and $0.01 \leq p < 0.05$, respectively ns shows not significant

In present experiment, fiber yield from flooding in the early season was significantly decreased to 13% of the control. This was due to reducing the leaf number, leaf area and core dry weight per plant. Yield was not reduced significantly in sugarcane by flooding as reported by Uraiwan (2002).

Soil flooding reduced grain yield of winter wheat by about 20 to 50% (Belford, 1981; Cannell *et al.*, 1984; Musgrave, 1994; Musgrave and Ding, 1998; Collaku and Harrison, 2002). Flooding just before harvest did not affect sweetpotato yield, but flooding at mid-season reduced yield by 36-53% (Robert, 1991). Yields from sweetpotato cultivars were variable in response to stress caused by flooding (Martin, 1983; Robert, 1991).

Adventitious rooting is one of the important adaptive responses of wetland plant for replacing the existing roots that have been killed or functionally suppressed under flooding conditions (Vatapetian and Jackson, 1997; Pezeshki, 2001). These adventitious roots usually emerge from the flooded stem base and elongate in the water on the soil surface, where relatively high content of oxygen is available (Jackson and Drew, 1984). These new roots might have a positive role in supporting shoot growth during prolonged flooding (Jackson, 1985; Amstrong *et al.*, 1994; Chen *et al.*, 2002; Shuwen *et al.*, 2006; Glaz *et al.*, 2004). Many studies describe an important role for ethylene in the process of adventitious rooting (Tany and Kozlowski, 1984; Voesenek *et al.*, 1990; Visser *et al.*, 1996; Liu and Reid, 1992).

Liu and Reid (1992) showed that enhanced production of ethylene in the cutting of sunflower seedlings by waterlogging involved the sensitivity for the existing auxin to induce adventitious roots. Chen *et al.* (2002) reported that there was a significant negative correlation between the number of adventitious roots and ethylene concentration in flooded roots 3 days after flooding. In the present study, the dry weight of adventitious roots in the early, mid and late season flooded treatments were 18, 11 and 6 g plant^{-1}, respectively. However, adventitious root dry weight did not show any significant difference among cultivars. No adventitious roots were found in the control.

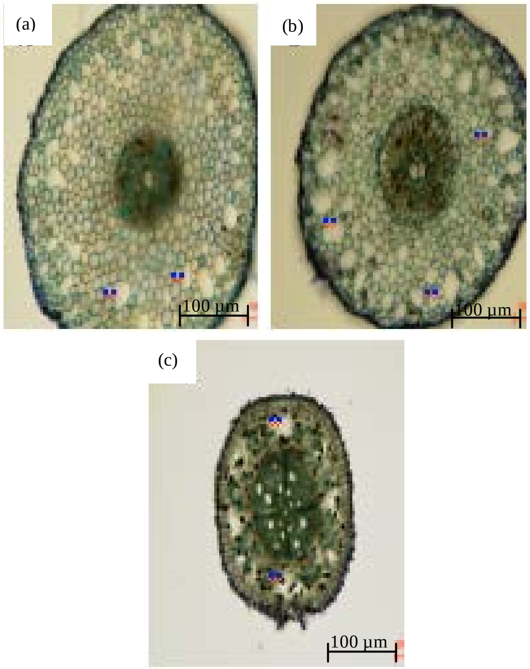

Fig. 1: Cross-sections at 5 cm from the root tip of adventitious root in water at different flooding times of cultivar KK60 (a) late-season flooding, (b) mid-season flooding and (c) early-season flooding) ae, aerenchyma

Flooding effects on Aerenchyma development: Aerenchyma developed in adventitious roots when the kenaf plants were subjected to flooding in early, mid and late seasons (Fig. 1a, b). Prolonged flooding (early season) caused casparian strips in the exodermis (Fig. 1c)

All the cultivars formed aerenchyma in their adventitious roots when the plant were subjected to flooding (Fig. 2a, d). Aerenchyma was more developed in adventitious roots of KK60 and KK977-044 cultivars (Fig. 2b, c).

Aerenchyma was observed in the tap (main) roots in soil both under flooded and non-flooded control treatments. At that time, aerenchyma was more developed in the flooded treatments compared with that in the non-flooded control (Fig. 3a, b).

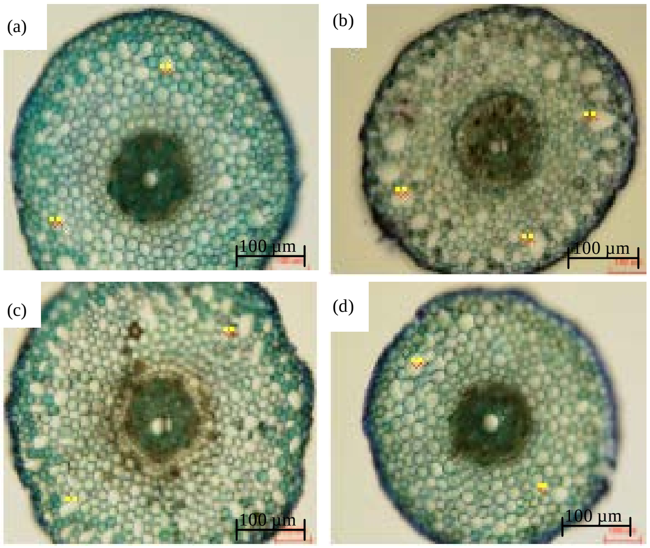

Fig. 2: Cross-sections at 5 cm from the root tip of adventitious root in mid-season flooding of different cultivars (a) KK, (b) KK60, (c) KK 977-044 and (d) Nongtakai) ae, aerenchyma

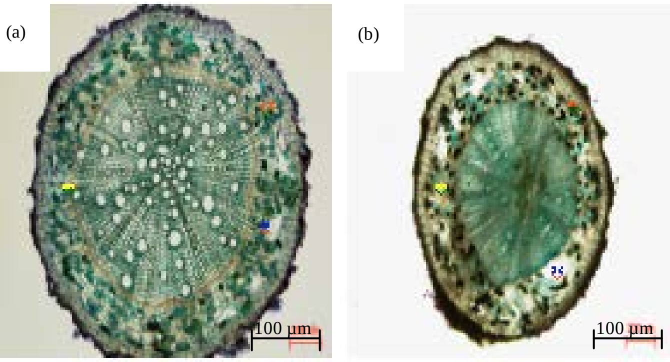

Fig. 3: Cross-sections at 5 cm from the root tip of tap root in soil of cultivar KK60 in control and mid- season flooding (a) control and (b) flooding ae, aerenchyma; en, endodermal and ex, exodermal.

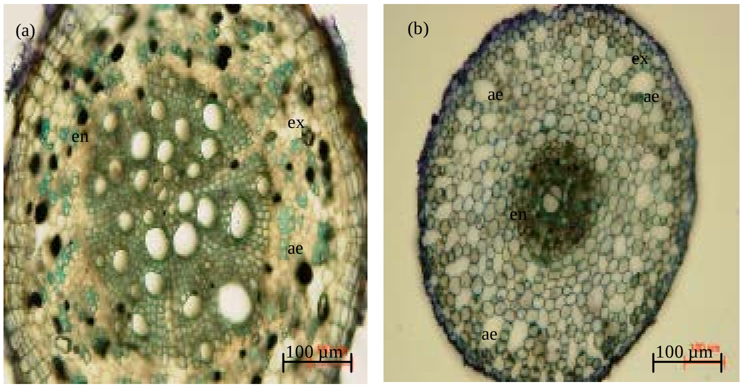

Fig. 4: Cross-sections at 5 cm from the root tip of tap root in soil and adventitious root in water of cultivar KK60 in mid-season flooding (a) tap root in soil and (b) adventitious root in water) ae, aerenchyma; en, endodermal

Aerenchyma was developed in adventitious roots both in soil and water above the soil surface in the flooded treatments where roots in water had more developed aerenchyma (Fig. 4a, b).

Aerenchyma developing in roots under soil flooding is thought to be an adaptive trait (Ray *et al*., 1996; Van Der Heyden *et al*., 1998; Jackson and Armstrong, 1999; Shimamura *et al*., 2003; Niki and Gladish, 2001; Chen *et al*., 2002; Mustroph and Albrecht, 2003; Setter and Waters, 2003; Shuwen Li *et al*., 2006). Aerenchyma development has been considered as a mechanism critical to a plant's ability to cope with anaerobiosis. This system allows plants to transport the atmospheric O_2 to the underground organs to maintain aerobic respiration and to oxidize various reducing compounds in the rhizosphere (Pezeshki, 2001). Aerenchyma forms in roots either lysigenously by cell separation and collapse or schizogenously by cell separation without collapse (Armstrong *et al*., 1991).

CONCLUSION

Early season flooding (30 days after planting) until harvest significant decreased in fiber yield of kenaf (*Hibiscus cannabinus*) but did not in mid-season (60 days after planting) and late season (90 days after planting) flooding. The fiber yield was not significantly affected by cultivar. However, the cultivar KK977-044 tended to produced the highest fiber yield. Adventitious roots were developed when the kenaf plant was subjected to flooding. Early season flooding formed the maximum adventitious roots located in water above the soil surface. All the cultivars formed aerenchyma in the adventitious roots. Aerenchyma was more developed in adventitious roots of KK 60 and KK 977-044 cultivars.

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