CHAPTER V

A FLOOD-FREE PERIOD COMBINED WITH EARLY PLANTING IS REQUIRED TO SUSTAIN YIELD OF PRE-RICE SWEET SORGHUM (*Sorghum bicolor* L. Moench)

Introduction

Sweet sorghum (*Sorghum bicolor* L. Moench) is a promising candidate as supplementary feedstock for ethanol production (Grassi et al., 1992). At present, cassava and sugarcane are main feedstock for ethanol production in Thailand, and the industry faces high competition with other well-established industries such as sugar, animal feed and cassava flour industries that use these commodities. The shortage of raw materials causes inefficiency of the industry because the factories can be operated for only four to six months a year. The production of sweet sorghum as a pre-rice crop might fill the gap during the shortage of feedstock and increase the efficiency of the industry.

In lowland rain-fed agro-ecosystem, most arable land is planted with rice as a mono crop in the mid-rainy season and the land is left fallow for several months during the dry-wet transition period. Therefore, there is the possibility for growing pre-rice crops. However, the success in introducing pre-flood crops into rice-based cropping systems is dependent on market potential of the crops, crop choice and the varieties used. The crops suitable for pre-rice conditions should be of appropriate maturity and able to tolerate drought and withstand flooding (Lantican, 1982), and sweet sorghum seems to meet these criteria (FAO, 2002; ICRISAT, 2004). Sorghum is intolerant of sustained flooding but can survive temporally. However, the ability of sweet sorghum to sustain yield under different waterlogging conditions has not been well researched.

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Growth and yield of many crops are affected by waterlogging and yield losses are dependent on growth stages of the crops (Orchard and Jessop, 1984; Zhou and Lin, 1995; Umaharan et al., 1997; Zaidi et al., 2004) and the duration of waterlogging (Orchard and Jessop, 1984; Malik et al., 2002). Under rain-fed conditions, waterlogging or flooding can occur in paddy fields at any growth stage of the crop cycle depending on location, soil texture and water table (Polthanee, 1989, 1997). Growth, yield and sugar content of sweet sorghum can be affected by different durations of flooding at any growth stage, particularly at late growing season (Lantican, 1982; Polthanee, 1997).

Previous investigation has demonstrated that waterlogging causes anaerobic conditions in root zone that leads to root death (Patwardhan et al., 1988). In water-loving species, intercellular gas-filled spaces that often extend from the shoots to near the root tips could enhance transport of oxygen, carbon dioxide and ethylene between plant parts above water and submerged tissues (Armstrong, 1979; Laan et al., 1990). Ability of plants to produce adventitious roots and nodal roots and aerenchyma formation in roots are important characters for flooding tolerant crops (Orchard and Jessop, 1985; McDonald et al., 2001; 2002; Zaidi et al., 2003; 2004; Matsuura et al., 2005; Mano et al., 2006; Henshaw et al., 2007; Kubo et al., 2007). Glaz et al. (2004) also reported aerenchyma formation in stalk that supports internal gas space resulting in enhanced cane yield and sucrose yield in sugarcane.

Sorghum roots are able to develop nodal roots and aerenchyma formation in response to waterlogging (Pardales et al., 1991; McDonald et al., 2002) however, the differential responses of diverse genotypes has not been reported, and the information on the morphological and anatomical acclimation of roots and stalks of sweet sorghum in response to flooding has not been well-documented. The questions focused on in this investigation are: how flooding of different durations initiated at different growth stages affects growth and yield of sweet sorghum, what characters confer flooding tolerance in sweet sorghum and are there differential responses to flooding among different genotypes for yield and other traits.

The objectives of this study were to investigate the effects of flooding of different durations on the growth of shoot, yield and sugar content and root and stalk morphology of sweet sorghum and to identify characters associated with flooding tolerance in sweet sorghum. It is hoped that the findings will prove valuable for the management of pre-flood sorghum.

Materials and methods

Plant materials and experimental procedures

The greenhouse experiment was conducted under natural sunlight and photoperiods in the rainy season during May to August 2006 at the Agronomy Farm of the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Thailand. In this growing environment the average temperature was 29 ±2 °C and 90% relative humidity during the experiment. Wire mesh was installed to protect the experiment from animal pests such as rodents and birds. Plastic containers with 37.5 cm diameter and 38 cm height were filled with 30 kg of sieved air-dried sandy loam soil that was taken from a rainfed paddy field. The chemical and physical soil properties were presented in Table 1. As major elements are not sufficient, chemical compound fertilizer (15-15-15 for N-P_2O_5-K_2O) was thoroughly applied to each container at the rate equivalent to 312.5 kg ha^{-1} . The containers were moistened at field capacity prior to planting the sorghum seeds, and the moisture was maintained constant until flooding was applied to the containers. The containers were weighted daily and water was added in equal amount to weight loss.

Three varieties (Bailey, Keller and Wray) of sweet sorghum were used in this study. All varieties were kindly donated by the National Research Center for Maize and Sorghum of Kasetsart University, Thailand. The choice of the sorghum varieties was because of their adaptability to a wide range of growing conditions (Smith et al., 1987; Dalianis et al., 1994; Curt et al., 2000; Tsuchihashi and Goto, 2004). Wray and Keller cultivars were selected because of their high yield, high content of sugar, adaptability and wide purposes (Broadhead et al., 1978; Smith et al., 1987) while Bailey has been known due to its high stalk production (Guiying et al., 2003).

The seeds were sown on May 26, 2006 and 50% emergence was reached on May 29, 2006. Four to Five seedlings were initially allowed to grow in the containers and the seedlings were later thinned to one seedling per container at 7th days after emergence (DAE).

The experiment was set up using a 3 x 5 factorial in a randomized complete block design with four replications. The three sorghum varieties were assigned as factor A, and the five water treatments were assigned as factor B. These included non-flooding control, flooding from 30 DAE, flooding from 45 DAE, flooding from 60 DAE and flooding from 75 DAE. The water level of the flooding treatments was kept at 5 cm above ground until harvest, whereas the soil moisture of the control treatment was kept at field capacity (13% v/v) until harvest. Prior to flooding, soil moisture was maintained at field capacity for optimum growth. Hand weeding, pesticide and insecticides were used as necessary.

Shoot and root measurements

The following data were recorded at physiological maturity stage (Broadhead, 1969). Days to harvest of control and all flooding treatments were also recorded.

The plants were cut at soil surface and the above ground samples were separated into main stem and tillers, which the development of tiller represents as an important yield compensation mechanism and is essential for crop leaf area development which in turn affects sorghum growth and grain yield (Lafarge and Hammer, 2002). Main stems and tillers were further divided into head, culm (stem and leaf sheath) and leaf components. Plant height was measured from the soil surface to the top of the leaf during the initiation stages and to the top of the head following panicle emergence. Stem diameter at first node was measured by Vernier.

Leaf area was measured by automatic leaf area meter, ACC-400 (Hayashi-Denko co, Ltd., Tokyo, Japan). Fresh weight yield was determined from the stripped stalks (leaf and leaf sheath were removed) of the main stem, tillers were discarded due to stunted growth, which were cut at above ground level and the upper most nodes. The juice was extracted from the stalks using a crushing machine, and the total soluble solid (degree) was determined using hand digital refractometer PAL-1 (ATAGO, Japan). The juice samples were stored in a deep freezer at –20 °C until sugar analysis was performed. Sucrose content (%) and total sugar content (%, TSC) consisting of sucrose, glucose and fructose were analyzed by a high performance liquid chromatography (HPLC) system.

After recording plant height, stem diameter, leaf area, the above ground parts were mixed and oven-dried at 80 °C for 48 hours or until the weight was constant and then shoot dry weight could be determined.

Roots were separated into two parts- one part in standing water and another part in the soil, and then the roots were carefully washed in tap water using a sieve of 2 mm mesh. Number of nodal roots was recorded and total root length was measured using WinRHIZO pro V2004a (Reagent Instruments Inc., USA). The roots were oven-dried as mentioned previously for shoots, and root dry weight was recorded.

Observation of root anatomy and aerenchyma formation in axes and stalks

Root anatomy was examined in nodal root and 1st order lateral roots of plants in both water (above soil surface) and flooded soil (below soil surface) at harvest stage. Segments of nodal roots were dissected from the 2nd - 3rd flooded node from the top at 3-5 cm from the root shoot junction whilst 1st order later root was sampled at 10-20 mm from that nodal root segment. Root segments in flooded soil were dissected 2-3 cm below soil surface. Nodal root and 1st order lateral root was sampled as same as root in stagnant water. The tissues were fixed in 70 % FAA (formalin, acetic acid, 70 % ethanol; 1:1: 18 parts by volume according to Pardaless et al. (1991). Free hand cross sections were stained by toluidine blue (0.01%) and observed under an Olympus biological microscope CX3 with a 4x and 10x objective lens and digitally imaged by an Olympus microscope digital camera system DP-12. The amount of aerenchyma in the root cortex was visually scored according to Mano et al. (2006). The amount of aerenchyma in the root cortex was visually scored: 0= no aerenchyma, 0.5 = partial formation, 1= radial formation, 2= radial formation extended toward epidermis and 3= well-formed aerenchyma.

The internal portion of axes, soil covered stem, and 3rd node of the stalk above water level of each plant were visually scored for relative size of pithy area with aerenchyma formation according to Glaz et al. (2004). Briefly, the ratings of pithy area with aerenchyma formation ranged from 0 for none to 5 for an area that was equal to about 70% of the stalk diameter.

Data analysis

Data for stalk yield, juice parameters, shoot and root traits were analyzed statistically using Statistix 8 software program (Analytic software, 2003). The analysis procedures followed a factorial design (Gomez and Gomez, 1984). Sorghum cultivar and water treatment were regarded as fixed effects and replication was considered to be random effect. The significances of mean differences were determined at 0.05 and 0.01 probability levels. Least significant difference (LSD) was used to compare means.

Results

Shoot growth and yield

Days to harvest of control and all flooding treatments of Bailey and Wray ranged from 86-91 DAE and were not considered significantly different. In the Keller flooding treatments the harvesting date of flooded plant was reduced, particularly in 45 DAE flooding treatment. The reduction was 77 DAE as compared to 89 DAE in control treatment. Plants ın the 30 DAE, 50 DAE and 75DAE flooding treatments had similar harvesting dates, ranging from 81-84 DAE.

It is clear from the results that flooding at any time of 15-day interval from 30 DAE to 75 DAE reduced shoot dry weight, stalk dry weight, stalk yield and number of nodes per stalk of sweet sorghum (Table 2). However, the significant reductions were observed in only 30 DAE flooding treatment for shoot dry weight, stalk dry weight, stalk yield and in 30 DAE and 45 DAE treatments for number of nodes per stalk. On the other hand, shoot dry weight, stalk dry weight, stalk yield and number of nodes per stalk were increased by delayed flooding incidence, as can be seen in Table 2. This means that flooding at early growth stages had severe detrimental effects on growth and yield of sweet sorghum, and the effects were minimized at late growth stages, especially at near harvest.

It should be noted that flooding did not affect plant height and stem diameter of sweet sorghum, and no significant effect of flooding was found on total soluble solid (Brix), sucrose content and total sugar content. Total soluble solid was on average 16-20% and it was consistent with 16.2% for total sugar content. The percentage of sucrose on average was 11-15%. However, differences among sorghum cultivars were significant for total soluble solid, sucrose content and total sugar content. Wray showed consistently the highest total soluble solid (17.25 degree Brix), sucrose (14.89%) and TSC (17.78%).

Flooding at any time during 15-day interval from 30 DAE to 75 DAE seemed to reduce leaf area and leaf dry weight (Table 2). The reductions of 60% and 70% in leaf area and leaf weight, respectively, were significant and most severe in 75 DAE flooding treatment, whereas the reductions of 22-42% and 10-30% in leaf area and leaf weight, respectively, in 30 DAE to 60 DAE flooding treatments were much lower.

Cultivar x flooding (G x E) interactions were not significant for all characters (Table 2). Significant differences among sweet sorghum cultivars, combined data across flooding regimes and control treatment, were observed for most characters except for leaf area (Table 2). The non-significances of G x E interactions for these characters also indicated that the cultivars responded similarly to flooding treatments. Wray, in general, performed better than Bailey and Keller for most characters, whereas Bailey and Keller performed similarly for most characters except for leaf dry weight in which Bailey was significantly better.

Root growth

The plants subjected to flooding from 30 DAE had significantly higher nodal root number than the plants that received flooding from 75 DAE and the plants without flooding (control) (Table 3). The results indicated that flooding induced the newly-emerging nodal roots, and the highest number of newly-emerging nodal roots occurred at early and long-term flooding.

When roots in water and roots in flooding soil were separated, root length and root dry weight increased towards no-flooding, whereas root length and root dry weight in water, in contrast, increased towards the flooding treatment with the longest duration (30 DAE flooding treatment) (Table 3). It is clear that roots in water were the newly-emerging roots induced by flooding, and the longer flooding duration the higher newly roots developed.

Cultivars were significantly different for all root characters evaluated (Table 3). The cultivar Wray was the best performer for root dry weight in water (5.83 g). It was also a good performer for nodal root number and root length in water. The cultivar Keller performed best regarding root length in flooded soil and root length in water. It also performed well for root dry weight in flooded soil. The cultivar Bailey was the best performer for nodal root number and root dry weight in flooded soil. It also performed well for root length in flooded soil and root dry weight in water.

The G x E interactions, though significant ($P \leq 0.05$), were low for nodal root number and root length in waterlogged soil (Table 3), and there were highly significant ($P \leq 0.01$) G x E interactions for root length and root dry weight in water (Fig. 1). Keller showed the best performance for these characters when flooding was imposed from 30 DAE, whereas Bailey and Wray had the highest root length and root dry weight in water when flooding was imposed from 75 DAE.

Root anatomy

At the end of the experiment, aerenchyma was formed in the cortex layer of the nodal root (Fig. 3a) and lateral root (Fig. 3b) of both flooding treatments and control. No significant difference in aerenchyma forming in nodal root and lateral root, penetrated into flooded soil was observed. The higher aerenchyma score was observed in NR in 30 DAE flooding treatment (Table 4). However, significant differences in aerenchyma-forming capacity of both roots in water were observed among flooding treatments. In water, scored aerenchyma of NR was found highest when flooding extended from 60 DAE and 75 DAE and lowest in 45 DAE flooding treatment. In contrast, aerenchyma scores of lateral roots were significantly highest in 30 DAE flooding treatment. It was interesting to note that less aerenchyma development in NR, while greater aerenchyma spaces were observed in lateral roots.

Significant differences among cultivars were found for aerenchyma score in NR under both positions and lateral root in water. Keller had highest nodal root aerenchyma, followed by Wray under both conditions, whereas no aerenchyma was observed in NR of Bailey in both positions. Keller and Wray had significantly higher aerenchyma developed in lateral roots under water than did Bailey. Highly significant G x E interaction was found for aerenchyma formation in NR and LR under water. High aerenchyma score was recorded in NR of Keller and Wray in 75 DAE flooding treatment, but aerenchyma was not present in Bailey (Fig. 2a). Aerenchyma score in

LR was consistently higher in Keller and Wray than that in Bailey in 75 DAE flooding treatment (Fig. 2b).

In some cases, nodal roots with no aerenchyma space produced many lateral roots with well developed aerenchyma (Fig. 3c) and a unique morphology of root anatomy was found. Small lateral roots were observed in aerenchyma channels of both NR and LR in both positions, especially in LR located in water (Fig. 3d).

Aerenchyma formation of axes and stalks

Internal pith areas of both axes and stalks developed air cavity, aerenchyma-space like. Flooding at all stages significantly increased aerenchyma (Table 4). This means that flooding stimulated aerenchyma development in plants' axes and stalks of sweet sorghum. Flooding extended from 30 DAE had the highest aerenchyma score in both parts. Aerenchyma formation in the pithy area in plants' axes was higher than in stalk. Differences in cultivar were significant for both parameters. The highest axe aerenchyma was found in Wray and the lowest axe aerenchyma was observed in Bailey. Significant highest stalk aerenchyma was noted in Keller, whereas similar response was found in Bailey and Wray. G x E interaction for aerenchyma score in plants' axes was significant ($P \leq 0.05$) but quite low compared to main effects.

Discussion

Flooding imposed at any growth stage reduced leaf area, leaf DW, shoot biomass and stalk dry weight (DW) and ultimately, stalk yield. However, the reductions in these characters remarkably depended on the onset and duration of flooding; an earlier and long-term flooding could result in the more severe reductions. Similar results have also been observed by Orchard and Jessop (1984); Zhou and Lin (1995); Linkemer et al. (1998); Zaidi et al. (2004). Flooding from 30 days after planting (DAP) and prolonged until harvest significantly reduced number of leaves per plant, leaf area, leaf DW and fiber yield of kenaf, whereas flooding imposed at later growth stages (60 DAP and 90 DAP) had little effect on growth and did not significantly reduce fibre yield (Polthanee et al., 2008). In the present experiment, similar responses were found. Flooding imposed from 30 DAE (early- season) resulted in the greatest reduced stalk node per plant, stalk DW, shoot biomass and stalk yield of sweet sorghum, ranging between 5 to 22%. This compares to a 1%-10% reduction when flooding was extended from the late growth stage (50 DAE and 75 DAE).

In this study, highest reductions in leaf dry weight and leaf area were found in 75 DAE flooding treatment (late season flooding), 60% and 70%, respectively, and smaller reductions were noted in 30 DAE flooding treatment (early- season), 42% and 30%, respectively. A significant leaf area reduction of grain sorghum was also noted when flooding was applied at late growth stage, anthesis stage, with longest flooding duration (Orchard and Jessop, 1984). Flooding applied at later growth stages accelerates leaf senescence influencing by a greater root mass at later growth stages has more rapid depleted oxygen (Orchard and Jessop, 1984). The ability to sustain leaf growth under prolonged flooding conditions (extended from 30 DAE) may be facilitated by the active nodal and lateral root growth and aerenchyma formation in roots, plant's axes and stalk base. The development of adventitious or nodal roots has been previously reported to support leaf growth of waterlogging tolerant soybean (Henshaw et al., 2007) and buckwheat (Matsuura et al., 2005).

Plant height and stem diameter were not affected by flooding and all studied cultivars had similar responses. Previous studies also found that flooding did not affect these features (Orchard and Jessop, 1984; Polthanee et al., 2008). In well-flooding adapted species such as deepwater rice (Kende et al., 1998) and *Rumex palustris* (Vosenek et al., 2003), the promotion of stem extension by flooding has been observed. This improves access to aerial or dissolved oxygen or to light for the generation of photosynthetic oxygen. Swelling of stem-base or water- covered portions is also found in many di-cot species such as soybean due to the development of secondary aerenchyma, which transfer oxygen into submerged parts (Shimamura et al., 2003).

No significant relationship was found between flooding treatments and cultivars in shoot growth and yield. Under the severe flooding conditions a reduction in shoot DW and stalk yield was found (20% and 22%, respectively). This may be due to the suppression of number of node per stalk or the inefficiency of aerenchymateous nodal roots. Nevertheless, in comparison to species susceptible to waterlogging such as: wheat, which only 3 d of waterlogging resulted in 43% shoot dry mass reduction and increase waterlogging duration to 28 d, greatly reduced shoot dry mass to 72%, compared to the control (Malik et al., 2002); maize, which only 10 d of excess soil moisture content reduced grain yield up to 80% (Zaidi et al., 2004); and soybean, which grain yield was reduced up to 98% when waterlogging extended from V3 onwards (Linkemer et al., 1998), there is reason to believe that sweet sorghum is relatively flooding tolerant species.

This may possibly be an attribute of grain, as exemplified by the major yield component of maize and soybean, whereas in sweet sorghum, stalk is the major yield component. This finding indicates that sweet sorghum is suitable for per-rice production since harvested yield in the vegetative organ is less affected by waterlogging than grain yield, such as recommend by Lantican (1982).

Under complete submerged conditions, there is an absence of free oxygen in the root zone (Musgrave, 1994). To survive under long-term anoxic conditions, oxygen must be conveyed into flooded roots. Ability of plants to produce adventitious roots or NRs carrying well-developed aerenchyma spaces is identified as an important character for flooding tolerance for well-adapted flooding species such as rice (McDonald et al., 2002; Colmer, 2003) or upland crop species such as maize (Zaidi et al., 2003; 2004; Mano et al., 2006), soybean (Henshaw et al., 2007), *Trifolium* spp. (Gibberd et al., 2001) kenaf (Changdee et al., 2008) or Tribe Triticeae (Kubo et al., 2007). Root aerenchymas or porosity enhance transport of oxygen, carbon dioxide and ethylene between plant parts above water and submerged tissues (Armstrong, 1979; Laan et al., 1990; Jackson and Colmer, 2005).

In the present experiment, flooding promoted the development of nodal root (NR) or adventitious roots in all studied cultivars and number of nodal root increased with duration of flooding. This is consistent with the response of pepper weed (*Lepidium latifolium*) to flooding (Chen et al., 2002). This study also found that the newly-developed NRs formed lysigeneous aerenchyma spaces. Aerenchyma was observed both in flooded roots and roots located in water, particularly under longest flooding duration (30 DAE). This finding further found that gas spaces also developed in flooded axes and above water stalk of plants referring that under long-term flooding duration sweet sorghum could tolerate to flooding conditions by development of interconnection of gas-filled spaces from flooded roots to above water plant parts, which was first revealed in sweet sorghum, as far as we know. Justin and Armstrong (1987) reported that in water-loving species, intercellular gas-filled spaces often extend from the shoots to near the root tips.

In addition, we observed that under flooding conditions aerenchyma is formed in both NRs and lateral roots. No aerenchyma spaces were observed in NR of Bailey but many lateral roots formed aerenchyma spaces that may partially account for gas transportation and nutrient uptake under flooding conditions. The lack of casparian strip of lateral roots might play a partial role in nutrient uptake with low energy supply. In addition, based on McDonald et al. (2002), *S. bicolor* had hexagonal or cuboidal cortical cell arrangement. Well- formed aerenchyma spaces in lateral roots may partially distribute to enhanced internal aeration in nodal roots through a higher porosity of non-aerenchymateous tissues of cuboidal cortical cell packing (Justin and Armstrong, 1987). Root branching and lateral roots under waterlogging conditions is found to correlate with waterlogging tolerance in Tribe Triticeae (Kubo et al., 2007) and Trifolium species (Gibberd et al., 2001). Pardales et al. (1991) indicated that these lateral roots have a short-living duration, but we found that newly-formed lateral roots were observed throughout flooding period.

It is interesting to note that during flooding sweet sorghum developed small lateral roots in aerenchyma channel of lateral roots. Similar behavior was also reported in flooding tolerant teosinte (*Z. nicaraguensis*) (Mano et al., 2006). With observed aerenchyma spaces- like in these small lateral roots, it may contribute to flooding tolerance of sweet sorghum. Therefore, as a result of this study, aerenchyma formation in various plant parts connected from flooded parts to above water level could be considered an important trait with regard to evaluating the ability of sweet sorghum to withstanding flooding stress.

While all studied cultivars had similar shoot and yield responses under flooding stress, different flooding tolerance mechanisms were observed. It is likely that the ability to withstand flooding stress in Keller was not only an avoidance mechanism (i.e. developing nodal root forming aerenchyma spaces) but also an escape mechanism via a shortened life cycle. In the other two cultivars this was not found. Less root development at late growth stage and a relative lower stalk yield than other two cultivars may be due to the flooding tolerance mechanism of this cultivar.

Unaffected juice quality under flooding conditions of sweet sorghum may be partly attributable to root aerenchyma formation. Aerenchyma spaces of newly-developed roots may enhance oxygen transportation and maintain functions of flooded roots to support leaf growth and photosynthesis. Therefore, in sweet sorghum, sucrose synthesis and the ability to transport sucrose to sink may not be affected by flooding conditions. A similar response has also been reported in sugarcane (Hansan et al., 2003; Glaz et al., 2004; Glaz and Gillbert, 2006) and sweet sorghum under drought stress (Massacci et al., 1996) indicating that sucrose synthesis and transportation are not affected by stress. The consistent higher juice quality of Wray may also be explained by its ability to form significant higher aerenchyma in plants' axes or lateral roots as well as ability to develop roots in water. This indicates that this cultivar is preferable for growing as a pre-rice crop or cultivating in waterlogging prone areas.

Growth and yield of all studied cultivars were found to be affected by flooding. This may be attributable to severely reduced soil conditions in this study. Aerenchyma formation in roots, axes and stalks may not provide sufficient oxygen to support aerobic respiration completely. The same finding was also reported in wheat (Malik et al., 2001) and *Spartina patens* (Burdick and Mendelssohn, 1990). This may be due to the relative low internal aeration efficiency within roots *of S. bicolor* as compared to well- wet adapted *O. sativa* as reported by McDonald et al. (2002).

Nevertheless, the implication of close relatives of crop species with the presence of high internal aeration efficiency traits (strong radial oxygen loss or small stele size) such as *V. zizanoides* and *V. filipes* (McDonald et al., 2002) and high stalk yielding performed cultivars under flooding stress such as Bailey or Wray may improve breeding programs for waterlogging tolerance wherein these traits are desirable. This would also decrease lodging problems since well root aeration system can increase root penetration into waterlogged soil (Gibberd et al., 2001). Furthermore, due to G x E interactions for root aerenchyma trait, Keller and Wray , forming aerenchyma spaces in 75 DAE flooding treatment, may prove to be crucial genetic sources for improving flooding tolerance at late season flooding. Improve flooding tolerance in sweet sorghum would further diversify production areas into waterlogging prone areas or marginal lands which finally avoid land competition between food and fuel crops.

Due to heavy rains combined with typically poor surface drainage, waterlogging or flooding is the current yield constrain for pre-rice crop production during the dry-wet transition period (Zantra et al., 1982), particularly at late growth stage (Lantican, 1982). Based on our results, a flood-free period of at least 30 DAE is required to sustain yield of pre-rice sweet sorghum, and early planting is highly recommended.

This study revealed that sweet sorghum could withstand prolong flooding imposed at early growth stage by the development of nodal and lateral roots in water and interconnection of aerenchyma formation in such roots at flooded soil and water above soil surface, plant's axes and stalks above water level. When flooding was applied at late growth stage, growth and stalk yield did not significantly affect. These indicate that early planting date is a valid strategy to improve yield of sweet sorghum grown as a pre-rice crop under low-land paddy fields. The investigation of sweet sorghum responses and the suitable planting date under field conditions are needed for further practical production. However, the variation in aerenchyma formation and aerenchyma efficiency are needed to repeat and compare among these cultivars. Combining cultivars or related genotypes with ability to exhibit high root and aerenchyma development capacity and quality and high-stalk yielding cultivars may be possible for improving yield and flooding tolerance in sweet sorghum.

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Table 1 Soil physiochemical characteristics of the experiment.

Soil characteristics	Values
Chemical properties	
pH[1]	4.95
Organic matter (g 100 g^{-1})[2]	0.47
Total N (g 100 g^{-1})[3]	0.03
Extractable P (ppm)[4]	3.00
Exchangeable K (ppm)[5]	27.00
Physical properties	
Clay (%)	7.50
Silt (%)	30.00
Sand (%)	62.50
Soil texture[6]	Sandy loam
Soil classification	Ultisols

[1]pH: pH meter (1:1 H_2O), [2]O.M.: Walkley and Black method, [3]Total N: Kjeldahl method, [4]Extractable P: Bray II and Molybdenum-blue method, [5]Exchangeble K: 1N NH4OAc pH7 and Flame photometry method, [6]Texture: Hydrometer method.

Table 2 Effects of flooding of different durations and cultivars on shoot dry weight, stalk dry weight, stalk yield, number of nodes per stalk, leaf area and leaf dry weight of sweet sorghum evaluated at final harvest.

	Shoot DW ($g\ plant^{-1}$)	Stalk DW ($g\ plant^{-1}$)	Stalk yield ($g\ plant^{-1}$)	No. of node per stalk	LA ($m^2\ plant^{-1}$)	Leaf DW ($g\ plant^{-1}$)
Flooding initiation (F)						
30 DAE	109.9(-20)	105.1(-20)	319.2(-22)	14.50(-5)	0.07(-30)	4.13(-42)
45 DAE	124.9(-8)	119.4(-9)	362.9(-12)	14.42(-5)	0.09(-10)	5.52(-22)
60 DAE	123.9(-10)	119.8(-9)	370.4(-10)	15.08(-1)	0.07(-30)	4.27(-40)
75 DAE	130.7(-5)	128.4(-2)	402.5(-2)	15.17(-1)	0.03(-70)	2.84(-60)
No flooding	137.0	130.7	412.5	15.25	0.10	7.12
LSD	16.8**	16.2**	54.1**	0.69*	0.04*	2.09**
Cultivars (C)						
Bailey	123.6	117.8	346.0	15.10	0.08	5.82
Keller	113.6	111.0	353.2	14.10	0.06	2.57
Wray	138.8	132.8	421.2	15.35	0.08	5.95
LSD	13.0**	12.6**	41.9**	0.71**	0.03ns	1.62**
F x C						
LSD	21.7ns	21.0ns	70.1ns	1.19ns	0.07ns	3.84ns

*,** Significant at $P \leq 0.05$ and 0.01 levels, respectively and ns not significant.

DAE=days after emergence.

LA= leaf area, DW= dry weight.

Number in parenthesis indicates percent increase (+) or decrease (-) of the treatment as compared to the control.

Table 3 Effects of flooding of different durations and cultivars on nodal root number per plant, root length in flooded soil, root length in water, root dry weight in flooded soil and root dry weight in water of sweet sorghum evaluated at final harvest.

	Nodal root number per plant	Root length (mm plant^{-1})		Root DW (g plant^{-1})	
		Soil	Water	Soil	Water
Flooding initiation (F)					
30 DAE	65.75(+25)	159064(-77)	243321	10.61(-67)	12.11
45 DAE	60.42(+15)	554568(-19)	85737	20.34(-37)	6.38
60 DAE	62.50(+19)	800909(+17)	39772	30.46(-6)	3.31
75 DAE	54.58(+4)	749156(+10)	4279	30.70(-5)	1.48
No flooding	52.50	681495	NA	32.45	NA
LSD	10.75**	149719**	49692**	4.35**	0.43**
Cultivars (C)					
Bailey	65.30	644064	58737	27.67	4.77
Keller	50.40	684329	134934	25.76	3.38
Wray	61.75	438722	86162	21.31	5.83
LSD	8.32**	115972**	43034**	3.37**	0.33**
F x C					
LSD	13.92*	193955*	86069**	5.64ns	0.62**

** Significant at $P \leq 0.05$ and 0.01 levels, respectively and ns not significant.

DAE=days after emergence.

NA=datum is not available for non-flooding control and was deleted from analysis of variance, DW= dry weight.

Number in parenthesis indicates percent increase (+) or decrease (-) of the treatment as compared to the control.

Table 4 Effects of flooding of different durations and cultivars on aerenchyma formation score of sweet sorghum measured in nodal roots, lateral roots, axes and stalks evaluated at final harvest.

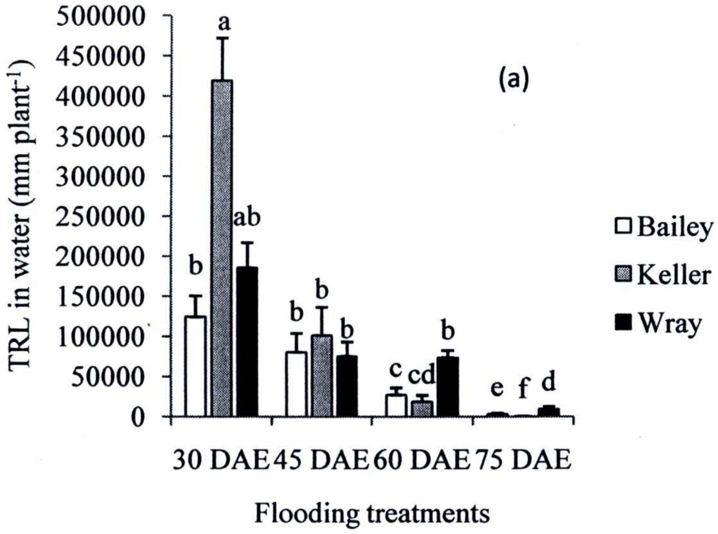
	Nodal root		Lateral root		Axe	Stalk
	Soil	Water	Soil	Water		
Flooding initiation (F)						
30 DAE	0.67(+131)	0.54	2.92(+1)	3.00	3.58(+186)	2.92(+403)
45 DAE	0.54(+86)	0.42	2.75(-4)	2.92	3.58(+186)	2.67(+360)
60 DAE	0.21(-28)	1.50	3.00(+4)	3.00	3.17(+154)	1.77(+205)
75 DAE	0.50(+72)	1.75	2.89(0)	2.61	2.08(+66)	1.50(+159)
No flooding	0.29	NA	2.89	NA	1.25	0.58
LSD	0.19ns	0.32**	0.07ns	0.10*	0.24**	0.44**
Cultivars (C)						
Bailey	0.00	0.00	2.84	2.64	2.30	1.60
Keller	0.90	1.59	2.84	3.00	2.90	2.40
Wray	0.43	1.56	3.00	3.00	3.00	1.60
LSD	0.19**	0.28**	0.06ns	0.11**	0.19**	0.26*
F x C						
LSD	0.72ns	1.34**	0.43ns	0.64**	0.99*	1.58ns

*,**Significant at P≤0.05 and 0.01 levels, respectively and ns not significant.

DAE= days after emergence.

NA=datum is not available for non-flooding control and was deleted from analysis of variance.

Number in parenthesis indicates percent increase (+) or decrease (-) of the treatment as compared to the control.



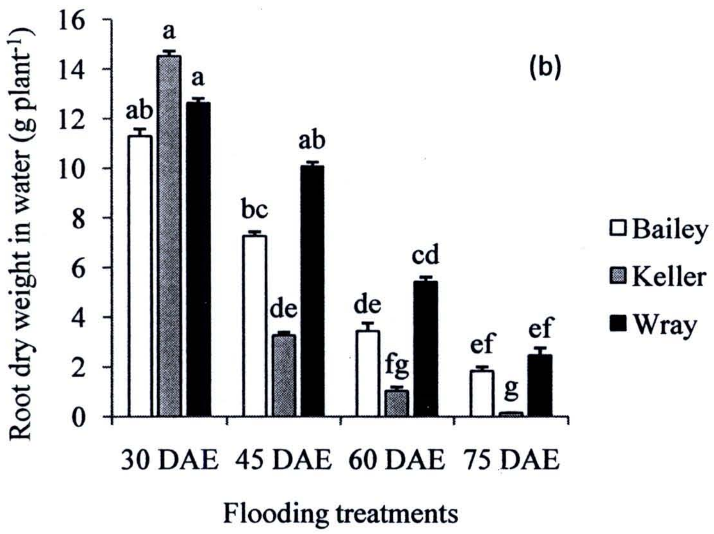


Figure 1 Effect of flooding treatments on total root length (TRL) (a) and root dry weight (b) of three sweet sorghum cultivars in water. Keller did not produced root at late growing season flooding. Treatment means followed by the same letter are not significantly different at $P \leq 0.01$ level. DAE= days after emergence. Bars represent S.E.

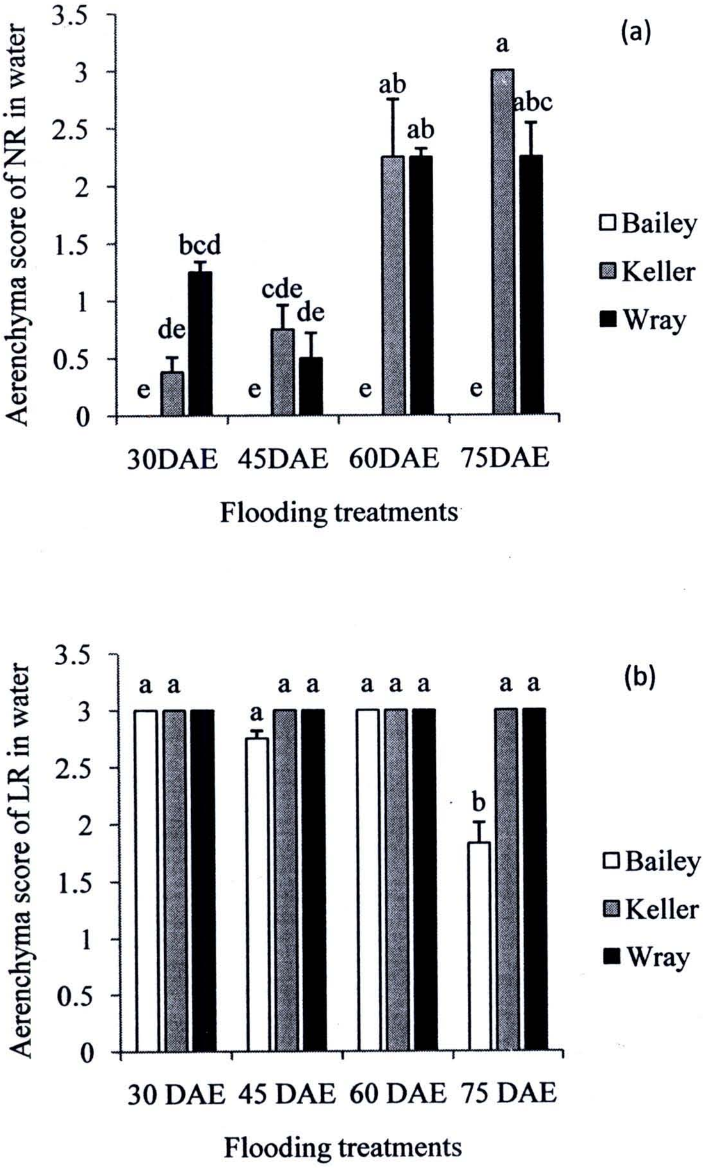
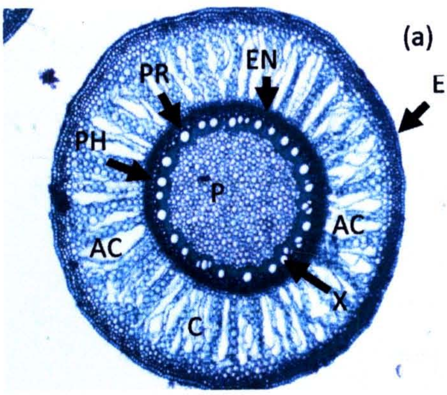
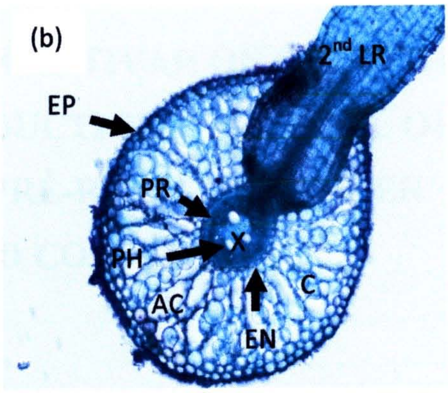


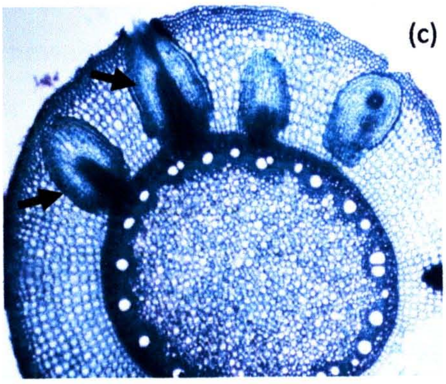
Figure 2 Effect of flooding treatments on aerecnhyma score of nodal root (a) and lateral root (b) located in water of three sweet sorghum cultivars. No aerenchyma space was observed in Bailey in all flooding treatments. Treatment means followed by the same letter are not significantly different at P≤0.01 level. DAE= days after emergence. Bars represent S.E.



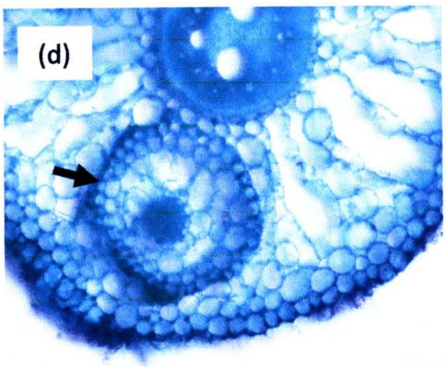
Nodal root of Keller in 60 DAE flooding treatment



Lateral root of Wray in 45 DAE flooding treatment



Nodal root of Bailey in 30 DAE flooding treatment



Lateral root of Wray in 45 DAE flooding treatment

Figure 3 Cross sections of adventitious root or nodal root and lateral root developed in water. (a) well- developed aerenchyma formed in nodal root, bar = 200 μm, (b) well- developed aerenchyma formed in 1st lateral root, bar = 39.9 μm (c) many first order lateral roots formed on nodal root (arrows), bar = 200 μm and (d) small later root developed in aerenchyma channels of later root (arrow), bar = 39.9 μm, EP, epidermis; AC, aerenchyma; EN, endodermis, PR, pericycle; PH, phloem; X, xylem; P, pith and 2nd LR, secondary lateral root. DAE= days after emergence.