CHAPTER IV

GROWTH, YIELD AND AERENCHYMA FORMATION OF TWO SORGHUM CULTIVARS *(Sorghum bicolor* L. Moench) SUBJECTED TO FLOODING AT DIFFERENT GROWTH STAGES

Introduction

In Thailand, ethonal use is being promoted in alignment with government objectives of reducing reliance on imported oil to the resulting reducing greenhouse gasses resulting from petroleum use. By 2022, ethanol production is projected to increase to 3.3 billion liters (DEDE, 2009a). However, current feedstock materials which are widely used such as cassava or limited materials such as sugarcane molasses (DEDE, 2009b) may not provide sufficient supply for future bioethanol demand. Subsidizing these materials with sorghum could strengthen the industry efficiency and ethanol production sufficiency security within the country.

Sorghum (*Sorghum bicolor* L. Moench) has aroused the interest of researchers throughout the world for its remarkable yield potential, even in marginal environments (Mariani et al., 1989) and its multipurpose potential for several non-food uses. It provides grain and stems for a feedstock for sugar, alcohol, syrup, fuel and paper production and as for animal feeding (Doggett, 1988). Current energy situation, sorghum is now being developed as a potential bioenergy crop. Sweet sorghum, grain sorghum and fibre or new-specific high biomass cellulose sorghum has been proved and projected as a dedicated bioenergy feedstock (Rooney, et al., 2007).

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The optimum types of sorghum to be grown for biofuel production mainly depend on the types of conversion process that will be used. Thus each type fits to different production systems. Energy production from sorghum has already been proven to economically attractive and has high energy efficiency (Dolciotti et al., 1998; Reddy et al., 2005; Sakellariou-Makrantonaki et al., 2007). It is environmentally safe (Barbanti et al., 2006) and impacts green house gas balance (FAO, 2008). However, when evaluating growing an energy crop one must keep in mind its competition with food crops. In Thailand, most arable land is occupied by staple crops, primarily rice, of which most farmers grow only a single crop annually in the mid-rainy season. The introduction of sorghum as a pre-rice crop has the potential to meet increasing demand for biomass feedstock without negatively impacting food crop production. This could directly increase farmers' incomes.

Nevertheless, due to land management practices and variable precipitation, flooding is a major constraint on crop growth and yield in pre-rice crop management (Polthanee, 1997). The ability to maintain production of sorghum under waterlogged conditions is necessary, but its response to flooding is not yet well understood. The evaluation of the effects of flooding on plant growth and yield parameters is an important factor in choosing the most suitable location for each variety.

Flooding causes a series of physiological, chemical and biological changes in soil (Zaidi et al., 2003). It also inhibits root and shoot growth, changes water and nutrient uptake and alters physiological properties (Zhuo and Lin, 1995; Ahmed et al., 2002; Pang et al., 2004). Ultimately, it reduces plant yield (Orchard and Jessop, 1984; Umaharan et al., 1997; Zaidi et al., 2003; 2004). The adverse effects of flooding on plant growth and yield mostly depend on the growth stage of plants before flooding commences (Orchard and Jessop, 1984; Zhuo and Lin, 1995; Zaidi et al., 2004) and plant species or genotype (Orchard and Jessop, 1984; Umaharan et al., 1997; Pang et al., 2004; Zaidi et al., 2004).

Formation of aerenchyma in root cortex (McDonald et al., 2002; Zaidi et al., 2003; Pang et al., 2004), stalk (Glaz et al., 2004; Gilbert et al., 2007) and adventitious root formation (Pardales et al., 1991; Zaidi et al., 2004) have been widely accepted as adaptive/acclimation responses to flooding stress and they have been found to be closely related to shoot growth and yield (Zaidi et al., 2004). Evaluation of flooding acclimation traits in both sorghum types could provide useful information for a flooding tolerance breeding program.

Growth, yield and root response to waterlogging has been previously observed in grain sorghum (Orchard and Jessop, 1984; Orchard et al., 1985; Pardales et al., 1991; McDonald et al., 2002). However, little is known about the response to flooding stress of sweet and multipurpose sorghum and comparative studies between both sorghum types under anoxic conditions are rare. Therefore, the aim of this study was to investigate and compare the effect of flooding on plant growth and yield between sweet and multipurpose sorghum at each growth stage. The focus in the evaluation is on the sensitivity of plants and on the identification of characteristics which may result in resistance to the aforementioned stresses with the perspective for specific variety selection and management under pre-rice production conditions as well as providing the possible useful flooding tolerance indicator(s).

Materials and methods

Plant materials and culture

The experiment was conducted from May to August 2005 under greenhouse conditions at the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Thailand, under natural sunlight and photoperiods. Pots (38 cm height, 37.5 cm wide) were arranged 15 cm apart. Each pot was filled with 30 kg of sieved air-dried loamy soil (taken from irrigated paddy fields in Thailand) containing 2.18% organic matter, 5.05 ppm of available phosphorus and 159.5 ppm extractable potassium. Fertilizer was thoroughly mixed in the soil of each pot to provide the equivalent of 47 kg ha^{-1} of N, P_2O_5 and K_2O. Each pot was then watered to approximate field capacity. This moisture content was maintained by weighing the pots every other day using a scale platform balance.

Into each pot, seeds of *S. bicolor* cv. Wray, sweet cultivar and cv. Supanburi 1, SP1, multipurpose cultivar, were sown on May 15, 2005. Seedlings emerged rapidly reaching 50% emergence on May 17, 2005. Four to five seedlings were initially planted per pot but these were thinned to one plant per pot at 7 days after emergence (DAE). Cv. Wray is reported to be a superior source of sugars along with good agronomic features (Ferraris, 1981), while multipurpose sorghum cv. Supanburi 1 (SP1) is grown for its grain and its stem for animal feeding. In addition, with its high sugar content stem, compatibility to the Thai growing environment and its relative waterlogging tolerance (Supanburi Field Crop Research Center, Thailand), this cultivar could suitable for waterlogging prone areas.

Experimental treatments

The experimental treatments consisting of 4 flooding treatments and 2 cultivars were arranged in a factorial experiment in a randomized complete block design with 4 replications. The 20 d of flooding treatments were applied at (i) early-vegetative stage, 10 DAE (Vanderlip and Reeves, 1972) (EV) (ii) early- reproductive stage, 30 DAE (ER) and (iii) mid- reproductive stage, 50 DAE (MR). During flooding periods, water level was raised to 3 cm above soil surface by adding water daily in to each pot. At the termination of each treatment, the pots were drained by making the hole at the bottom of the pot. In the control treatment (CK), soil moisture was kept at field capacity during the whole growth period. Pesticide and insecticides were used as needed.

Shoot and root growth analysis

Each treatment has three sets for three occasions of samplings. The first sampling was taken one day before flooding. The second sampling was taken at the end of each flooding treatment and the third samples were taken after plant recovery from flooding at 80 DAE (i.e. 50, 30 and 10 days after the end of flooding in the three respective treatments).

Plants were cut at soil surface and samples were separated into main stem and tillers. These 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 early reproductive stages and to the top of the head following panicle emergence. Stem diameter at first node was measured.

Leaf area was measured by automatic leaf area meter, ACC-400 (Hayashi-Denko co, Ltd., Tokyo, Japan). Shoot material was dried at 80 °C for 48 hours to determine dry weight.

After the shoots were harvested, roots were carefully washed from the soil over a sieve (mesh, 2 mm). The nodal root (NR) number per plant was counted. Roots were divided into those arising above soil level (standing water) and below soil level (flooded soil). Total root length from each pot was measured using WinRHIZO pro V2004a (Reagent Instruments Inc., USA). Roots were dried at 80 °C for 48 hours to determine dry weight.

Aerenchyma observation

Twenty days after flooding for each treatment root anatomy was examined in nodal and 1st order lateral roots of flooded plants in both water above soil surface and soil below ground surface. Roots of control plants were similarly examined. Segments of nodal roots were dissected from the 2nd - 3rd flooded node from the top at 3-5 cm long from the root-shoot junction, whilst the 1st order later root was sampled at 10-20 mm from that nodal root segment. Root segments in waterlogged soil were dissected 2-3 cm below soil surface. Nodal root and 1st order lateral root were sampled the 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-section of the sections stained by toluidine blue (0.01%) were observed using 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 using: 0= no aerenchyma, 0.5 = partial formation, 1= radial formation, 2= radial formation extended toward epidermis and 3= well-formed aerenchyma. In addition, at harvest, stalk bases of the first node of plants were transverse cross-sectioned to observe air space or stalk aerenchyma in its pithy area.

Yield and Quality analysis

At final harvest the stalk of sorghum (above soil surface to the uppermost internodes) was stripped and weighed as the yield of plant. Following that, juice was extracted using crushing equipment and the Brix value (soluble solid, %) of the juice was measured by hand digital refractometer PAL-1 (ATAGO, Japan).

Data analysis

Analyses of variance for all collected data were performed using Statistix 8 software (Analytical software, Tallassee, USA). To analysis significant differences of treatment effects the data set was partitioned into separated growth stage of each flooding treatment and harvest stage (recovery) with replications as random effects and cultivar and flooding as fixed effects. Least significant difference test was used to evaluate the differences between the treatment means.

Results

Shoot growth response

Plant height was significantly reduced to 35% and 22% as compared to the control when 20 d of flooding was applied at early-vegetative stage (EV) and early-reproductive stage (ER) but plant height was not significantly different from control when flooded at mid-reproductive stage (MR) (5% of the control) (Table 1). There was no significant difference between cultivars when flooded at EV but significant differences were found at ER and MR (Table 1). The significant interaction of flooding x cultivar for height was noted when flooding was applied at MR. Cv. Wray under non-flooded and flooded conditions had similar height, while height was increase over control in cv. SP1 (Fig. 1a). At final sampling, the significant effects of flooding treatments, cultivars were found. Plants subjected to flooding at EV had lowest high, while plants subjected to flooding at ER and MR could recover to control levels (Table 2).There were significant flooding x cultivar interactions. Cv. SP1 flooded at ER had the lowest plant height (also lower than its respective control), while height was increased over its respective control in cv. Wray (Fig. 1a).

Stem diameter was significantly reduced by flooding at EV (83%) but not at ER and MR. Cultivar effects were significant when water was applied at EV and ER but not at MR (Table 1). The significant flooding x cultivar interactions were found only when flooding was applied at EV and ER (Table 1). Cv. SP1 flooded at EV had lowest stem diameter (Fig. 1b). At final sampling, adverse effects on stem diameter by flooding at EV persisted, resulting in a 38% reduction compared to the control but sorghum flooded at ER and MR had similar stem diameter to the control. There were significant cultivar effects and flooding x cultivar interactions for stem diameter (Table 2). Damages from flooding at EV of cv. SP1 could not be reduced, whereas flooding at MR in cv. Wray increased stem diameter over the control (Fig.1b).

LA and leaf dry weight were significantly lowest when flooding was applied at EV (80% and 81% reduction, respectively), while flooding at ER these reductions decreased (24% and 23%, respectively). Flooding at MR resulted in similar LA and leaf dry weight to the control (Table 1). Both cultivars had similar LA and leaf dry weight when flooded at EV but cv. Wray had significant higher leaf traits than cv. SP1 when flooded at ER and MR (Table 1). The significant flooding x cultivar interactions were noted for LA when flooding applied at EV. Cv. SP1 had highest LA in non-flooding treatment and lowest in flooding treatment (Fig. 2a). At recovery period, there was no significant difference among flooding treatments for LA. Significant cultivar effects and flooding x cultivar interactions were found in both leaf traits (Table 2). Leaf traits of cv. Wray flooded at MR were similar to its respective control. Cv. Wray flooded at EV and ER maintained their leaf traits higher than cv. SP1. LA (Fig. 2a) and leaf dry weight (Fig. 2b) were significantly lowest in cv. SP1 in all treatments (Fig. 2a and b).

Shoot dry weight was significantly lowest when flooding at EV (83%), followed at ER (22%) but shoot dry weight was similar to the control when flooded at MR (Table 1). Both cultivars had similar response when subjected to flooding at EV. But cv. Wray had significantly higher shoot dry weight than cv. SP1 when flooded at ER and MR (Table 1). Nevertheless, there was no significant flooding x cultivar interaction.

At harvest, plants flooded at EV and ER could not completely recover from flooding damages, giving 53% and 37% lower shoot dry weight than the control, respectively, however, plants flooded at MR had no significantly different shoot dry weight from the control. Cv. Wray showed significant higher shoot dry weight than cv. SP1 (Table 2). There was no significant flooding x cultivar interaction in shoot dry weight (Table 2 and Fig. 2c).

Primary root growth response

Twenty days of flooding significantly reduced root length and root dry weight in soil or primary root growth when sorghum was subjected to flooding at EV and ER.

Root dry weight and root length were more severe reduced when flooded at EV, 88% and 74%, respectively and the reduction was decreased to 69% and 58% at ER. Flooding significantly reduced root length (89%) only when plants were subjected to flooding at MR (Table 1). There were significant cultivar effects on root traits when plants subjected to flooding at EV and ER as well as for root length when flood applied at MR. Significant flooding x cultivar interactions were noted for root dry weight and root length when flooding was applied at EV and ER as well as for root length at MR (Table 1). Root length and root dry weight were highest in non-flooded SP1 at both growth stages, while flooded SP1 showed lowest root traits. Root length in mid-reproductive flooding treatment also showed similar response to previous growth stage (Fig. 3a).

At recovery, plants flooded at EV increased root length to plants flooded at MR, while sorghum flooded at ER had similar root length as the control. Nevertheless, root dry weight of sorghum flooded at EV could not completely recover, while there was no significant difference from the control in other treatments (Table 3). Both cultivars had similar root length but significant higher root dry weight was found in cv. Wray (Table 3). There were no significant flooding x cultivar interactions for root length (Fig. 3a) and root dry weight (Fig. 3b).

Root developed in water

Flooding at ER and MR significantly increased newly-nodal root number to 7% and 43%, respectively but not at EV (Table 1). Both cultivars had similar nodal root development when flooded at EV and ER but cv. Wray produced significantly higher nodal root number than cv. SP1 at MR (Table 1). There was no significant flooding x cultivar interaction for nodal root number (Table 1 and Fig. 3c). At recovery, plants subjected to flooding at EV had significantly lowest nodal root number, while in other treatments had similar root number to the control. No cultivar effect and flooding x cultivar interaction were noted (Table 3).

During the flooding period, growth of newly-nodal roots and lateral roots are presented in terms of root length and root dry weight (Table 4). Plants subjected to flooding at ER had significant highest root traits, followed by MR and lowest at EV (Table 4). Cv. Wray showed significantly higher root traits than cv. SP1. Significant flooding x cultivar interactions were found for both root traits. Cv. Wray flooded at ER had the highest root length and root dry weight while flooded cv. Wray at EV showed the lowest root length (Fig. 4a). Cv. SP1 had the lowest root dry weight (Fig. 4b).

Aerenchyma formation

In flooding treatments, nodal and lateral roots of both cultivars developed in water above the soil surface and the parts that penetrated into the soil formed aerenchyma spaces. These aerenchyma spaces were identified as lysigeneous aerenchyma, gas spaces occurred due to the breakdown of the cell wall in cortex layers (Fig. 5). The aerenchyma spaces were also observed in roots of control plant but in small amounts (data not shown). Roots developed in water formed higher aerenchyma than that penetrated into flooded soil. Nodal roots of plants flooded at EV had significantly highest aerenchyma scores, with a similar degree of development observed between the other two treatments (Table 4). Nodal root of cv. Wray, penetrated into flooded soil and lateral root floating in water developed significant higher aerenchyma than cv. SP1 (Table 4). The significant flooding x cultivar interaction was noted; flooding at EV giving the highest aerenchyma scores in both cultivars as well as flooding at MR of cv. SP1 while cv. Wray flooded at MR had the lowest aerenchyma score (Fig. 4c). In addition, cross- sections of sorghum stalk bases also showed the air spaces or stalk aerenchyma in the pithy area of both control and floodedplants. The highest stalk aerenchyma was observed in plants subjected to flooding at MR, followed by ER and lowest in EV. Cv. Wray developed relative higher stalk aerenchyma than cv. SP1 (data not shown).

Stalk yield and Brix value responses

Stalk yield of plants subjected to flooding at MR was almost the same as in the control. However, sorghum subjected to flooding at EV produced significantly lowest stalk yield, 48% reduction, followed by flooding at ER, 32% reduction. Cv. Wray produced higher stalk yield (517.06 g plant^{-1}) than cv. SP1 (247.03 g plant^{-1}) (Table 2). The significant flooding x cultivar interaction was noted for stalk yield. Cv. SP1 flooded at EV had the lowest stalk yield. Nevertheless, as compared to its respective control, percent reduction was similar between both sorghum types (46% and 50% lower than the control in cv. SP1 and cv. Wray, respectively). When flooding was applied at ER, stalk yield was 2X higher in cv. Wray than cv. SP1, but the similar reduction was found (31% and 32% for cv. SP1 and Wray, respectively). Even though flooding at MR in both cultivars gave similar stalk yield as the control; stalk yield was significant lower in cv. SP1 than cv. Wray both in terms of absolute values or reduction percentage (13% and 5%, respectively) (Table 5). This indicates that both sorghum types response similarly when flooding at EV and ER but multipurpose sorghum is more susceptible to flooding at reproductive stage than sweet sorghum.

Flooding had no significant effect on Brix value (total soluble solids in juice). Cv. Wray had higher brix value (17%) than cv. SP1 (14%). No significant flooding x cultivar interaction was noted for Brix value (Data not shown).

Discussion

Our results indicate that 20 d of flooding applied at early vegetative stage, severely reduced primary root and shoot growths, followed by early-reproductive stage , while it had no significant effect at late growth stage i.e. mid-reproductive stage. These findings confirmed the previous studies that adverse effects of flooding on crop growth and yield depends on crop growth stage (Orchard and Jessop, 1984; Umaharan et al., 1997; Linkemer et al., 1998). Furthermore, early-growth stage is the most susceptible growth stage and crop susceptibility decreases gradually at later growth stages (Zhou and Lin, 1995; Zaidi et al., 2004). In this study, the high susceptibility to flooding at EV may be partly due to the restricted nodal root development concomitant with the finding in maize (Zaidi et al., 2004).

In the present study, few occurrences of flooding x cultivar interactions for shoot traits at the end of each flooding treatment indicate that both sorghum types had similar shoot growth suppression. Regarding previous reports of the relative flooding tolerance of cv. SP1, mentioned earlier and, and based on photosynthetic capacity (Promkhambut et al., 2010a), prolong flooding in this experiment lead to a greater degree of root anoxia and may result in no different cultivar performance, particularly shoot growth.

In sugarcane, Gilbert et al. (2007) also reported similar results. However significant interactions for all shoot traits, except shoot and stalk biomass at harvest stage, indicate that sweet and multipurpose sorghum differ in height, stem diameter, leaf area and leaf dry weight recovery. In general, with similar growth reduction at the end of flooding at each growth stage, cv. Wray had higher shoot traits than cv. SP1 at harvest. It is noted that flooded cv. Wray at late growth stage had the ability to increase plant height and stem diameter over the control but cv. SP1 did not. This may partly result in less stalk yield reduction when flooding was applied at MR.

In wheat (Malik et al., 2001) and barley (Pang et al., 2004) waterlogging has higher adverse effects on root growth than shoot growth. This is similar to our findings. The significant flooding x cultivar interactions in root dry weight and root length indicating that there is variation in root responses between sweet and multipurpose sorghums. In general cv. SP1 had higher root growth under non-flooded conditions indicating that it had deeper root growth. Therefore, when subjected to flooding, root growth reduction was higher than cv. Wray, particularly when subjected to flooding at mid-reproductive stage. Higher root length reduction of cv. SP1 when flooded at mid-reproductive stage while exhibiting no significant root dry weight from control may be explained by the death of fine roots at deeper soil layer, let alone bigger root at soil surface. MacFarlane et al. (2003) also found similar response in ryegrass. Pardales et al. (1991) indicated that the dieback of older roots in sorghum is concomitant with new root development in the upper node of the stalk. This shows the plasticity acclimation of sorghum to flooding. Nevertheless, this finding indicates that high root growth sorghum may not be suitable for production in waterlogging prone areas.

Matsuura et al. (2005) suggested that adventitious root development during waterlogging replaces the function of dieback primary roots in waterlogged soil and plays a crucial role in supporting water and nutrient uptake in waterlogging tolerant buckwheat. These adventitious roots of flooding tolerant plants develop aerenchyma or air porosity that functions as an alternative source of oxygen supply under anaerobic conditions (McDonald et al., 2002). Pardales et al. (1991) also reported that in sorghum nodal root development is a crucial trait for waterlogging tolerance. In sweet sorghum, Promkhambut et al. (2010b) also found that nodal root number increases with duration of flooding and plays the important role of sustaining leaf growth in flooded plants. In this study, the similar trend response of nodal root number and shoot and yield support previous studies. The significantly highest nodal root number found at MR but lower root length indicates that the length of these new roots was restricted. This is similar to the report of Pardales et al. (1991).

In addition, the observed aerenchyma development in these roots indicates that these roots could function under flooding conditions. Orchard et al. (1985) indicated that in grain sorghum root aerenchyma development is an important trait giving higher waterlogging tolerance than sunflower. The higher observed aerenchyma space at vegetative stage than late growth stage is consistent with that found in grain sorghum reported by Orchard et al. (1985). Overall, aerenchyma scores to root cross-section area in cv. Wray were significantly larger than that in cv. SP1. There have also been reports of the positive correlation between the percentage of aerenchyma in adventitious root and shoot growth (Huang et al., 1994) and yield (Setter and Waters, 2003) of wheat as well as higher root porosity and yield in maize (Zaidi et al., 2004).

Based on this concept, cv. Wray may possess relatively higher flooding tolerance than cv. SP1. Nevertheless a high quantity of aerenchyma may be a less important flooding tolerant trait if radial oxygen losses from the root (Setter and Waters, 2003). The significantly higher score aerenchyma in cv. SP1 flooded at mid-reproductive stage but higher stalk yield reduction may support this point of view. McDonald et al. (2002) reported low radial oxygen loss in sorghum but there is no variety comparison. Further research is needed to compare the ability to form barrier(s) to radial oxygen loss among various sorghum genotypes. This would help to identify the genotypic possessing high flooding tolerance in sorghum.

No significant flooding x cultivar interaction for nodal root number was found both at the end of flooding treatments and at final sampling. This could imply that nodal root is the general flooding root acclimation trait for sorghum, particularly under prolong flooding. Nevertheless, relatively higher nodal root development of cv. Wray than cv. SP1 when flooding was applied at mid-reproductive stage may result in the significant lower stalk yield reduction at harvest. However, based on our results, nodal rooting may not be a useful tool to identify genotypic flooding tolerance in sorghum. In the case of upland crop species with relatively high flooding tolerance or subjected to long-term flooding such as sugarcane, adventitious root development may also not be a flooding tolerant screening tool (Gilbert et al., 2007).

Gilbert et al. (2007) indicated that stalk aerenchyma is the important indicator in differentiate genotypic flooding tolerance in sugarcane. They found that cultivar with constitutive stalk aerenchyma had higher flooding tolerance. The observed higher aerenchyma development in stalk bases of cv. Wray than cv. SP1 may be one of the important flooding tolerant traits in sweet sorghum. However, in this experiment the study in this trait was only preliminary and used only two cultivars. Further researches should concentrate on this particular trait with a larger number of genotypes.

Significantly different flooding x cultivar interactions for root development in water indicate that growth stage of plant is important for the study on root acclimation to flooding. Significant cultivar differences when flooding was applied at early-reproductive stage but not at early-vegetative stage and mid-reproductive stage indicate that morphological root acclimation traits may not be useful flooding tolerant indicators at too early and late growth stage. Umaharan et al. (1997) reported that cowpea waterlogged at reproductive stage lost its root acclimation ability and they mentioned that ability to maintain leaf area and leaf growth are more important. With respect to this concept lower leaf area and leaf dry weight reduction than the control of cv. Wray than cv. SP1 when flooding was applied at mid-reproductive stage may imply that sweet sorghum is more tolerant to flooding at late growth stage than multipurpose sorghum. This may be one of the factors supporting lower stalk yield reduction at late growth stage of sweet sorghum. Nevertheless, the relative higher grain of cv. SP1 may compete photosynthates with stalk when flooding was applied at mid-reproductive. Under pre-rice crop production conditions, flooding often occurs at mid-rainy season due to rainfall intensity and rising water table level. The early- rain in the rainy season could be successfully used to grow sweet sorghum by early planting date selection to avoid the early growth stage to meet flooding incidence. Therefore, sweet sorghum is preferable for this cropping system over multipurpose sorghum.

No significant difference in juice quality as indicated by Brix value between flooding and control plants in both sorghum types indicating that sucrose synthesis and allocation during flooding is unaffected by flooding. This is similar to the response of sweet sorghum to drought stress (Massacci et al., 1996) and sugarcane response to flooding (Gilbert et al., 2007).

In conclusion, both sorghum types are susceptible to flooding at early-vegetative stage and the susceptibility is decreased at late growth stage. Nodal root development plays a crucial role for flooding tolerance in both sorghum types. However, this root trait may not be a useful trait to identify genotypic flooding variation under severe flooding or relative flooding tolerant genotype. Aerenchyma development in roots and stalk may be more important traits. Cv. Wray showed significantly higher flooding tolerance based on stalk fresh weight than cv. SP1 when flooding was applied at late growth stage. It also had the capacity of the recovery of plant height and stem diameter, relative higher nodal root number and aerenchyma formation in root during flooding and observed higher stalk aerenchyma. This indicates that cv. Wray is more suitable for pre-rice crop production conditions. In addition, with 2X higher stalk yield in sweet sorghum compared to multipurpose sorghum, sweet sorghum production in paddy field is recommended. In addition, early sowing to avoid growth stages that are susceptible to flooding is the most economical solution for energy crop production in paddy fields.

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Table 1 ANOVA table of individual flooding treatment at different growth stages and cultivars of shoot and root growth.

Treatments	SDW	Height	SD	LA	LDW	RDW	RL	Root no.
EV (F)								
Control	23.89a	139.88a	1.52a	0.45a	12.93a	2.92a	167.00a	25.50
Flooding	4.15b	91.38b	0.26b	0.09b	2.40b	0.36b	42.84b	23.00
F-test	**	**	**	**	**	**	**	ns
Cultivar (C)								
Wray	13.54	119.25	1.26a	0.24	7.11	1.14b	56.56b	22.88
SP1	14.50	112.00	0.50b	0.30	8.21	2.13a	153.27a	25.63
F-test	ns	ns	**	ns	ns	**	**	ns
F x C								
F-test	ns	ns	*	*	ns	**	**	ns
ER (F)								
Control	111.76a	249.75a	1.93	0.59a	28.43a	13.58a	403.91a	45.38b
Flooding	87.54b	195.38b	1.80	0.45b	22.00b	4.24b	171.44b	72.38a
F-test	**	**	ns	**	**	**	**	**
Cultivar (C)								
Wray	105.93a	247.00a	2.20a	0.58a	27.42a	7.85b	255.71b	59.13
SP1	93.36b	198.13b	1.80b	0.46b	23.01b	9.97a	319.64a	58.63
F-test	*	**	**	**	*	*	*	ns
F x C								
F-test	ns	ns	ns	ns	ns	*	**	ns
MR (F)								
Control	184.56	254.25	2.01	0.56	28.35	17.90	566.06a	48.34b
Flooding	177.67	267.75	1.97	0.45	22.41	15.13	63.11b	69.00a
F-test	ns	ns	ns	ns	ns	ns	**	**
Cultivar (C)								
Wray	225.70a	293.75a	2.24a	0.60a	30.90a	16.78	218.82b	66.00a
SP1	136.53b	228.25b	1.74b	0.41b	19.87b	16.25	410.35a	51.44b
F-test	**	**	**	*	**	ns	**	**
F x C								
F-test	ns	*	ns	ns	ns	ns	**	ns

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

Flooding = control and flooding treatment

EV; early- vegetative stage, ER; early- reproductive stage and MR; mid- reproductive SDW=shoot dry weight (g plant^{-1}), Height (cm), SD= stem diameter (cm plant^{-1}), LA= leaf area (m^2 plant^{-1}), LDW= Leaf dry weight (g plant^{-1}), RDW= root dry weight (g plant^{-1}), RL = root length (m plant^{-1}) and root no.= root number per plant

Table 2 Effects of flooding treatments at different growth stages and cultivars on shoot growth and yield of sweet sorghum at final sampling (recovery).

Treatments	Shoot dry weight (g plant^{-1})	Stalk yield (g plant^{-1})	Height (cm)	SD (cm)	Leaf area (m^2 plant^{-1})	Leaf dry weight (g plant^{-1})
Flooding	(F)					
EV	88.75c[1]	252.89c	249.25b	1.27b	0.32	13.62b
ER	119.22b	333.65b	258.63ab	1.94a	0.34	16.67ab
MR	175.35 a	452.52a	269.75a	2.01a	0.34	18.34a
Control	189.72a	489.11a	259.00ab	2.04a	0.35	19.01a
F-test	**	**	*	**	ns	**
Cultivar	(C)					
Wray	167.87a	517.06a	290.31a	2.07a	0.47a	23.93a
SP1	118.66b	247.03b	228.00b	1.56b	0.20b	9.88b
F-test	**	**	**	**	**	**
F x C						
F-test	ns	**	**	**	**	**

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

[1]Means followed by the same letter are not significantly different.

EV; early- vegetative stage, ER; early- reproductive stage and MR; mid- reproductive stage and control; free drainage.

SD= stem diameter

Table 3 Effects of flooding treatments at different growth stages and cultivars on root growth of sorghum at final sampling (recovery).

Treatments	Nodal root no. per plant	Root length (m $plant^{-1}$)	Root DW (g $plant^{-1}$)
Flooding (F)			
EV	40.00b[1]	265.00c	10.54b
ER	67.63a	391.51a	23.10a
MR	72.13a	267.76bc	22.26a
Control	63.88a	342.71ab	23.32a
F-test	**	**	**
Cultivar (C)			
Wray	60.06	326.26	22.05a
SP1	61.75	307.23	17.56b
F-test	ns	ns	**
F x C			
F-test	ns	*	ns

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

[1]Means followed by the same letter are not significantly different.

EV; early- vegetative stage, ER; early- reproductive stage and MR; mid- reproductive stage and control; free drainage.

Table 4 Effects of flooding treatments at different growth stages and cultivars on root length, root dry weight and root aerenchyma scores after 20 days of flooding.

	Root growth[1]		Root aerenchyma scores[1]			
Flooding	Root length (m $plant^{-1}$)	Root dry weight (g $plant^{-1}$)	NR in water	LR in water	NR in soil	LR in soil
Flooding (F)						
EV	42.84b[2]	0.39b	3.00a	3.00	2.00a	2.09
ER	171.44a	5.68a	1.63b	2.63	0.13b	1.63
MR	63.11b	1.21b	1.67b	2.75	0.06b	2.13
F-test	**	**	**	ns	**	ns
Cultivar (C)						
Wray	106.69a	2.78a	1.92	3.00a	0.96a	1.81
SP1	78.23b	2.07b	2.28	2.58b	0.50b	2.08
F-test	*	*	ns	*	*	ns
F x C						
F-test	**	*	*	ns	ns	ns

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

[1]Data are analyzed only in flooding treatments

[2]Means followed by the same letter are not significantly different.

NR= nodal root and LR= lateral root

EV; early- vegetative stage, ER; early- reproductive stage and MR; mid- reproductive stage and control; free drainage.

Table 5 Stalk yield (stalk fresh weight, g plant^{-1}) of sweet and forage sorghum at final sampling.

Flooding	Stalk yield (g plant^{-1})			
	EV	ER	MR	Control
Wray	333.13c[1] (50)	448.05b (32)	627.05a (5)	660.00 a
SP1	172.65e (46)	219.25de (31)	278.00cd (13)	318.23c

Figures in parenthesis indicate percent decrease as compared to the control.

[1]Means followed by the same letter are not significantly different at 0.01 probability level.

EV; early- vegetative stage, ER; early- reproductive stage and MR; mid- reproductive stage and control; free drainage.

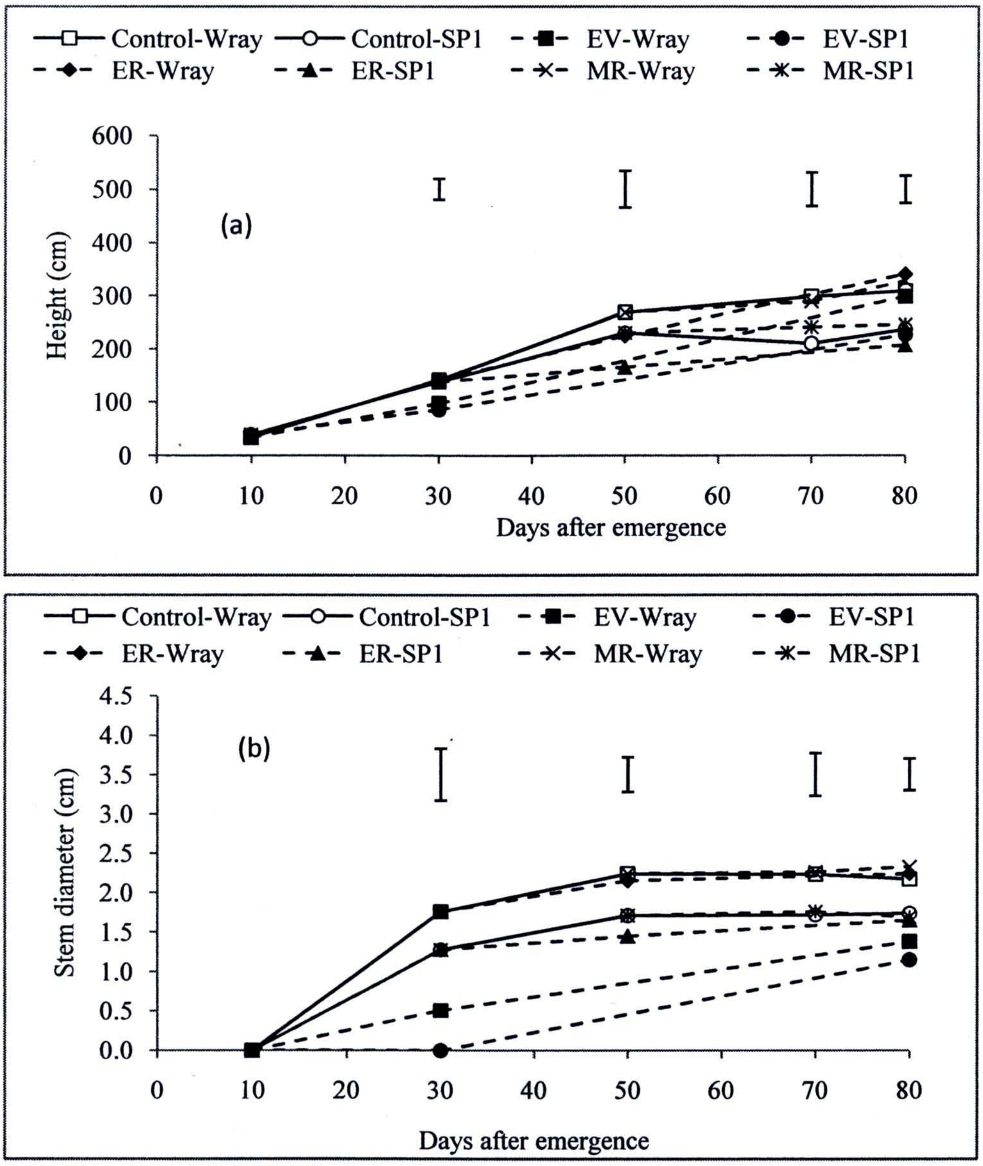

Figure 1 Change in plant height (a) and stem diameter (b) for cv. Wray and cv. SP1 after 20 days of flooding at different growth stages and at harvest. EV= flooding at early- vegetative stage, ER= flooding at early-reproductive stage and MR= flooding at mid-reproductive stage.

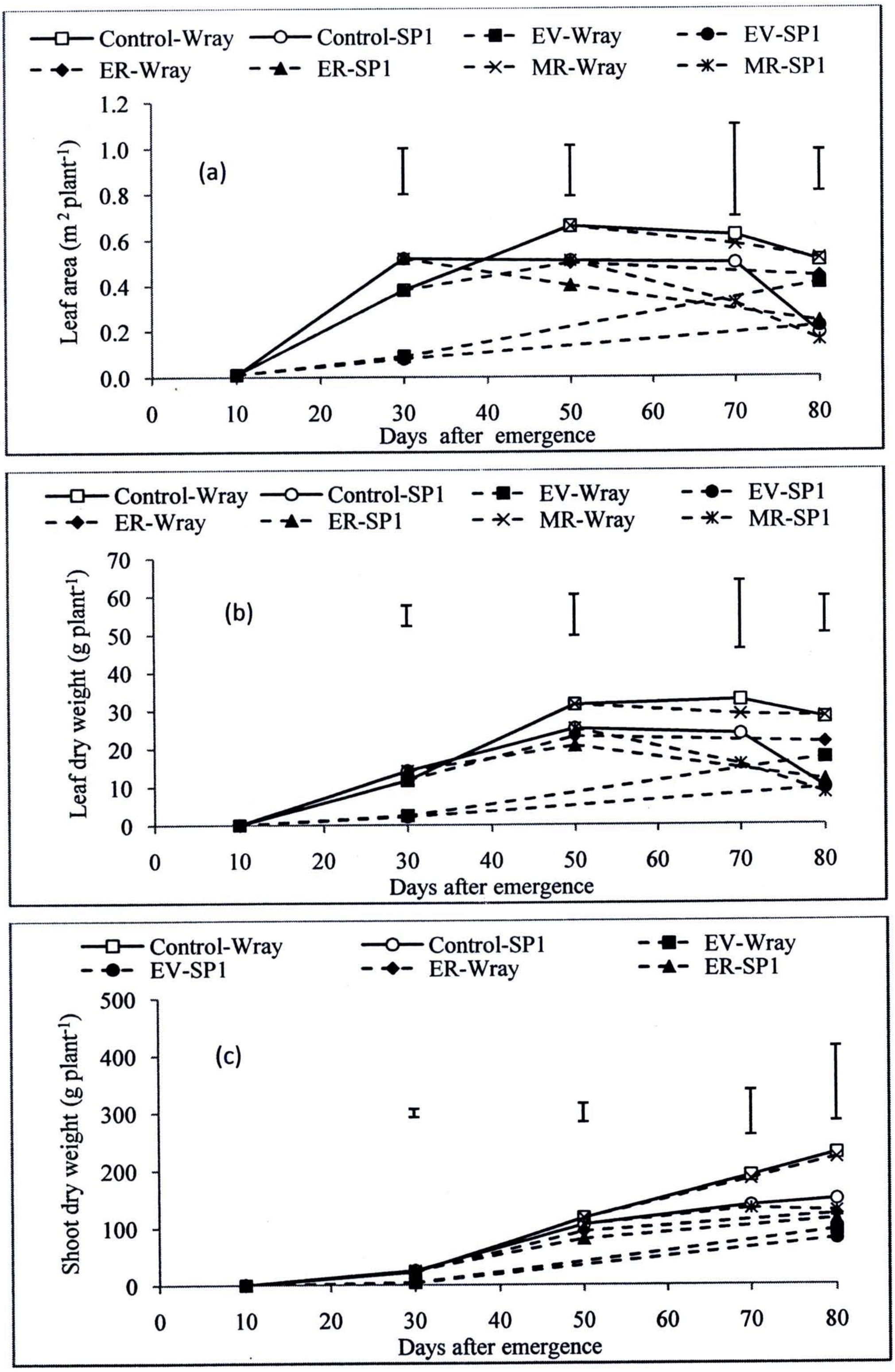

Figure 2 Change in leaf area (a), leaf dry weight (b) and shoot dry weight (c) for cv. Wray and cv. SP1 after 20 days of flooding at different growth stages and at harvest. EV= flooding at early- vegetative stage, ER= flooding at early-reproductive stage and MR= flooding at mid-reproductive stage

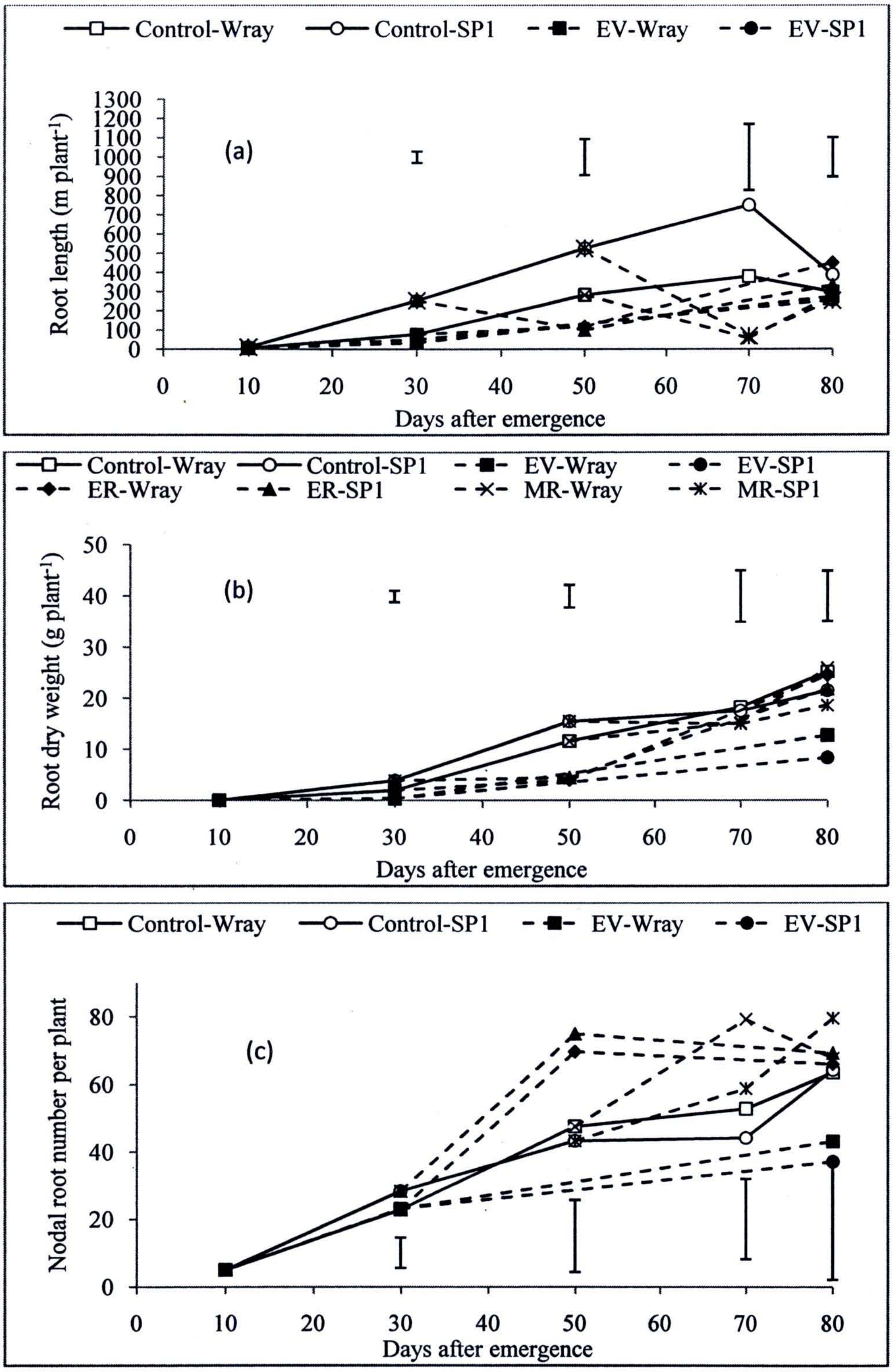

Figure 3 Change in root length (a), root dry weight (b) and nodal root number (c) for cv. Wray and cv. SP1 after 20 days of flooding at different growth stages and at harvest. EV= flooding at early- vegetative stage, ER= flooding at early-reproductive stage and MR= flooding at mid-reproductive stage.

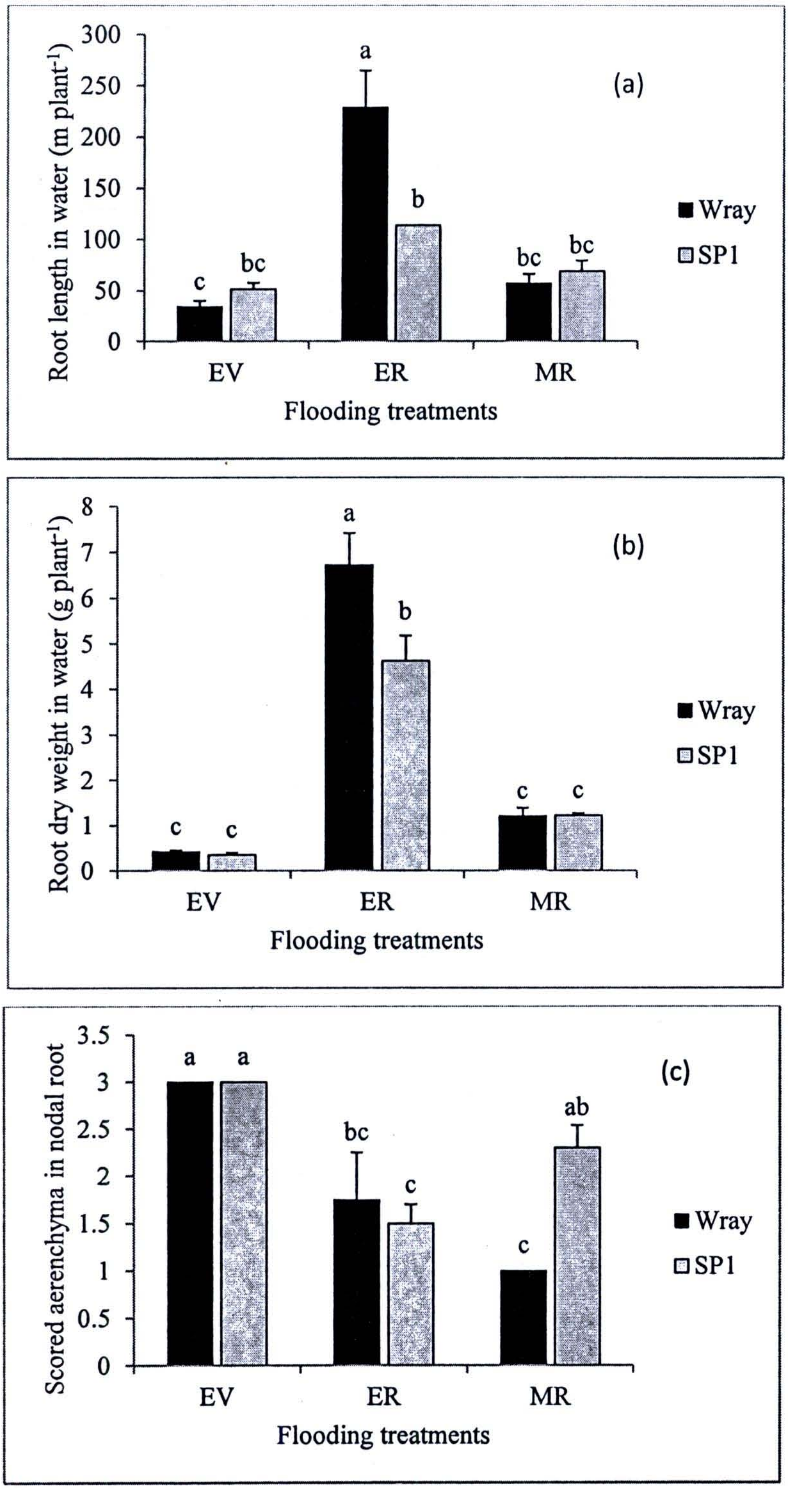

Figure 4 Root growth parameters developed in standing water after 20 d of flooding, root length ($p \leq 0.01$) (a), and root dry weight ($p \leq 0.05$) (b) and scored aerenchyma of nodal roots developed in water (c). EV= flooding at early-vegetative stage, ER= flooding at early-reproductive stage and MR= flooding at mid-reproductive stage.

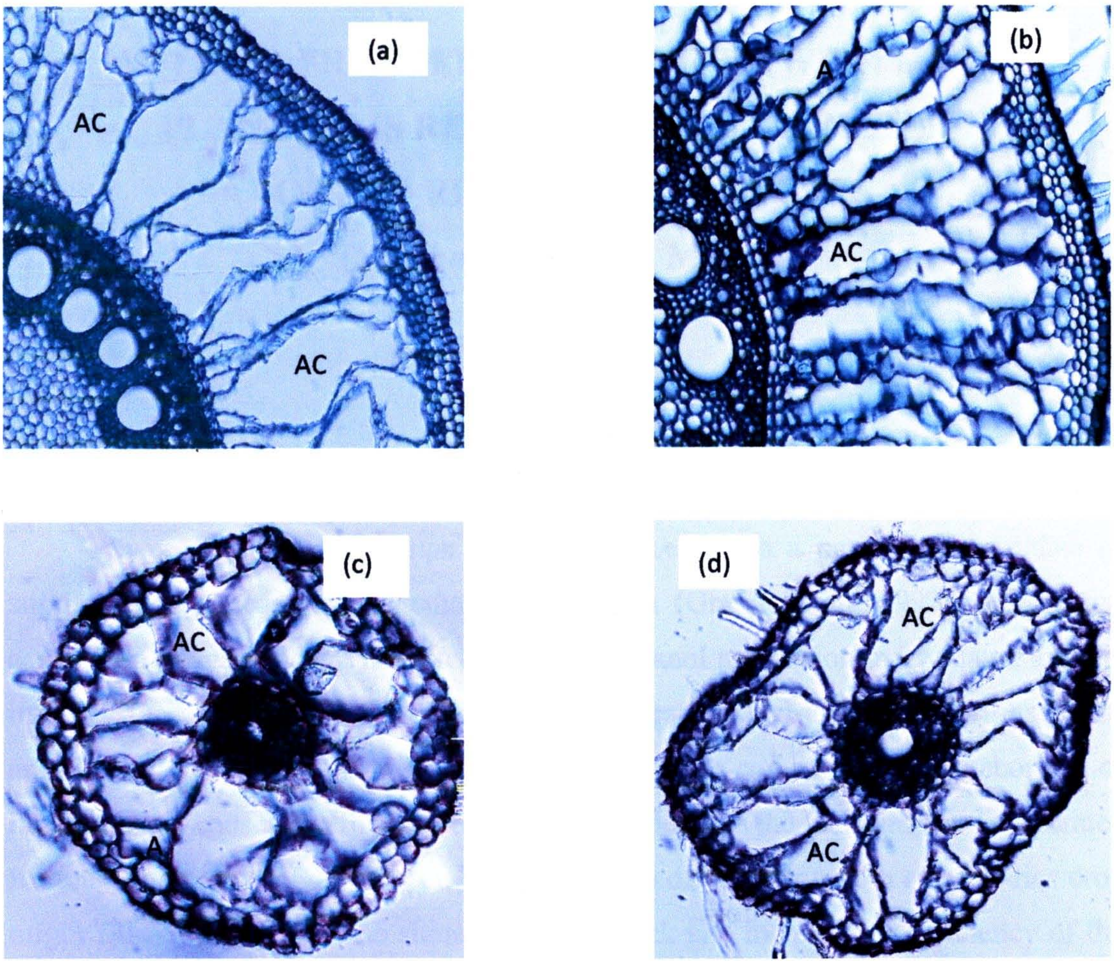

Figure 5 Cortical aerenchyma in nodal root (NR) and lateral root (LR) that penetrated in flooded soil of cv. Wray (a and c) and cv. SP1 (b and d) when 20 d of flooding was applied at early vegetative growth stage. AC = aerenchyma space.