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The Effects of Aluminum on Fiber and Protein Bound Condensed Tannin, Polyphenols and Some Growth Index in Two *Sorghum* Cultivars

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Abstract: The effect of Al on fiber and protein bound condensed tannin, polyphenols, tannin and some growth index in two cultivars of *Sorghum* (cult. 132 and 552) has been investigated. The medium culture was river sand and peat in 3:1 ratio, respectively. All pots received Hoagland's nutrient solution with and without AlCl₃. The first plant samples were obtained 30 day after sowing and the others were obtained each two weeks interval (days, 30, 44, 58, 72, 86, 90, 114 and 128) up to the end of plant's vegetative growth. Plant dry weight, growth rate and leaf area were measured and then total tannin, fiber and protein bound condensed tannin, total polyphenols in eight successive harvests were compared. The results indicated that the mean growth rate for cultivars 132 and 552 in control plants were 255 and 196 mg/plant/day, respectively and when Al contributed in the growth medium, the mean growth rates were reduced to 224 and 170 mg/plant/day in cultivars 132 and 552, respectively (almost 36% reduction). In average, leaf expansions were 6.4 and 4.5 cm² day⁻¹ in cultivar 132 and 552, respectively and Al significantly decreased (p<0.001) the leaf area by 11.3 and 7.1% in cultivar 552 and 132, respectively (p<0.001). Al causes to increase the concentration of Protein Bound Condensed Tannin (PBCT) in leaves of two cultivars with different patterns. In cultivar 132, PBCT was accumulated almost in a rate of 0.075 mg g⁻¹ DW day⁻¹ in plant during growing season. In contrast, in cultivar 552 the amount of PBCT was the same at early stage of growth in plants treated with Al and then increased slightly afterward. Although the root's PBCT were lower in cultivar 552, their amounts were decreased up to the end of growing season. This behavior was completely different in cultivar 132. Adding Al into nutrient media would change the pattern of PBCT in root. The amounts of PBCT in control plants were higher than fiber bound condensed tannin. However the trend was different in different cultivars respect to Al toxicity. Amount of total polyphenols in control plants were higher in cultivar 132 (90.9 mg g⁻¹ DW) than cultivar 552 (52.6 mg g⁻¹ DW) during growing season however Al has no significant effect on the amount of total polyphenols except at late stage of growth in which Al increased total polyphenols in both cultivars. Total tannin in cultivar 132 was peaked at middle stage of growth and was lower at younger and elder leaves. Al causes to increase the total tannin at elder leaves.

Key words: Fiber bound condensed tannin, protein bound condensed tannin, tannin, polyphenols, leaf area, *Sorghum*

INTRODUCTION

It is usually assumed that plants defend themselves against a biotic stresses by producing secondary metabolites (Barcelo *et al.*, 1996). The type of defense may be a chemical defense in which plant producing components such as terpenes alkaloids, polyphenols and tannin. These products are often regarded as an index of chemical defense (Kochian *et al.*, 2004). Simultaneously plant can change leaf toughness, trichoma density, leaves area and relative growth rate as an index of physical defense (Chen *et al.*, 2002).

Al toxicity is widely considered to be the most important plant growth limiting factor in most strongly acid soils and the most widespread problem of ion toxicity stress in plant (Barcelo *et al.*, 1996; Barcelo and Poschenrieder, 2002). In contrast, Al decreases nutrient efficiency especially for P and Ca. Al resistance is a fundamental trait for plant to fit into sustainable systems of crop production in acid soil (Guo *et al.*, 2007). Several studies find a correlation between Al resistance and the amount of polyphenols and condensed tannin (Stoutjesdijk *et al.*, 2001). Polyphenols apparently involved in metal detoxification and thus they can

complex different heavy metals with more or less efficiency (Schmohi *et al.*, 2000). Experimental data from Lavid *et al.* (2001) suggested that condensed tannin can also complex Cd. In contrast to the other observed cell wall polyphenols and flavonoids, the increase in condensed tannins was specific to Cd exposure. The increase in polymerized proanthocyanidin in the palisade parenchyma towards the leaf edge corresponded well with the gradient in mineral nutrients often observed in leaves and resulting from the leaf transpiration. A direct involvement of polyphenols, including hydrolysable tannin, in Cd sequestration has been demonstrated in two semi-aquatic species (Vollenweider *et al.*, 2006).

Respectively tannin compound are often regarded as excellent ion chelators, in part, they can act as effective group scavengers (Ma *et al.*, 1998; Riedl *et al.*, 2001). The similarity between siderophore ortho-dihydroxy substitution patterns and the substitution patterns on condensed tannin suggests that tannin may also have very high affinities for Al (Stoutjesdijk *et al.*, 2001). Recent investigations found Al induced exudation of the flavonoids type tannins (catechin and quercetin) from 10 mm root types in an Al resistant maize variety (Chen *et al.*, 2002). In Al resistant maize variety, the Al induced exudation of catechin reached rates above 100 mol/tip/h while that of citrate did not exceed 1 nmol/tip/h (Stoutjesdijk *et al.*, 2001). The ability of pentahydroxy-flavones to bind Al and ionic strength conditions of the apoplast of tips root exposed to Al is demonstrated by many studies (Stoutjesdijk *et al.*, 2001; Kochian *et al.*, 2004).

Sorghum bicolor L. Moench is the staple cereal in sub-saharan Africa and India where 300 million people rely on its grain (Dick *et al.*, 2002). In *Sorghum's* species tannin is an abundant component as high as 8-15% of dry weight which prevents damage from biotic and a biotic stress. Polyphenols compounds specialized condensed tannins play an important role in plant defense by oxidation of endogenous phenolic compound into quinines (Koupai *et al.*, 1993). The resulting quinines may undergo non enzymatic auto polymerization or covalent hetro condensation with proteins and carbohydrates to produce colored compounds. These compounds may also constitute a physical barrier against biotic and a biotic stresses (Hartley *et al.*, 1989; Schmohi *et al.*, 2000). Thus defensive effects of polyphenols compounds (tannins) reflect the risk of stress biotic and abiotic of particular tissue and its value for the future fitness of the plant.

The Growth/Differentiation Balance (GDB) hypothesis attempt to explain patterns of polyphenols allocation suitable with type of stress (Dewar, 1993; Carolyn *et al.*, 2007). On the other hand Jones and Hartley (1999) assume that the syntheses of defensive

compounds are constrained by the external availability of resources and internal trade-off in resource allocation between growth and differentiation.

The aim of this experiment were to investigation: (1) can Al effect the rate of polyphenols compound through the growing season, (2) can Al changes the partitioning of dry matter between the leaf and root in *Sorghum* cultivars, (3) can Al changes relationship between total polyphenols, total tannin, protein bound condensed tannin and fiber bound condensed tannin in two cultivars of *Sorghum* and (4) to evaluate the GDB hypothesis for *Sorghum* cultivars.

MATERIALS AND METHODS

Plant material: Seeds of two *Sorghum* (*Sorghum bicolor* L.) cultivars (552 and 132) were obtained from Seed Research Center of Isfahan, Iran. The seeds were sterilized for 20 min in a 10% sodium hypochlorite solution. The seeds were planted in pots (30 cm in diameter and 60 cm depth) on April 22nd, 2006 in University of Bu-Ali Sina experimental station. During growing season the average day/night temperatures ranged between 23±5/15±8°C and the average relative humidity was 60±5, respectively. The medium culture was river sand and peat in 3:1 ratio, respectively. Two small holes were obtained in pots, the top hole for nutrient balance and the bottom one to drain the nutrient. All pots received Hoagland's nutrient solution (Hoagland and Arnon, 1950) once in two week. Simultaneously, the AlCl₃ were applied in different concentration (0 and 30 mg L⁻¹) as treatment using a liter of 30 mg L⁻¹ AlCl₃ solution according to thresholds fixed by Barcelo *et al.* (1996). The first plant samples were obtained 30 day after sowing and others were obtained each two weeks interval (days, 30, 44, 58, 72, 86, 90, 114 and 128) up to the harvesting the plants (early September).

Plant dry weight, leaf area: The plant parts were separated and roots were washed free of soil then the fresh weights of different plant parts were measured. The fresh plant parts were placed in oven at 70°C for 4 days then the dry weight of different plant parts were measured separately. The leaf area per plant for different plant samples was determined using leaf area meter (LI 3100; Li-Cor, Lincoln, NB, USA).

Sample preparation: The homogenized fresh material of leaves, shoot and root samples were used for different analysis. The 100 mg of fresh materials were extracted with 10 mL acetone (70%) using a magnet stir and centrifuged for 5 min at 2500 g. The extraction procedure repeated

three times. The three extraction fractions were combined into a final volume of 50 mL. Purified extract was used for determination of total polyphenols and total condensed tannin.

The chemical analysis such as protein bound condensed tannin and fiber bound condensed tannin, total tannins, total polyphenols were measured using different methods described as follow.

Protein bound condensed tannin analysis: For better extract protein bound condensed tannin as Hagerman 2002 described, 15 mL Sodium Dodecyl Sulfate (SDS) solution (10 g L⁻¹ SDS and 50 g L⁻¹, 2-mercaptoethanol in 10 mM tries/chloride buffer, pH 8) was added to the residual remaining from previous step, tubes were shaken at 100°C for 45 min, then after they cooled to room temperature, centrifuged at 5000 g for 15 min and the supernatant was poured into another 50 mL conical flask. The pellet was re-extracted three times and the supernatants combined. One milliliter of the obtained aqueous solution was added to 6 mL of freshly prepared BuOH-HCl solution (950 mL of BuOH, 50 mL of HCl 37%) and heated under reflux (95°C for 75 min) finally absorbance measured at 550 nm were read on Spectrophotometer (Perkin Elmer, Lambda 45, uv/vis D6484. USA). Purified tannin was used to obtain standard liner curve.

Fiber bound condensed tannin analysis: Fiber bound condensed tannin was determined (Hagerman, 2002) directly on the residue remaining from the extraction of protein bound condensed tannin. The residue was washed into a 50 mL glass centrifuge tube with 3 mL SDS solution, 21 mL butanol/HCl solution was added and maintained at 100°C for 75 min. Then tubes were cooled on ice, centrifuged at 5000 g for 15 min and the absorbance was read at 550 nm on a Spectrophotometer. The purified tannin was used to draw standard curve.

Total polyphenols analysis: The total polyphenols was analyzed according to Prussian blue method (Hagerman, 2002). The purified extract from the first step (0.1 mL) was transferred into a 125 mL Erlenmeyer flask and 50 mL DI water plus 3 mL FeNH₄(SO₄)₂ was added and swirl. Additions should be timed; 1 min intervals are convenient. Exactly 20 min after the addition of the ferricyanide, absorbance was read at 720 nm, making readings at 1 min intervals using Gallic acid monohydrate as standards (0.094 g Gallic acid monohydrate per 50 mL methanol).

Total condensed tannin (TT) analysis: Total condensed tannin was determined with acid butanol assay (Hagerman, 2002). In a screw cap tube 6 mL of the acid

butanol reagent was added to a 1 mL of purified extract from the first step, then 0.2 mL of iron reagent was added and shook by vortex and the absorbance was read at 550 nm. A blank containing only sample solvent, acid butanol and iron was used. The purified tannin was used to obtain standard curves.

Statistical analysis and model growth interpretation:

To evaluate the relations between total polyphenols and total tannin with growth index such as leaf area, plant dry weight a multiple regression analysis was used. Simultaneously, analysis of variance was used to evaluate the effect of different treatments and their interactions on measured index. SPSS and Excel were used for statistical analysis and drawing graphs, respectively.

RESULTS

Plant dry weight: Total dry weights of two cultivars were different, however the growth rate was higher in cultivar 132 than 552 (Fig. 1). At the last harvest (end of vegetative growth) the mean growth rate over growing season for cultivars 132 and 552 were 255 and 196 mg/plant/day, respectively. From statistical point of view the effect of Al, cultivars and their interactions on dry weight of plant were significant (Table 1). Although

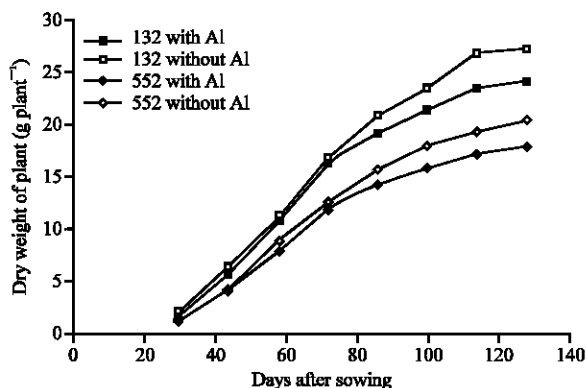


Fig. 1: Changes in total dry weight of two *Sorghum* cultivars in media with and without Al

Table 1: The F-values resulted from analysis of variance for leaf area, leaf and root dry weigh and relative growth rate under different treatment

| Source of variation | df | Leaf area (cm ² plant ⁻¹) | Leaf dry wt. (g plant ⁻¹) | Root dry wt. (g plant ⁻¹) |
|---------------------|----|--|---------------------------------------|---------------------------------------|
| Harvest | 7 | 23.41*** | 42.00*** | 14.30*** |
| Cultivars | 1 | 4.54** | 4.79** | 3.15** |
| Al | 1 | 6.79*** | 5.27** | 3.46** |
| Harvest×cultivar | 7 | 4.37** | 3.28* | 4.51** |
| Harvest×Al | 7 | 5.12** | 5.71** | 3.70* |
| Cultivar×Al | 1 | 3.54** | 2.13* | 3.86** |
| Harvest×cultivar×Al | 7 | 1.46 | 1.03 | 2.40* |

F-values are significant at ***p<0.001, **p<0.005 and *p<0.01

Table 2: Addition (+) or reduction (-) in root, stem and leaf dry weight (g plant^{-1}); Leaf area ($\text{cm}^2 \text{plant}^{-1}$) of two *Sorghum* cultivars due to addition of Al in nutrient solution

| Harvest times, days after sowing | Cultivar 132 | | | | Cultivar 552 | | | |
|-------------------------------------|--------------|-------|-------|-----------|--------------|-------|-------|-----------|
| | Root | Stem | Leaf | Leaf area | Root | Stem | Leaf | Leaf area |
| 30 | -0.21 | -0.04 | -0.16 | -49 | -0.06 | 0.00 | -0.21 | -110 |
| 44 | -0.33 | -0.37 | -0.73 | -139 | -0.66 | 0.29 | -1.54 | -89 |
| 58 | -0.14 | 0.28 | -1.39 | -510 | -0.45 | -0.63 | -1.30 | -513 |
| 72 | -0.38 | 0.15 | -2.22 | -930 | -0.44 | -0.58 | -1.75 | -1023 |
| 86 | -1.03 | -0.69 | -2.95 | -966 | -0.66 | -0.07 | -2.09 | -1203 |
| 100 | -1.02 | -0.79 | -2.67 | -940 | -1.09 | -0.30 | -2.80 | -1224 |
| 114 | -1.20 | -1.79 | -2.73 | -940 | -1.08 | -0.46 | -3.40 | -1336 |
| 128 | -1.80 | -1.73 | -2.80 | -1035 | -1.68 | -0.92 | -3.50 | -1263 |
| Average over season | -0.63 | -0.62 | -1.95 | -688 | -0.75 | -0.33 | -2.07 | -845 |

Table 3: The F-values resulted from analysis of variance for protein (PBCT) and fiber (FBCT) bounded tannin, total tannin and total phenols

| Source of variation | df | PBCT (mg g^{-1}) | FBCT (mg g^{-1}) | Total tannin (mg g^{-1}) | Total phenols (mg g^{-1}) |
|---------------------|----|-----------------------------|-----------------------------|-------------------------------------|--------------------------------------|
| Harvest | 7 | 8.10*** | 6.70*** | 11.60*** | 16.20*** |
| Cultivars | 1 | 3.12** | 1.20 | 3.51** | 5.60** |
| Al | 1 | 4.16** | 6.40*** | 5.20*** | 3.14** |
| Harvest×cultivar | 7 | 3.11* | 3.40* | 4.21** | 5.40** |
| Harvest×Al | 7 | 2.54 | 2.56 | 2.58 | 2.02 |
| Cultivar×Al | 1 | 2.32 | 3.40** | 1.14 | 2.18* |
| Harvest×cultivar×Al | 7 | 0.48 | 0.12 | 4.11* | 2.42* |

F-values are significant at *** $p < 0.001$, ** $p < 0.005$ and * $p < 0.01$

the dry weights of two cultivars were decreased due to Al in nutrient culture but reduction of dry weights due to Al were almost the same in two cultivars (36%) (Table 2). When Al contributed in the growth medium, the mean growth rates were reduced to 170 and 224 mg/plant/day for cultivars 552 and 132, respectively.

There was a significant difference in root, stem and leaf dry weight of treated plants with Al in compared to the control. The most remarkable reduction in dry weights was observed in root of cultivar 552 and stem of cultivar 132 (Table 2). The rate of root dry weight reduction were 29.3 and 40.6% in cultivars 132 and 552, respectively due to Al ($p < 0.001$). However the reduction for stems in cultivars 132 and 552 were 39.5 and 23.9%, respectively.

Leaf area: Leaf expansion in cultivar 132 was faster than cultivar 552, therefore; the leaf area was higher in cultivar 132 than in 552. In average leaf expansion were 46.75 and 36.26 $\text{cm}^2 \text{day}^{-1}$ in cultivar 132 and 552, respectively (Table 2). Al significantly decreased ($p < 0.001$) the leaf area by 5.5 and 8.9% for cultivar 552 and 132, respectively. The average leaf area in which reduced due to Al was 42.57 $\text{cm}^2 \text{day}^{-1}$ in cultivar 132 and 34.12 $\text{cm}^2 \text{day}^{-1}$ in cultivar 552. The effect of Al in leaf area reduction was statistically significant in cultivar 552 during growing season however in cultivar 132 this effect was not significant.

Protein Bound Condensed Tannin (PBCT): The effect of different treatments such as cultivar, Al and their interaction on the amount of PBCT was statistically significant (Table 3). The amount of leaf's PBCT in different cultivars were different and cultivar 132 has

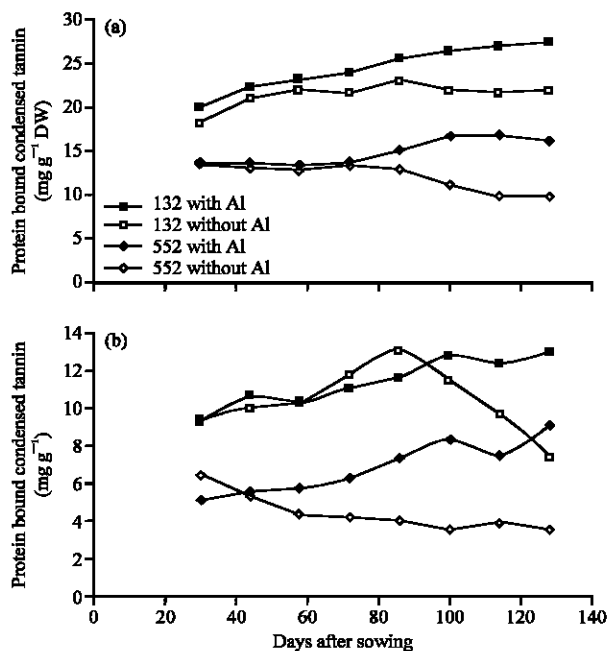


Fig. 2: Changes in leaf and root protein bound condensed tannin (PBCT) of two *Sorghum* cultivars in growth media with and without Al, (a) Leaf and (b) Root

higher PBCT than 552. In control plants, the amount of PBCT was constant in cultivar 552 up to the days 80 and then started to decrease up to the end of growing season (Fig. 2a,b). However in cultivar 132 the amount of PBCT increased up to the days 80 and then were constant up to the end of growing season ($p < 0.001$, Table 4). Although Al causes to increase the concentration of PBCT in leaves

Table 4: Addition (+) or reduction (-) in PBCT, FBCT, TT and TP (mg g⁻¹) due to addition of Al in nutrient solution in two *Sorghum* cultivars 132 and 552

| Harvest times, days after sowing | PBCT | | FBCT | | TT | | TP | |
|-------------------------------------|--------------------|---------------------|--------------------|---------------------|---------------------|--------------------|--------------------|---------------------|
| | 132 | 552 | 132 | 552 | 132 | 552 | 132 | 552 |
| 30 | 1.65* | 0.20 ^{ns} | 1.70 ^{ns} | 0.38 ^{ns} | 1.06 ^{ns} | -5.30*** | -0.29 | -0.93 ^{ns} |
| 44 | 2.33* | 1.30 ^{ns} | 1.75 ^{ns} | 1.60** | 2.71** | -4.10*** | 1.50** | -2.30** |
| 58 | 1.24 ^{ns} | 0.60 ^{ns} | 4.40*** | 1.62** | 0.40 ^{ns} | 5.34*** | 1.20 ^{ns} | 0.11* |
| 72 | 2.34* | -0.90 ^{ns} | 4.34** | -0.30 ^{ns} | -0.96 ^{ns} | 3.70** | 3.06** | 2.37** |
| 86 | 2.40*** | -0.90*** | 3.51 ^{ns} | -0.90 ^{ns} | 2.75*** | 2.11 ^{ns} | 7.70*** | 3.32** |
| 100 | 4.30*** | 1.50*** | 3.72 ^{ns} | -1.40* | 4.31*** | 1.20 ^{ns} | 8.10 ^{ns} | 2.74 ^{ns} |
| 114 | 5.50*** | 4.60*** | 2.87 ^{ns} | -1.90** | 6.30*** | 3.50 ^{ns} | 9.60*** | 4.11*** |
| 128 | 5.60*** | 5.20*** | 3.36 ^{ns} | -2.80*** | 5.60*** | 3.64 ^{ns} | 9.80 ^{ns} | 3.40 ^{ns} |
| Average over season | 3.05 | 1.19 | 3.20 | -0.48 | 2.76 | -1.30 | 4.70 | 1.50 |

F-values are significant at ***p<0.001, **p<0.005 and *p<0.01, ns: Not significant

of two cultivars with different patterns, the growth rate of PBCT production were the same in leaves of cultivar 132 and PBCT was accumulated almost in a rate of 0.075 mg g⁻¹ DW day⁻¹ in plant during growing season. In contrast, in cultivar 552 the amount of PBCT was the same at early stage of growth in plants treated with Al and then were increased slightly.

The amount of root's PBCT was higher in cultivar 132 than 552 in control plants and its concentration were also different during growing season (Fig. 2). Although the root's PBCT were lower in cultivar 552, their amounts were decreased up to the end of growing season. This behavior were completely different in cultivar 132 in which at early stage of growth the PBCT were increased up to the mid-season (days 80) and then decreased up to the end of growing season. Adding Al into nutrient media would change the pattern of PBCT production in root of two cultivars. In both cultivars the PBCT of the root were increased up to the end of growing season at almost in a constant rate (0.051 and 0.041 mg g⁻¹ DW day⁻¹ in cultivars 132 and 552, respectively).

Fiber Bound Condensed Tannin (FBCT): The amount of leaf's FBCT were almost the same in cultivar 552 (6 mg g⁻¹ DW) during growing season, however, their amounts were lower than cultivar 132. Amount of FBCT in leaves of cultivar 132 peaked at mid-season and were lowered at younger and elder age (Fig. 3a,b). The difference was not statistically significant (Table 3). Addition of Al in growth media would change the trend of leaf's FBCT in such a way that the concentration of FBCT in leaves were increased in cultivar 132 but decreased in cultivar 552. In this condition, at early stage of growth the FBCT of leaves were almost 6.35 and 7.93 mg plant⁻¹ in cultivar 552 and 132, respectively then its amount was decreased to 3.16 in cultivar 552 but increased to 12.8 mg plant⁻¹ in cultivar 132.

The average amount of root's FBCT in cultivar 132 was higher than 552 during growing season, except at the beginning and the end of growing season in which their amount were the same (Fig. 3a,b). Although the

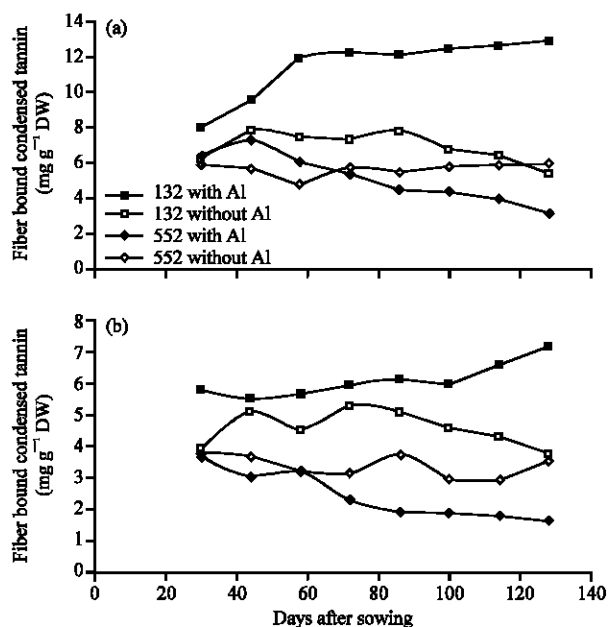


Fig. 3: Changes in Leaf and root fiber bound condensed tannin (FBCT) of two *Sorghum* cultivars in growth media with and without Al, (a) Leaf and (b) Root

concentration of root's FBCT in cultivar 552 was lower compared to 132 in media contain Al, their patterns were different up to the end of growing season. The pattern of FBCT in cultivar 132 was rising and in cultivar 552 was falling up to the end of growing season.

Comparing the amount of PBCT and FBCT indicated that the amount of PBCT in control plants in two cultivars were higher than FBCT (p<0.001). However the trend was different in different cultivars respect to Al toxicity. The average amount of FBCT was increased due to Al in cultivar 132 compared to the control but in cultivar 552 its amount were lower compare to the control plants. Al significantly increased concentration of PBCT in leaves and roots in two cultivars (p<0.001). This was in contrast with FBCT in which its concentration in cultivar 552 peaked in early growth and declined thereafter in its leaves and roots (p<0.001).

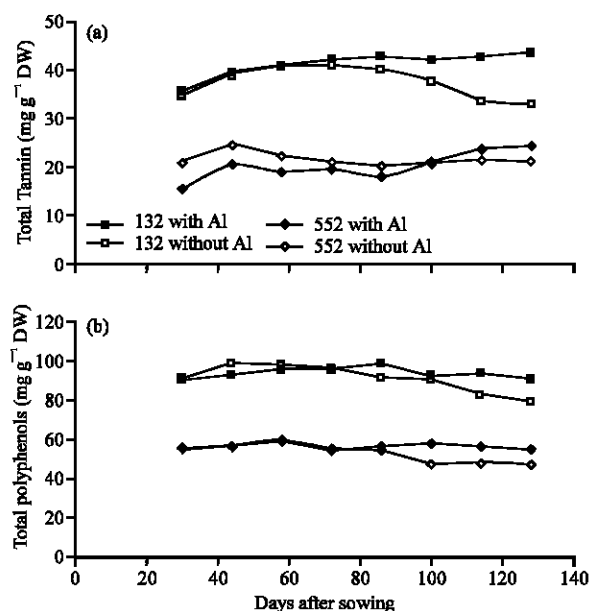


Fig. 4: Changes in total tannin and total polyphenols of leaf of two *Sorghum* cultivars in growth media with and without Al, (a) Leaf and (b) Root

Leaf total polyphenols (TP): The amount of total polyphenols in control plants were different in two cultivars and its amounts were higher in cultivar 132 (90.9 mg g⁻¹ DW) than 552 (52.6 mg g⁻¹ DW) ($p < 0.001$) during growing season (Table 4). Although the amounts of TP were almost the same at early stage of growth in cultivar 552, its amounts were lower at elder leaves (Fig. 4a,b). This behavior was almost the same in a cultivar 132 except the rate of reduction in TP was higher in this cultivar. Although the amount of TP was higher in cultivar 132 compared to 552, Al has no significant effect on the amount of TP except at late stage of growth in which Al causes an additional accumulation of TP in both cultivars compared to the control plants. The concentrations of total polyphenols in leaves of treated plants were almost 93.8 and 56.3 mg g⁻¹ DW in cultivar 132 and 552, respectively (Table 4).

Leaf total tannins (TT): The amounts of total tannin were different in two cultivars at different stage of growth and also its concentration were different in treated and non treated plants with Al (Fig. 4a,b). In control plants, Total Tannin in cultivar 132 was peaked at middle stage of growth and was lower at younger and elder leaves (Table 4). However in cultivar 552 their amounts were almost the same at different harvest. In cultivar 132, TT in treated and none treated plants with Al was the same at early stage of growth but after days 80 their

concentrations were higher in treated plants with Al. This was different compare to 552 where TT was higher in none treated plants at early stage of growth then was lower afterward.

DISCUSSION

The effect of Al on plant growth and development was evaluated by different researchers and their results indicated that Al injury could affect in different organs and different plant parts in different ways (Barcelo *et al.*, 1996; Ahn *et al.*, 2001; Collet *et al.*, 2002). Present results also indicated that different plant parts and different chemical components in plant would be affected by Al in growth media. The behaviors of *Sorghum* plant (cultivars 132 and 552) in this experiment were different in different measured index. This means that the ability of adjustment in these cultivars with respect to environmental stresses is different. The plant dry weight was reduced due to Al in growth media however the rate of reduction was faster in cultivar 552 in compare to cultivar 132 (Fig. 1). The biomass allocation into roots and leaves would be affected by Al in such a way that the root dry weight was reduced more that leaf dry weight. Same result was reported by Chen *et al.* (2002) when plant shoot was evaluated to Al toxicity.

According to our result, the damage of biomass reduction was much higher than leaf's surface reduction in cultivar 132 however in cultivar 552 the reduction of leave surface was much higher that leaf dry weight (Table 2). In detail, the leaf dry weight of two cultivars would be affected more at early stage of growth; however the leaf area of plants would be damaged at the late stage of growth. This conclusion is agreed with results of Dicko *et al.* (2002). This may be suggested that, the higher reduction rate of leaf area at late stage of growth is because of accumulation of different chemical components in leaf such as tannin and polyphenols and consequently, causes more leaf hardness. Although Ma *et al.* (1998) reported that in low concentration of Al the leave dry weight and leave surface of wheat plant would be increased. However, similar result was not obtained in this experiment. The less transformation of synthesis chemicals into root causes to have higher shoot/root ratio and consequently, the uptake of minerals especially, nitrogen from root would decreases. This condition causes the adsorption of higher CO₂ in compare to the plant N-nutrient. The consequent of this process would be higher production of hydrocarbons compared to proteins (Yin and Schapendonk, 2004). The allocation of synthesized chemicals into roots and shoots may be interpreted according to the functional equilibrium theory

(Koricheva *et al.*, 2002). It is believed that contribution of dry matter allocation for better fitness in different environments has happened in changing in root/shoot ratio.

The results of Dick *et al.* (2002) indicated that the variation of polyphenols in plant's leaves is related to the age of plant. However, in this experiment, the amount of polyphenols in leaves are adjusted with the growth rate of plant; therefore, when the growth rate is high the synthesis of polyphenols is high too and consequently polyphenols accumulated in leaves. In details, the relation between the rate of polyphenols synthesis and the age of plant was positive and significant up to the mid-stage of growth and then this relation was negative afterward. Adding Al into growth media was changed the pattern of this relation in such a way that the relation was positive up to the end of growing season. The same trend was observed for leaf's tannin except that the peak of maximum synthesis delayed respects to polyphenols, because tannin is more stable than polyphenols (Riipi *et al.*, 2002).

The results of Wen *et al.* (2003) indicated that most polyphenol compounds (proanthocyanidin and gallotannin) and some flavonoids (epicomaryl and paracomaryl) were increased in spring and then decreased in summer. This may happened due to the reverse relation of lignifications and polyphenols production in plant. In this experiment, the amount of polyphenols decreases as the leaves' expansion increases. This is a reasonable result due to consumption of polyphenols in lignification process.

According to the results of Forkner *et al.* (2004), tannin can reduce the efficiency of plant for grazing and therefore; they concluded that protein can bound with tannin and produce protein bound condensed tannin in which cause the reduction of grazing's efficiency.

Terrill *et al.* (1992) reported that the amount of protein bound condensed tannin in forage plants is higher in compared to other tannin compounds. According to our results, the amount of protein bound condensed tannin in *Sorghum* plant is higher than other tannin compounds and when aluminum added into growth media, this complex would increase in roots and leaves.

According to the role of tannin as chelator of proteins and aluminum in plant, it can be expected that the higher tannin in cultivar 132 can reduce the toxicity of aluminum more than that of low tannin cultivar 552. This conclusion is agreed with results of Stoutjesdijk *et al.* (2001), McDonald *et al.* (1999) and Terrill *et al.* (1992). They indicated that tannin can chelate with aluminum and reduce plant toxicity. This mechanism increases the plant tolerance relevant to aluminum toxicity.

In contrast, when aluminum added into growth media, the amount of fiber bound condensed tannin decreases relevant to the age of plant, however the amount of protein bound condensed tannin would increase. This is a reverse relation with the amount of lignification against time.

The variation and allocation of different chemical compound such as (protein and fiber bound condensed tannin, total tannin and total polyphenols) within plant parts during the growing season with aluminum is different. The amount of fiber bound condensed tannin is decreased since early stage of growth. This means, the synthesis rate is lower than the transformation rate into different compounds. This is in contrast with the others and therefore, the accumulation rate is higher due to the less transformation in compared to the synthesis rate. This conclusion is agreed with results of Gebrehiwot *et al.* (2002) and Stoutjesdijk *et al.* (2001).

The result of multiple regression analysis for prediction of the injury's effect of Al on leaf area, root and leaf dry weight indicated that the injury of Al on root dry weight and leaf area was higher than other growth index in two cultivars and also the effect of Al on PBCT and FBCT was higher than other polyphenols.

Regardless of actual leaf and root age clear difference exist in strength of defense between early and late leaves and roots in two cultivars. These results are agreed with finding of Stoutjesdi *et al.* (2001). Change in leaf and root biochemistry during Al stress is an important factor for *Sorghum* resistance and its value for the future fitness of *Sorghum* as an adapted plant in acid soil.

The result could be explained according to Jones *et al.* (1999). They explained, tannins are produced in the shikimate pathway via the aromatic amino acid phenylalanine and thus their synthesis may compete directly with the synthesis of proteins. According to the protein competition model hypothesis, phenylalanine should be highest during leaf expansion in spring (early stage of growth) and allocation of phenylalanine to those tannin content and their derivative is very low. Therefore the amount of tannin in early stage of growth should simultaneously decrease.

CONCLUSION

According to growth differentiation balance GDB (Lorio, 1988) growth processes dominate over differentiation or allocation to polyphenols compound which potential defensive should be low when plant growth is high. Therefore, at early stage of growth in which the leave expansion in *Sorghum* cultivars is so high, the allocation of carbon-sources into polyphenols

and tannin would be low. In this case, we expected that tannin and polyphenols concentration increases in relevant to the age of leaves due to less leaf expansion. This might be due to contribution of more leaves in synthesis of tannin and polyphenols. This conclusion is in agreement with the results obtained by Dicko *et al.* (2002).

According to these result, accumulation of polyphenols in control plants was proportional to leave expansion in which this is in contrast to GDB's theories (Carolyn *et al.*, 2007). When aluminum added into the growth media, the allocation and accumulation of polyphenols is relevant to the GDB's predication. Present finding shows, *Sorghum* can expand its leaves and at the same time can synthesis and accumulate polyphenols and tannin into plant. This behavior was different in two cultivars.

If total biomass is important factor, cultivar 132 has low injury than 552. However leaf area in cultivar 132 was higher than cultivar 552 but leaf dry weight of cultivar 132 was lower than 552. This means, cultivar 132 is able to expand its leaf area rapidly and simultaneously produce abundance secondary compounds in early stages of growth (Foy *et al.*, 1993).

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