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THE EFFECTS OF ALUMINUM ON CHEMICAL DEFENSE AND PHYSICAL TRAITS IN TWO CULTIVARS OF SORGHUM (*SORGHUM BICOLOR*)

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ABSTRACT

The effect of aluminum (Al) on some defensive compounds and physical traits in two cultivars (cultivar 132, 552) of sorghum was investigated. Plants were cultivated in two Al concentrations (0 and 30 mg L^{-1}) before flowering in mixture of river sand and peat. Physical (toughness, relative growth rate, leaf area) and chemical defense (total tannin, protein-bound condensed tannin (PBCT), fiber-bound condensed tannin (FBCT), total polyphenols) traits in eight harvests were considered. The traits of tannin in leaves and roots were more suitable than polyphenols with growth differentiation balance (GDB) model. The results revealed that Al can trade off between decrease in leaf dry weight and production of tannins, which might be due to an increase in protein-bound condensed tannin (PBCT). Leaf area in cultivar 132 was higher than cultivar 552, but leaf dry weight in cultivar 132 was lower than 552. As cultivars developed, stems mass fraction in cultivar 132 and leaves mass fraction to the total plant biomass in cultivar 552 increased. Leaf toughness during various developmental stages increased in cultivar 552 but not in cultivar 132. Leaf expansion in cultivar 132 was greater than in cultivar 552. Al steadily increased PBCT, but decreased FBCT, which was apparently involved in Al detoxification. In both the cultivars, allocation to defense varied in both amount and type. The fitness of cultivars 132 and 552 was due to chemical resistance. Fiber-bound condensed tannins were detected in young leaves; and in cultivar 552, the levels were higher in stages 1-2 than in later stages. In cultivar 132, the FBCT decreased after five harvests. Although mechanical properties were relatively poor in cultivar 132, Al content was high in the leaves though not significant. The transformation of tannin in cultivar 132 was much less than the rate of synthesis; however, in cultivar 552 the rate of transformation was similar to the rate of synthesis. In two cultivars 132 and 552, when the leaf growth rate was high, Al increased PBCT and decreased FBCT, in which the rate of changes was higher in 552 than 132. When leaf growth rate was low, the amount of production of PBCT and FBCT increased in both cultivars. With increasing duration of treatment, Al toxicity increased with subsequent decrease in the amount of FBCT in

shoot and root, suggesting that section of tannins have transformed, which could be bound to Al.

Keywords: fiber bound condensed tannin, protein bound condensed tannin, tannin, toughness, leaf area

INTRODUCTION

Al toxicity is widely considered to be the most important growth limiting factor for plant in most strongly acid soils and the most widespread problem of ion toxicity stress in plant (Kochian et al. 2004). Al resistance is a fundamental trait for plant to fit into sustainable systems of crop production in acid soil and Al decreased nutrient efficiency, especially for P and Ca (Ryan et al. 1993; Kochian et al. 2004). Several studies find a correlation between Al resistance and the amount of polyphenols; and condensed tannin is a polyphenol that is apparently involved in metal detoxification. Polypheno, thus, can complex different heavy metals with more or less efficiency (Peter et al. 2001). Experimental data from Lavid et al. (2001) suggested that condensed tannin could 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 corresponds well with the gradient in mineral nutrients, which is 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 (Lavid et al. 2001).

Tannin compound are often regarded as excellent ion chelators and they can act as effective group scavengers (Bores et al. 1996). The similarity between siderophor ortho-dihydroxy substitution patterns and the substitution patterns on condensed tannin suggests that tannin may also have very high affinities for Al (Chimi et al. 1991). 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. In Al resistant maize variety, the Al induced exudation of catechin reached a value of above 100 mol per tip h^{-1} while that of citrate did not exceed 1 nmol per tip h^{-1} (Juan et al. 2002). 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 (Juan et al. 2002; Ryan et al. 1992).

Sorghum [*Sorghum bicolor* L. Moench] is the staple cereal in sub-Saharan Africa and India where 300 million people rely on its grain (Mamoudou et al. 2002). In Sorghum species, tannin is an abundant component as high as 8-15% of dry weight, which prevents damage from biotic and abiotic stress. Polyphenols compounds and specialized condensed tannins play an important role in plant defense by the oxidation of endogenous phenolic compound into quinones (Riedl et al. 2001). The resulting quinones 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 abiotic stresses (Chimi et al. 2004). 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 (Carolyn et al. 2007) attempts to explain patterns of polyphenols allocation suitable with type of stress. On the other hand, assume that the synthesis of defensive compounds is constrained by the external availability of resources and internal trade-off in resource allocation between growth and differentiation (Riipi et al. 2002). The functional equilibrium theory (Carolyn et al. 2007) states that root:shoot ratio changes to maintain the activity ratio between the shoot and root, and does not involve in Al resistance. Model Dewar (1993), on the other hand, represents an optimal allocation of biomass to the root and shoot, which maximizes the relative growth rate (RGR) and other factors.

The aims of this experiment were to investigate: (1) the possibility of Al effect on producing polyphenols compound during the growing season; (2) the changes in Al partitioning of dry matter between the leaf and root in sorghum; (3) the changes in Al relationship between toughness and defense compound, in two cultivars of sorghum; and finally (4) to challenge the GDB hypothesis.

Therefore, two cultivars of sorghum were cultivated in nutrition medium with none and high concentration of Al as threshold for tolerance (Barcelo et al. 1996). During the growing season at eight successive harvests, the concentration of major groups of defense compounds such as protein-bound condensed tannin (PBCT), fiber-bound condensed tannin (FBCT), total tannin (TT) and total polyphenols (TP) were measured. In addition, the dry weight of leaf, root and shoot, toughness and leaf area were measured.

MATERIALS AND METHODS

Plant materials

The seeds of two sorghum cultivars (552 and 132) were obtained from Seed Research Center of Isfahan. The seeds were sterilized for 20 min in a 10% sodium hypochlorite solution. The seeds were then planted in pots (30 cm in diameter and 60 cm depth) on April 22nd in a glass house in Isfahan University. During the growth period the temperature ranged between 20±5°C. The medium culture was river sand and peat in 3:1 ratio, respectively. Two small holes were made in the growing pots, the top hole for nutrient balance study, and the bottom one for drainage. The Hoagland's nutrient solution was added to each pot once in two weeks (Hogland 1950). Simultaneously, the $AlCl_3$ was applied in different concentration (0 and 30 mg l^{-1}) as treatment using a liter of 30 mg l^{-1} $AlCl_3$ solution according to thresholds fixed by Barcelo et al. (1996).The first plant sample was obtained at 30

days after sowing and other plant samples were obtained at two weekly interval up to the last harvesting of the plants (early September).

Plant dry weight (dwt), leaf area (LA) and toughness (TN) analysis

The plant parts were separated and roots were washed free of soil; then the fresh weight of different parts and leaf area per plant were determined using leaf area meter (LI 3100; Li-Cor, Lincoln, NB, USA). The toughness of fresh leaf using a push-pull gauge (CPU gauge; AIKOH, Nagoya, Japan) was measured at three points on each leaf and the mean value was determined. The samples were placed in oven at 60 °C for 4 days, then the dry weights were measured separately. Plant dry weights for different parts were calculated for two successive harvests. The chemical analyses such as protein-bound condensed tannin, fiber-bound condensed tannin, total tannins and total polyphenols were measured using different methods described as below.

Sample preparation

The homogenized fresh materials of leaves, shoots and roots samples were used for different analyses. A 100 mg of samples were suspended in 10 ml of 70% aqueous acetone, continuous stirring and centrifuged for 5 min at 2500 rpm. The pellet was re-extracted twice. All aqueous phase was combined to a 50 ml conical flask and the purified extract was used for determination of total polyphenols and total condensed tannin.

Protein-bound condensed tannin (PBCT) analysis

For better extract of protein-bound condensed tannin (PBCT) a (Hagerman et al. 2002; Bores et al. 1996) 15 ml 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 pellet from the above. Tubes were shaken at 100 °C for 45 min. after which they were cooled to room temperature, centrifuged at 5000 rpm for 15 min., and the supernatant was poured into another 50 ml conical flask. The pellet was re-extracted three times and the supernatants were combined. A 1 ml 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) pellet, finally absorbance measured at 550 nm were read on Spectrophotometer (Perkin Elmer, Lambda 45, uv/vis D6484. USA). The purified tannin was used as a standard.

Fiber-bound condensed tannin (FBCT) analysis

Fiber-bound condensed tannin (FBCT) was determined (Hagerman et al. 2002) directly on the residue remaining from the extraction of protein-bound CT (pellet). The pellet was washed into a 50 ml glass centrifuge tube with 3 ml SDS solution, and 21 ml butanol/HCL solution was added and maintained at 100 °C for 75 min. Tubes were cooled on ice, centrifuged at 5000 rpm for 15 min, and the absorbance

at 550 nm was read on a Spectrophotometer. The purified tannin mg g^{-1} was used for standard curves and all analyses were carried out in three replicates.

Total polyphenols (TP) analysis

The 5 ml of supernatant flask was used for measuring total polyphenols with the Prussian blue method (Hagerman et al. 2002). A 0.1 ml sample was dispensed into a 125 ml Erlenmeyer flask. Then 50 ml distilled water was added. Poor quality water, especially iron-containing water, was not used as it gave high blanks and unacceptable results.

Total condensed tannin (TT)

Total condensed tannin was determined with acid butanol assay (Hagerman et al. 2002). In a screw cap culture, 6 ml of the acid butanol reagent was added to a 1 ml aliquot of the sample. The a 0.2 ml of the iron reagent was added and the sample was vortexed. The tube was capped loosly, and put in a boiling water bath for 50 min. The tube was cooled and the absorbance was read at 550 nm. The absorbance containing only sample solvent, acid butanol and iron was subtracted from a blank. The purified tannin mg g^{-1} was used for standard curves.

Statistical analyses and model growth interpretation

For growth index analysis, in each harvest three replicates of each treatment of four pots (two cultivars and two Al concentrations) were randomly selected, and analyzed. For the dynamic growth models of the plants, typical sigmoid curves were assumed for two cultivars. The model of Carolyn et al. (2007) was used after modification. In this model average LA, dwt, FBCT, PBCT, and TP were estimated between each two successive harvests. The means of these parameters for different stages of growth were estimated by assuming exponential growth at each harvesting interval.

$$X= (X_2 - X_1)/ (\ln X_2 - \ln X_1) \qquad (1)$$

Where X could be any variable between time 1 and time 2, and is derived from $X_1$and X_2, the values at times 1 and 2, such as mean dwt., LA, TT, PBCT and FBCT for a period between time 1 and 2.

The relative growth rate was calculated using the following equations.

$$RGR_{1\text{-}2}=1/M_1 \times (M_2 - M_1)/ (T_2 - T_1) \qquad (2)$$

$$RGR_{3\text{-}2}=1/M_2 \times (M_3 - M_2)/ (T_3 - T_2) \qquad (3)$$

Where RGR_{1-2} denotes the RGR from first to second harvest, RGR_{3-2} denotes the RGR from second to third harvest, M_1 is the total dry mass at the first harvest, M_2 is the total dry mass at the second harvest, M_3 is the total dry mass at third harvest, and t_1, t_2 and t_3 are the respective dates of these harvests. Analysis of variance, ANOVA, using System SPSS 13 for Windows was used to test the effects of Al on cultivars, harvest, and their interactions on variables of cultivars. The linear regression models were interpreted as the estimated mean response in mid-stage, at any time of the plant growth since the variable time (t) was centered on the mean variables.

For relations between dynamics of the TP, TT with LA, dwt leaves of the plant, a multiple regression with four degrees was used. The polyphenols and tannin dynamics assumed to be characteristics for two cultivars were allowed to differ in the intercept with leaf area and dwt leaves.

$$Y_i = \beta_0 + \beta_2 \times x_{i2} + \beta_3 \times x_{i3} + \beta_4 \times x_{i4} + \beta_5 \times x_{i5} + \beta_1 \times x_{i1} + e_i \qquad (4)$$

Hence the multiple regression of linear models were allowed to interact with any characters consistent with the independent variables against dependent variables; β1,β2,β3,β4 were coefficients of the regression.

RESULTS

Plant dry weight (dwt) and relative growth rate (RGR).

Al significantly decreased total plant dwt in both cultivars, and with prolonging of time the effects became larger (Table 1). With comparison among means of control and Al treatments at late harvest in both cultivars, Al decreased total plant dwt ($P<0.001$) of cultivar 552 by 13.3 %, and cultivar 132 by 10.68 % (Table 3 and 4). There was a significant difference in dry weights of root, shoot, and leaf against the control, and the decrease was found most remarkable in dwt of root. With the comparison between mean control and Al treatments recorded at late harvest, dry root weight of cultivar 552 decreased by 21.5 % compared to that of cultivar 132, which declined by only 13.5% ($P<0.001$; Table 3 and 4; Figure 1 and 2). With passing of time, RGR decreased; Al was observed to significantly reduce the RGR ($P<0.001$). But the decline in cultivar 552 (Table 3) was faster than in cultivar 132 ($P<0.001$: Table 4).

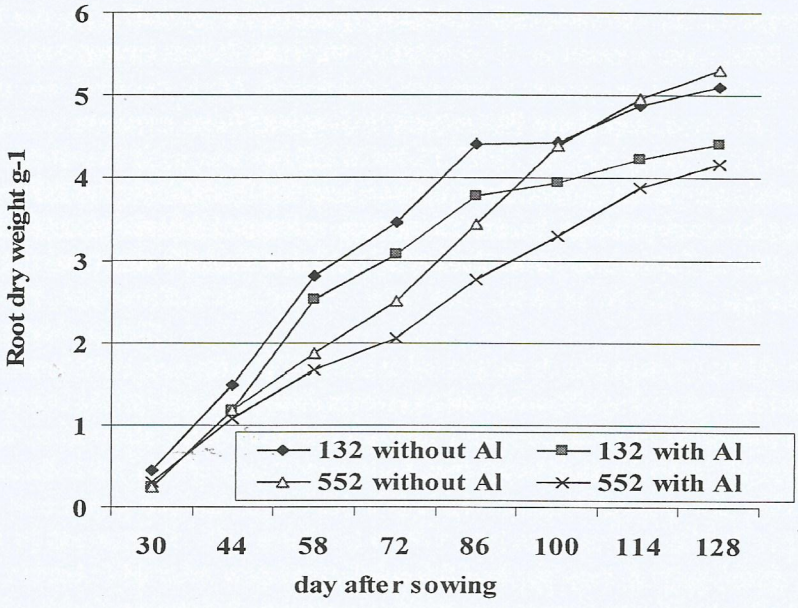

Figure 1. Changes in root dry weights with time to Al exposure for two cultivars of sorghum, 132 and 552.

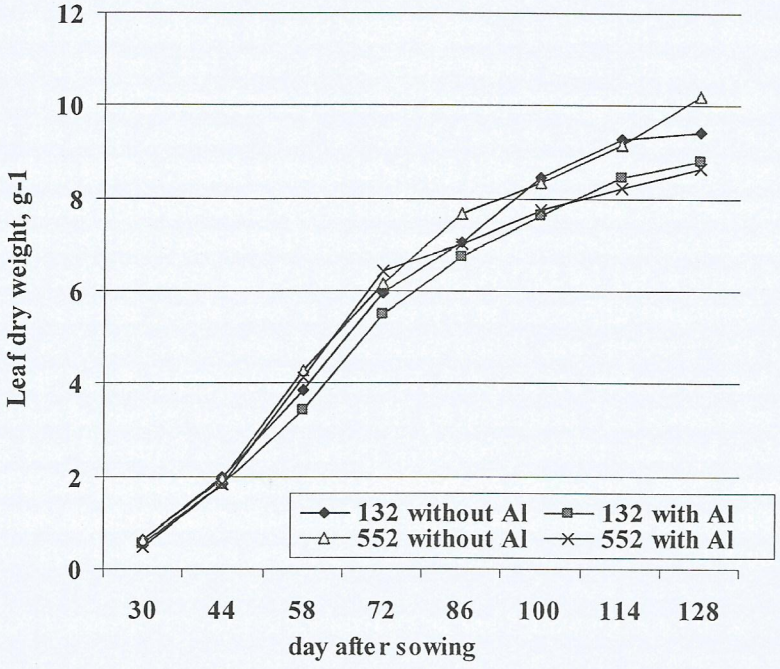

Figure 2. Changes in leaf dry weights with time to Al exposure for two cultivars of sorghum, 132 and 552.

Leaf area and toughness

Leaf expansion in cultivar 132 (6.43 mm day^{-1}) was faster than cultivar 552 (4.51 mm day^{-1}). At late harvest, leaf area significantly decreased compared to controls and Al treatment (cultivar 552 by 11.3 %; cultivar 132 by 7.1; %; $P< 0.001$; Figure 3; Table 3 and 4). The relative growth rate of leaves generally decreased with leaf maturation but leaf dry weight increased until early seventh harvest ($P<0.001$; Table 1). The dry weight leaf in cultivar 552 was bigger than 132 but leaf area in cultivar 132 was higher than 552 ($P<0.001$).

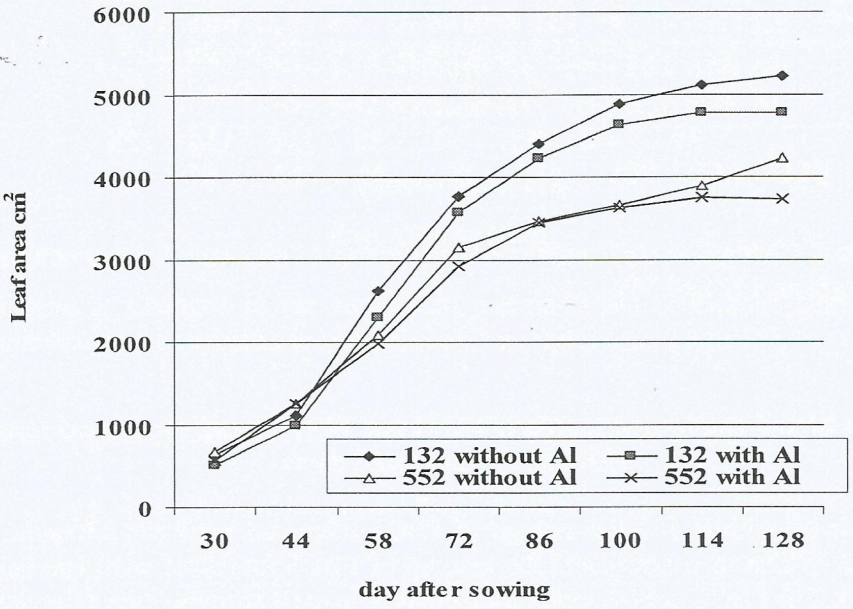

Figure 3. Changes in leaf area with duration of Al exposure in two cultivars of sorghum, 132 and 552.

Leaf toughness differed significantly with time of harvest and between species (Table 3, 4). With comparison of means, late leaves were significantly tougher than early formed leaves in both cultivars; in cultivar 552 it was significant (df 3, f= 7.15, $P<0.01$; Figure 4; Table 1 and 3), but not in cultivar 132 (Table 4). Although Al increased the toughness in cultivar 132, the difference was not significant. Leaf toughness was positively correlated with leaf dry weight and negatively with leaf area in cultivar 552 ($p= 0.004$; Table 2).

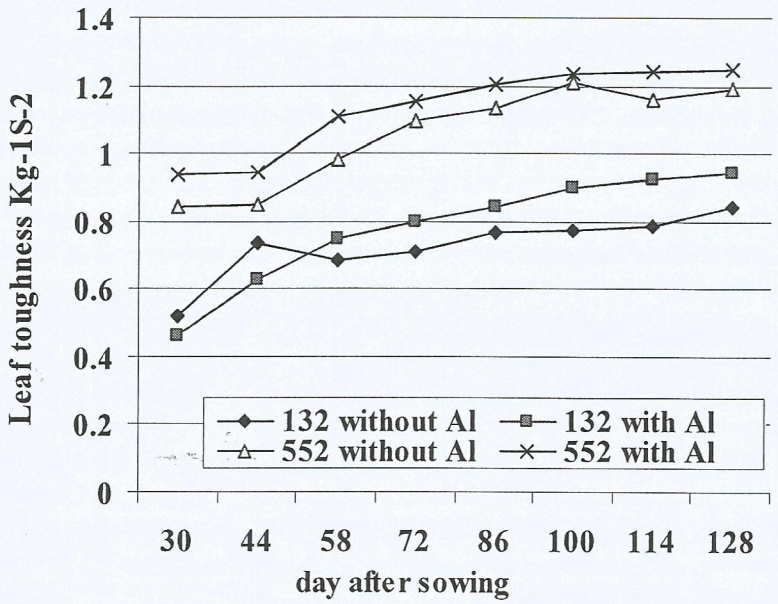

Figure 4. Changes in leaf toughness with time to Al exposure for two cultivars of sorghum, 132 and 552.

Protein-bound condensed tannin (PBCT) and fiber-bound condensed tannin (FBCT) in Leave and roots

In controls of cultivar 552 the amount of PBCT increased until two harvests, then decreased. Whilst in controls 132 the amount of PBCT increased until late harvest (Figure 5). The FBCT in both cultivars increased in early growth, then decreased as growth progressed (Figure 6). When comparing PBCT and FBCT, the amount of PBCT in leaves of both cultivars was higher than FBCT ($P<0.001$: Figure 5, 6). Al significantly increased the concentration of PBCT in the leaves of cultivar 552 (df.1, $f=16.46$; $P<0.001$) and cultivar 132 (df.1, $f=8.93$; $p<0.001$; Figure 5; Table 1); and in the roots of cultivar 552 (df.1, $f=4.43$, $P<0.005$) and cultivar 132 (df.1, $f=5.72$; $P<0.005$; Table 1). This was in contrast with FBCT; the concentration of which the cultivar 552 peaked in early growth and Al significantly declined thereafter in the leaves of cultivar 552 (df. 1, $f=6.25$; $P<0.001$; Figure 6; Table 1) and roots (df 1, $f=3.24$; $P<0.001$; Table 1, 3, 4). But in cultivar 132, Al significantly increased FBCT and together, they declined at fifth harvest. Meanwhile, Al significantly increased FBCT in the root ($P<0.001$; Table 1), whilst there was a significant decrease in FBCT of cultivar 132 by Al after fifth harvest in the leaves (Figure 6) and third harvest in roots (Figure 5; Table 1, 3, 4).

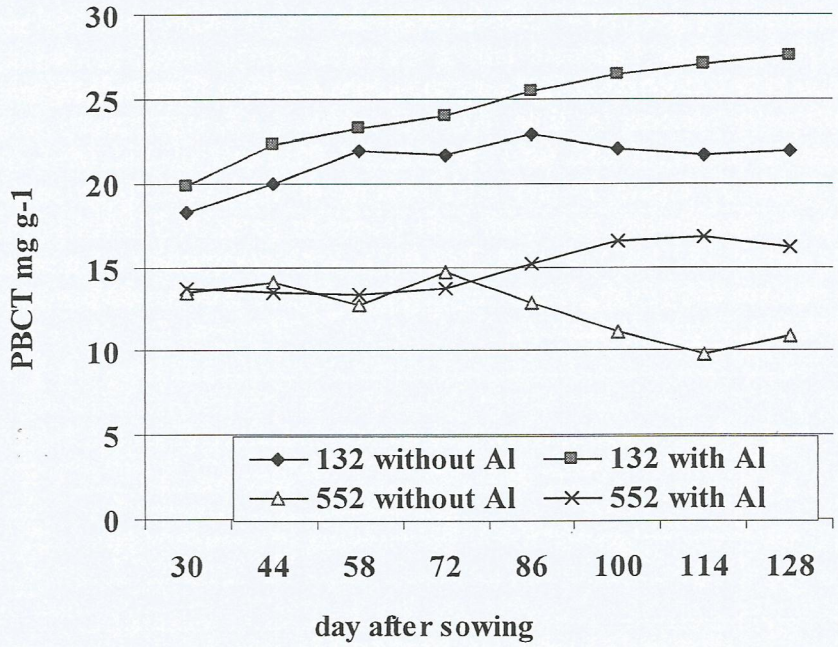

Figure 5. Changes in the amount of protein-bound condensed tannin with duration to Al exposure in two cultivars of sorghum, 132 and 552.

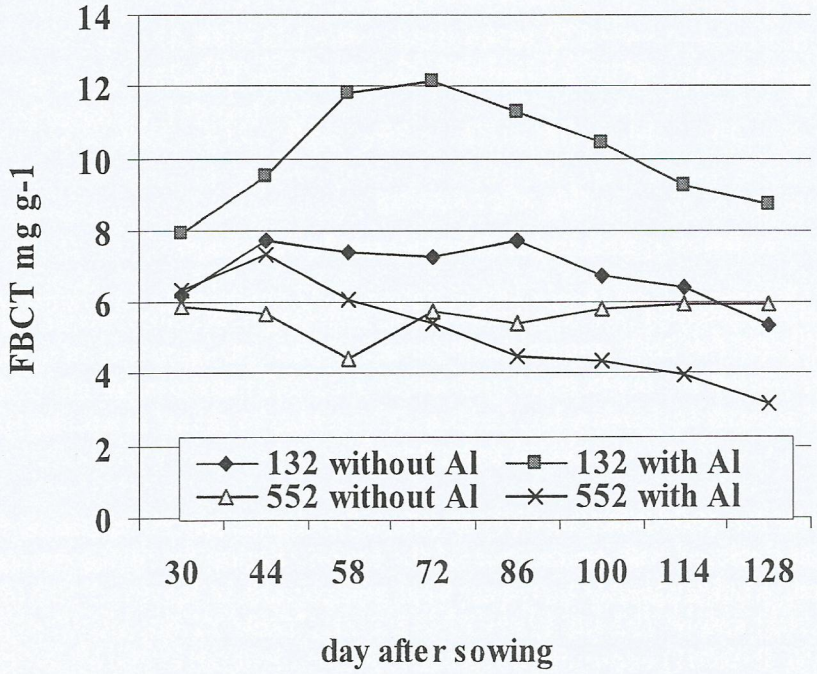

Figure 6. Changes in the amount of fiber-bound condensed tannin with duration of Al exposure in two cultivars of sorghum, 132 and 552.

Total tannins

Total tannin in two cultivars at first increased, then declined (Figure 7). Total tannin was significantly affected by time, AL and cultivars. AL significantly increased total tannin in leaves of 552 (df.1, f= 5.2; P< 001) and of 132 (df.1, f=3.49, P<0.001; Table 1; Figure 7) and with passing of time the effects was larger (P<0.001). Al significantly increased total tannin in roots of 552 (df.1, f=14.82; P<0.001), and of 132 (df 1, f=11.8; P<0.001). With comparison of means, total tannin in cultivar 132 (Table 4) was significantly higher than in cultivar 552 (df.1, f=8.32; P<0.001; Figure 7; Table 3).

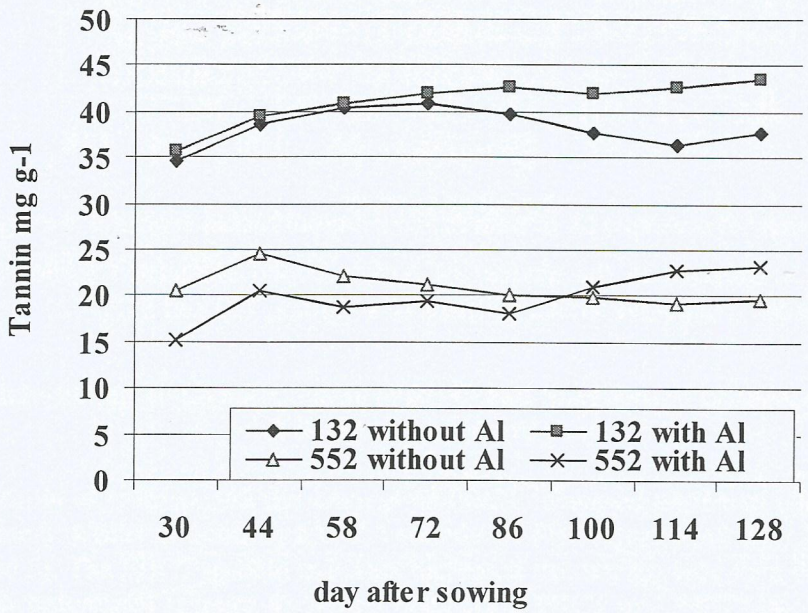

Figure 7. Changes in total tannin with duration to Al exposure in two cultivars of sorghum, 132 and 552.

Total polyphenols

In the controls for both cultivars the amount of polyphenols in leaves at first increased, then declined with senescence of the leaves (Figure 8). In the course of the experiment, Al significantly increased total polyphenols in leaves of 552 (df.1, f= 2.83, P<0.001) and of 132 (df 1, f = 3.14, P< 001; Table 1), but less in root of 552 (de.1=8.26, P<0.001) and root of 132 (df.1, f=5.61, P<0.001). The effect of Al on leaves (Figure 1) was higher than on roots (Figure 2). With comparison of means the concentration of total polyphenols in early leaves was about 60.22 mg g^{-1} in cultivar 132 (Table 4), and 39.17 mg g^{-1} in cultivar 552 (Table 3), and in late leaves,

cultivar 132 was 70.29 mg g^{-1} and cultivar 552, 43.11 mg g^{-1}. The amount of polyphenols in leaves of two cultivars at first increased until six successions of harvests, after which it became constant (Figure 8).

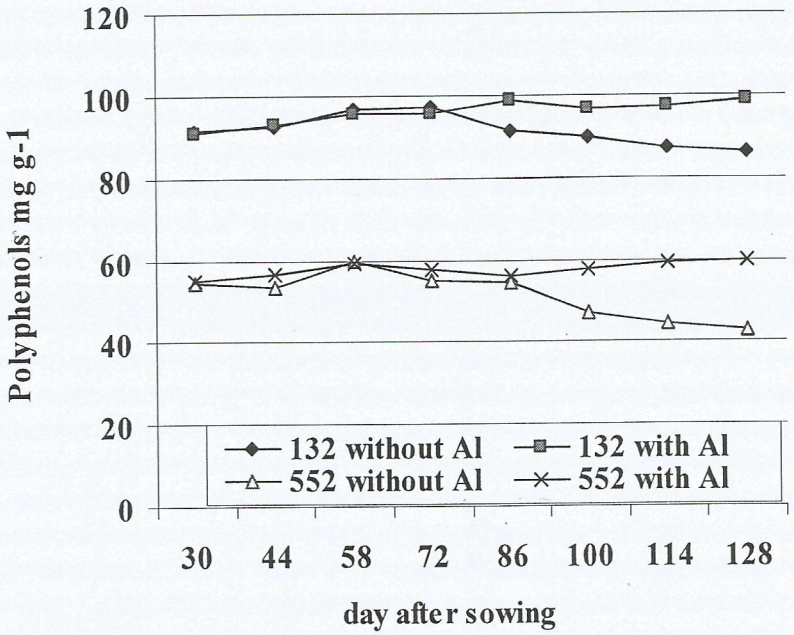

Figure 8. Changes in total tannin with duration to Al exposure in two cultivars of sorghum, 132 and 552.

Dynamics of tannins with regression prediction

With linear multiple regression in equation (4), we estimated relation between change in leaf area, dry weight leaves, total tannins, total polyphenols and PBCT, FBCT in experiment (Table 2). According to equation (4) below, the changes in total tannins in leaves of cultivar 552 with Al and without Al were as followed.

Total tannin = .63LA (2.76) + (1.92DL (2.73) + 3.81TP (8.36)
with Al (8.91)

Total tannin = .534LA (-5.47) +1.054dwt L (-6.73) + 2.42TP (12.36)
without Al (7.34)

(4)

Where LA: leaf area, dwt L: dwt leave, TP: total polyphenol. In the equation, total tannin was a dependent variable and total phenol, leaf area, and dwt leaves were independent variables. With comparison between two equations, Al decreased LA and dwt of the leaves. Therefore, all factors in the equations were positive. Any changes with Al, was observed to increase total tannin in cultivar 552 (Table 2).

In other comparison for tannins with Al and without Al, the transformation activity of tannin in cultivar 132 was much less than the rate of synthesis. But in cultivar 552 transformation of tannin was parallel with the rate of synthesis (Table 2). To predict the effects of Al on index of injury on LA, dwt leaf and dwt root, multiple regressions was done. The result showed that the injury of Al on dwt roots and leaf area was higher than other injuries on cultivars. The effects of Al on PBCT and FBCT were higher than other polyphenols, which function was defense.

To study the relationship between leaf dwt. and accumulation of tannins (PBCT and FBCT), we calculated the changes in the sum amount of all tannins between adjacent sampling dates, and the changes in the dwt of leaves during the same time. Since tannins form a large portion in cultivar 132 (>11%; Table 2; Table 4) and in cultivar 552 (<8 %; Table 2, 3) in leaves dwt, we subtracted the change in the amount of tannins from the change in leaf dwt within each time interval, and used the residual dwt as an indicator of leaf growth in the subsequent calculations. We used the mean values from this calculation to perform two kinds of comparisons, temporal and among four correlations.

In the temporal correlations residual growth and the change in the amount of tannins were averaged for the study of cultivars and divided by the number of days between the two consecutive sampling dates to obtain a mean RGR and a mean rate of accumulation. These relations were then correlated to discover whether a higher growth rate is connected to a low accumulation rate of tannins or polyphenols during the season because tannin was stable. Among four correlations, residual growth and the change in tannins we correlated within each time period between adjacent sampling dates, and found that both cultivars with actively growing leaves accumulated less tannins than slow growing leaves during the same time period (Table 2). It was observed that when leaves growth was active in both cultivars 132 and 552, Al increased PBCT and decreased FBCT and it was higher in cultivar 552 than in 132. When leaves growth was not active, the amount of tannin accumulation in both forms increased and was parallel (Table 3 and 4). The prediction for Al injury, Al effects, and the effects on LA, dwt root and total tannin were higher than other factors (Table 1 and 2).

DISCUSSION

Regardless of actual leaf and root age, clear difference exists in strength of defense between early and late leaves and roots in these two cultivars. Changes in leaf and root biochemistry during Al stress are important for sorghum resistance and its

value for the future fitness of sorghum, which is in agreement with the finding of Peter et al. (2001). For explanation of result we used hypotheses of the growth differentiation balance, GDB by Lorio (1988) and Carolyn et al. (2007).

According to these hypotheses, growth processes dominate over differentiation or allocation to polyphenols compound in which potential defensive should be low when plant growth was active. Our results support the hypothesis that Al decrease dwt of leaves and roots, and leaf area in two cultivars, and growth was decreased (Table 3 and 4). Therefore, in the accumulation of polyphenols, tannins is most active when leaf growth is declining (Carolyn et al. 2007). On the contrary, in controls of two cultivars, amounts of polyphenols in leaves increased until second harvest when leaves were actively growing, then decreased (Table 3 and 4). In late harvest when leaf growth had slowed down, the amount of polyphenols became constant (Gebrehiwot et al. 2002).

With GDB, the traits of tannins in leaves and roots were better than polyphenols because tannins were stable than polyphenols. The effects of Al were fitted with hypotheses GDB. Al increased allocation to polyphenols and tannins, which are potential defensive compounds. The amount should be low in the spring during rapid growth of two cultivars while during the summer it should increase (Karolewski et al. 1994). Among three correlations of leaf dry weight and accumulation of tannin between successive sampling dates, they were positive for most of stages and weekly, even in the early stages of both cultivars (Gebrehiwot et al. 2002). The week correlations in the early season hinted that there may be some physiological constraints on the production of tannin in the first growing leaves (Bennett et al. 1994). The result could be explained with the new method of transcription, as 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 (Jones et al. 1999). According to the protein competition model hypothesis, phenylalanine should be highest during leaf expansion in spring early growth, and allocation phenylalanine to those tannin content and derivative was very low. Therefore, the amount of tannin in early growth should simultaneously decrease. The amount of phenylalanine in late growth of leaf is fast declining because a section of the former has transformed into tannins, and that synthesis is lower than transformation process. In the case of tannins, which are stable end products, the accumulation directly corresponds to the synthesis of dwt of leaves (Vollen Weider et al. 2006).

The results of statistical regression ($R2 = (-0.41; F(3, 47) = 9.35, P<0.001$; Table 2) confirms that there was sign that Al is involved in the trade off between decrease leaf dwt, leaf area and increase production of tannins. The trade off may be due to the decreased recycling of tannin because tannin was stable. According to Riipi et al. (2002) at the interval of first harvest threshold for toxicity, chemical defense such as some terpens and alkaloides has rapid turnover and high cumulative to be cost effective than polyphenols for leaves. We conclude that Al toxicity could have changed the relations between syntheses of two groups of defense terpens and

alkaloids, and polyphenols. Thus, Al increased polyphenols in early growth. In Al treatment, at first the amount of tannin in cultivars 552 was low and then it increased (Table 3); therefore, with additional toxicity from Al chemical, defense system would alter as the leaf developed, and a fraction of product allocated for the synthesis of tannin (Juan et al. 2002).

If biomass allocation was the basis of resistance, we have focused mainly on cultivar 132, which had lower injury than 552. The RGR of leaves and LA in cultivar 132 were higher than 552, but the reversed occurred for dwt leaves. With passing of time, leaf toughness in cultivar 552 increased but not in cultivar 132 (Table 3, 4). Therefore, leaves expansion in cultivar 552 was retarded compared to 132 because toughness of the leaves became constraints to cell expansion and cell wall development. The FBCT in cultivar 552 in leaves and roots also decreased (Table 3) compared to cultivar 132 (Koupai et al. 1993).

In this study changes in dynamics of group tannins, FBCT and PBCT, together with total polyphenols were examined (Koupai et al. 1993). FBCT after an initial increase in concentration declined rapidly in the early growth of leaves and roots, and remained low until late harvest (Figure 6; Table 3 and 4). PBCT represents the major group of tannins and increased steadily from the first harvest until early harvest (Figure 5; Table 3). This was in contrast with the FBCT, the compound of cell wall; and with expanded leaf and root the amount was decreased, in agreement with the finding of Peter et al. (2001) and Mamoudou et al. (2002). FBCT were apparently involved in Al detoxification. They can, thus, complex with different heavy metals having more or less efficiency (McDonald et al. 1996). We have observed with passing of time Al toxicity increased and at the same time FBCT in leaves and roots decreased (Table 3, 4). Together with our observations and others (e.g. Hagerman et al. 1995), authors suggest that tannins have site, which could bind with Al, and that more than one sites was effective in binding Al in the cell walls of the analyzed samples. Recently, Vollenweider et al. (2006) described the ability of Al to be bound with plant cell walls. We suggest that FBCT can bind with Al.

We also observed that the polyphenols and tannins defense can clearly be very dynamic within developing leaves and roots as shown in this study. With regard to tannin concentration, approximately twofolds increase in leaves of cultivar 132 obtained, whilst the concentration in cultivars 552 was low but stable. We conclude that the allocation to defense can vary in both amount and type as observed in the two cultivars studied. The mechanism of fitness in cultivar 132 and 552 exhibited the mechanical resistances of the cultivars, as in agreement with the findings from Peter et al. in 2001. Concentration obtained in the developing leaves in both cultivars showed the influence of different growth stages (Table 3, 4) on chemical concentration, and each stage varies in resource concentration and protection. In cultivar 552, which leaf toughness in early growth stage was higher than cultivar 132, did not need to invest more in chemical defense compared to cultivar 132. In the former cultivar, the leaf also became tougher and could not

expand compared to the latter cultivar, hence, the level of putative defense compound increased in cultivar 132 as the less toughened leaves matured. Subsequently, the ratio of chemical to mechanical defense increased with a strong negative correlation between total polyphenols and leaf toughness, which also occurred in cultivar 552; there was strong negative correlation between leaf toughness and total polyphenols (Table 2; Bennett et al. 1994; Peter et al. 2001).

The amount of chemical defense by Al changed with growth stages. The FBCT were detected higher in young leaves (mobile defense) at growth stages 1-2 compared to leaves in the later stages entirely due to dilution by cell wall, which caused leaves of cultivar 552 to be tough; but in cultivar 132, the FBCT decreased after fifth harvest and the leaves were not toughened. Conversely, the amount of PBCT at all growth stages in both cultivars increased. AL increased in the leaves of controlled cultivar 132 although it was not significant with subsequent relativel poor mechanical properties. Similar results were also reported by Vollenweider et al. in 2006.

CONCLUSION

Our results indicated that the allocation to defense can vary in both amount and type in two cultivars. The mechanism of fitness for chemical resistances in two cultivars of sorghum, 132 and 552, invested highly their defense in the late leaves. In cultivar 552, leaf was tougher than in 132 during early expansion, hence, invested less in its chemical defense. Simultaneously, the FBCT, compound of cell wall, and time of leaf and root expansion were all reduced. We suggest that FBCT can bind with Al, and that PBCT represents the major group of tannins, which increased steadily from the first harvest until early harvest.

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Table 1. F-values from the analysis of variance for the effects of Al on leaf dry weight (dwt. L), root dry weight (dwt R), relative growth rate (RGR), leaf area (LA), protein- bound condensed tannin (PBCT), fiber- bound condensed tannin (FBCT), total tannin (TT), total polyphenol (TP) and leaf toughness (TN) in two cultivars.

Factors	d.f.	LA cm^{-2}	dwt. L g^{-1}	dwt. R g^{-1}	RGR $g^{-1} d^{-1}$	PBCT mg/g^{-1}	FBCT mg/g^{-1}	TT mg/g^{-1}	TP mg /g^{-1}	TN $Kg\ m^{-1}\ s^{-2}$
harvest	7	23.41***	42.***	14.3***	3.45*	8.1***	6.7***	11.6***	16.2***	6.4**
cultivars	1	4.54**	4.79**	3.15**	4.15**	3.12**	1.2	3.51**	5.6**	7.15**
Al	1	6.79***	5.27**	3.46**	3.15**	4.16**	6.4***	5.2***	3.14**	1.3
harvest× cultivar	7	4.37**	3.28*	4.51**	2.45*	3.11*	3.4*	4.21**	5.4**	1.02
harvest ×Al	7	5.12**	5.71**	3.7*	2.73	2.54	2.56	2.58	2.02	2.15
cultivar Al	1	3.54**	2.13*	3.86**	3.82**	2.32	3.4**	1.14	2.18*	1.24
harvest× cultivar× Al	7	1.46	1.03	2.4*	1.15	0.48	0.12	4.11*	2.42*	0.14

*** P<0.001, ** P<0.005 and * P<0.01

Table 2. Correlations between two variations and partial variations predicators with index dependent variable Al injury.

Variables	Correlation between some indicators and index effect of Al	P	Correlation between some indicators and effects of Al with control and other variables	P
leaf area	-0.39	***	-0.31	**
d wt. leaf	-0.23	**	-0.18	
d wt. root	-0.26	***	-0.13	*
d wt. total	-0.18	*	-0.1	
total phenol	-0.36	**	-0.27	**
total tannin	-0.41	***	-0.31	**
protein bound CT	-0.26	**	-0.18	*
fiber bound CT	-0.23	*	-0.11	

*** P<0.001, ** P<0.005 and * P<0.01

Estimated coefficients and P-values of multiple linear regression models presented in equation for each factor.

Table 3. The comparison mean values of PBCT concentration, FBCT concentration, total tannin (TT), total polyphenols (TP), toughness (TN), leaf area (LA), dry weight leaves (dwt), dry weight root (dwt) in cultivar 552. The initials without Al, with Al and day after sowing are briefly indicated as W.O Al, W. Al, and D.A.S, respectively.

Cultivar 552

PBCT mg. g^{-1}				FBCT mg. g^{-1}			LA cm^{-2}			d wt.leaf g^{-1}			d.wt root g^{-1}			toughness kg m^{-1} s^{-2}			total phenol mg. g^{-}			total tannin mg. g^{-1}		
D.A.S	W.Al	W.o.Al	P	W. Al	W.o Al	P	W. Al	W.o.Al	p	W. Al	W.o.Al	p	W.Al	W.o.Al	p	W.Al	W.o. Al	p	W.Al	W.o.Al	p	W. Al	W.o.Al	p
33	13.7	13.5	n.s	6.4	5..9	n.s	563	672	*	0.5	0.6	n.s	0.3	0.2	n.s	0.9	0.8	**	55.2	54.6	n.s	15.3	20.6	***
44	13. 5	14.1	ns	7.3	5.7	**	1259	1248	n.s	1.8	1.9	n.s	1.1	1.2	n.s	0.9	0.8	**	56.2	56.1	n.s	20.5	24.6	***
58	13.7	12.3	n.s	6.1	4.4	**	1981	2094	*	4.1	4.3	n.s	1.7	1.9	n.s	1.2	1.1	***	59.4	59.6	n.s	18.8	22.2	**
72	14.6	13.2	n.s	5.4	5.7	n.s	2928	3150	***	6.1	6.6	*	2.1	2.5	*	1.2	1.1	**	56.3	54.5	**	19.4	21.1	**
86	15.1	12.9	***	4.5	5.4	n.s	3460	3463	n.s	7	7.7	**	2.8	3.4	**	1.23	1.1	***	56.7	47.1	***	18.8	20.2	**
100	16.6	11.1	***	4.4	5.8	*	3636	3698	n.s	7.8	8.4	**	3.3	4.4	**	1.28	1.1	***	57.9	47.	***	21.3	20.8	*
114	16.8	9.7	***	4.1	5.9	**	3752	3889	**	8.2	9.3	***	3.9	5	***	1.28	1.1	***	56.4	47.2	***	23.8	21.3	**
128	16.1	10.8	****	3.2	6.1	***	3743	4225	***	8.6	10.2	***	4.2	5.3	***	1.28	1.11	***	55.2	47.3	***	24.3	20.8	***

Estimate mean values of variation between two means: significant in ***, $P<0.001$, **, $P<0.005$ and *, $P<0.01$; and n.s., not significant.

Table 4. The comparison mean values of PBCT concentration, FBCT concentration, total tannin (TT), total polyphenols (TP), toughness (TN), leaf area (LA), dry weight leaves (dwt), dry weight root (dwt) in cultivar 552. The initials without Al, with Al and day after sowing are briefly indicated as W.O Al, W. Al, and D.A.S., respectively.

Cultivar 132

	PBCT mg. g^{-1}			FBCT mg. g^{-1}			LA cm^{-2}			d wt.leaf g^{-1}			d.wt root g^{-1}			toughness kg m^{-1} s^{-2}			total phenol mg. g^{-}			total tannin mg. g^{-1}		
D.A.S.	W. Al	W.o.Al	P	W. Al	W.o Al	P	W. Al	W.o.Al	P	W. Al	W.o.Al	P	W.Al	W.o.Al	P	W.Al	W.o. Al	P	W.Al	W.o.Al	P	W. Al	W.o.Al	P
33	19.9	18.2	n.s	7.9	6.2	n.s	635	507	**	0.5	0.6	n.s	0.2	0.4	n.s	0.52	0.5	n.s	90.9	91.7	n.s	35.6	34.6	n.s
44	22.3	21.0	n.s	9.5	7.8	**	1122	983	**	1.8	2	n.s	1.2	1.5	*	0.5	0.5	n.s	93.1	98.6	**	39.4	38.2	n.s
58	23.2	22	n.s	11.8	7.4	***	2618	2308	***	3.4	3.9	n.s	2.5	2.8	*	0.8	0.75	n.s	95.9	97.8	n.s	40.8	41.4	n.s
72	24	21.7	**	12.1	7.2	***	3763	3583	***	5.3	6	*	3.1	3.5	n.s	0.8	0.76	n.s	95.8	96.3	n.s	41.9	40.2	**
86	25.7	23	***	11.3	7.8	***	4403	4237	**	6.8	7.1	**	3.8	4.6	**	0.81	0.8	n.s	99.1	91.4	***	42.5	39.6	***
100	26.4	22	***	10.4	6.7	***	4883	4643	***	7.6	8.5	***	3.9	4.4	***	0.9	0.86	n.s	92	89.2	***	42.3	37.6	***
114	27	21.6	***	9.3	6.4	***	5111	4772	***	8.5	9.3	**	4.2	4.9	***	0.9	0.87	n.s	93.3	82.7	***	42.6	33.4	***
128	27.5	21.9	***	8.7	5.4	***	5217	4782	***	8.8	9.5	**	4.4	5.1	***	0.9	0.86	n.s	91.2	79.2	***	43.4	32.8	***

Estimate mean values of variation between two means: significant in ***, $P<0.001$, **, $P<0.005$ and *, $P<0.01$; and n.s., not significant.