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## Genetic gain of Al tolerance and contribution of agronomic traits on Al tolerance in the early stage of sorghum breeding program

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### ABSTRACT

Genetic gain (GG), heritability and contribution of the agronomic traits on Al tolerance are precious information for selection strategy in the breeding of Al-tolerant sorghum. The objectives of this study were (i) to estimate the GG of Al tolerance in sorghum and (ii) to determine the direct effect of agronomic traits on Al tolerance through path analysis. Evaluation of agronomic characters used  $F_2$ , and  $F_3$  sorghum progenies in field experiment and screening of Al tolerance used the hematoxylin-staining method. Three sorghum populations showed the low GG of Al tolerance ( $GG = 0.558-1.108$ ). This result indicates that high allocation of progenies in the early stages of the Al-tolerant breeding program and high selection differential ( $sd$ ) of Al tolerance should be applied to obtain a higher gain of Al tolerance. Selection pressure for early maturity ( $sd = -2.942$ ) and short plant height (PH) ( $sd = -38.165$ ) resulted in lower improvement of GG for Al tolerance. The high dry weight and tall PH provided a more genetic contribution to the advancement of Al tolerance. Based on the overall path analysis data, the increase of plant biomass, the significant number of progenies in the early stage of breeding and strong  $sd$  of Al tolerance would be more appropriate to improve the Al tolerance in sorghum.

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### Introduction

The breeding of sorghum tolerant to the Al toxicity is the primary goal for the improvement of sorghum yield in acid soil. Acid soil can indicate a high abundance of  $Al^{3+}$  in the land which is very toxic to plants. Studies on the genetic control of Al tolerance showed some different results even in the same crop species (Ezaki, Katsuhara, Kawamura & Matsumoto, 2001; Shu et al., 2015). There were also various reports on the mechanisms of Al tolerance within and among plant species (Simões, Melo, Magalhaes & Guimarães, 2012). These reflect variation in the genetic control of Al tolerance of some plants (Caniato et al., 2007). Sometimes, there was inconsistency in explanation of the number of gene-controlled Al tolerance in some species. This information creates difficulties in the selection strategy of Al tolerance.

The breeders are faced with a difficult decision of screening time, and how many genetic resources should be maintained in the early stages of Al-tolerant breeding program. Allocation of genetic resources in each generation of collection needs genetic gain (GG) information of Al tolerance. Testing of genotypes in a massive amount of progeny is costly, and handling of selection will be more difficult if the number of

plants and genotypes increases. Use of GG information enables breeders to improve efficiency in selection methods (Daetwyler, Hayden, Spangenberg & Hayes, 2015; Engel, Higa, Andrejow, Flôres Junior & Soares, 2016; St. Martin & Futi, 2000). However, there is limited GG information of Al tolerance that has been reported to improve the efficiency of sorghum breeding program.

The knowledge on the strength of association among important traits is proven a useful tool to improve the quantitative characters. Since multiple alleles affected Al tolerance of sorghum, simultaneous selection and partition of characters into direct and indirect influence on Al tolerance is more proper to accelerate Al-tolerant character. Some researchers reported the relationship between yield components and yield in various crops including rice, maize, soybean and wheat (Ball, McNew, Vories, Keisling & Purcell, 2001; Cooper et al., 2012; Mohammadi, Prasanna & Singh, 2003). There was also the relationship between drought tolerance character and physiological characters in sugar beet (Ober et al., 2005).

Since the excellent phenotypic performance of the plant in the acid soil represents a tolerant plant to the Al toxicity, we should optimize these agronomic traits

to improve Al tolerance. Al-tolerant plants showed better phenotypic performance than susceptible plants in acid soil or tissue culture, and different response of agronomic traits in acid soil was also reported (Anas & Yoshida, 2002; Flores, Clark & Gourley, 1988; Zaifnejad, Clark & Sullivan, 1997). However, there is no report so far of the direct and indirect effect of agronomic traits on Al tolerance in sorghum. Therefore, the combined information of the GG of Al tolerance and the direct and indirect effect of agronomic characteristics to Al tolerance is essential information for the breeder to accelerate and determine a selection strategy.

This present study aimed (i) to estimate the GG of Al tolerance in three breeding selection (BS) sorghum populations, i.e. Al tolerance, early maturity and short plant height (PH) sorghum population and (ii) to observe the direct and indirect effect of agronomic traits on the enhancement of Al tolerance in sorghum.

## Materials and methods

### *Development of recombinant inbred (RI) population*

RI populations were developed in this study by the crossing of parents with contrasting aluminum tolerance that was selected for their differential response to aluminum toxicity (Anas & Yoshida, 2000). C9/H11 and C9/H13 parents are Al-susceptible inbred lines, and ICR3 parent is an Al-tolerant line (Anas & Yoshida, 2004). The C9/H11 parent also showed excellent performance for early-maturity and medium-PH, while the C9/H13 parent is a late-maturity and tall PH inbred line. The RI populations developed from two sets of the crossing of C9/H11//ICR3 and C9/H13//ICR3.

The seed of the RI population used for experiments was grown under standard nursery conditions with the planting size 25 cm spacing between plants and 65 cm spacing between rows and generated in the same season. Soil pH was around 6.7–7.0 and fertilizers of 90 kg N ha<sup>-1</sup>, 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 35 K<sub>2</sub>O ha<sup>-1</sup> were applied for all RI populations (parents, F<sub>2</sub> and F<sub>3</sub> population). One-third of nitrogen fertilizer was given when planting, and the rest two-third was applied before the flowering stage. The phosphorus and potassium fertilizers are provided at once when planting.

The RI populations were developed using the modification of pedigree selection method, in which only selected plants of the F<sub>2</sub> segregating population are grown to form the next generation. The hematoxylin-staining screening method was applied to screen Al-tolerant plant in all of RI populations.

The minimum number of F<sub>2</sub> plants to be evaluated has considered the equation of Muller (1923). One hundred eighty-three F<sub>2</sub> plants from each set of the crossing were planted in the subsequent generation of selfing. These RI populations refer to grain sorghum 1 (GS1) population and grain sorghum 2 (GS2) population, respectively. There were two GS blocks for F<sub>2</sub> populations and blocks for parents.

The F<sub>3</sub> population was generated from selfing of selected Al-tolerant F<sub>2</sub> plants that were scored above three by the hematoxylin-staining screening method. Fifty-seven plants of the GS1 population and 70 plants of the GS2 population have been selected, and 30 seeds of each selected plants were grown in separate plots to form the next generation.

### *Evaluation of agronomic characters*

Each sorghum population (parents, F<sub>2</sub>, and F<sub>3</sub>) were grown in a separate plot for observation of agronomic performance. PH (from the ground to the tip of a panicle of the main stalk) and days to flowering (DF – days of panicle appear from flag leaf) were recorded before plant harvested. Dry weight (DW), grain weight (GW), length of head (LH – from the first branch of panicle inflorescence to the panicle tip), and harvest index (HI – the ratio of GW to the total above-ground weight) were obtained after plant harvested. Agronomic traits data were recorded from an individual plant of both GS sorghum populations for GG and path analysis. Three groups of parent populations were also grown to record agronomic traits data.

### *Evaluation of Al tolerance*

Hematoxylin-staining screening method according to Anas and Yoshida (2000) was applied to determine Al tolerance of individual plant in F<sub>2</sub>, F<sub>3</sub> and parent population. The hematoxylin-staining screening method was well-established as Al-tolerant screening method for some crop species (Giaveno & Filho, 2000; Ma, Zheng, Li, Takeda & Matsumoto, 1997; Polle, Konzak & Kittrick, 1978). Since hematoxylin-staining screening method showed a significant genetic correlation with some agronomic characters of sorghum under field condition, selected plant by hematoxylin-screening method might affect the performance of plants in the field (Anas & Yoshida, 2004). There was also a high correlation of hematoxylin-screening method with root growth of sorghum seedling (Anas & Yoshida, 2000), seedling of maize (Giaveno & Filho, 2000) and Brachiariagrass seedling (Wenzl et al., 2006). Meanwhile, the root growth indices in nutrient solution were highly associated with plant performance in acid soil under field conditions (Pereira, 2018).

Ten seeds of each plant from  $F_2$ ,  $F_3$  and parent population were harvested for evaluation of Al tolerance. The seeds were then germinated directly on the cork-planting tray (19.3cm × 25.3cm with 560 seed holes) in the plastic container filled with a nutrient solution (4.0 mM  $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ , 6.5 mM  $\text{KNO}_3$ , 2.5 mM  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.1 mM  $(\text{NH}_2)\text{SO}_4$ , 0.4 mM  $\text{NH}_4\text{NO}_3$ ) that was adjusted to pH 4.0 with 0.25 M HCl.

Seedlings were grown for 48 h, and 53.60 ppm Al was added to the nutrient solution from 0.1 M  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  stock solution after 31 h seedling grew in nutrient solution. The planting tray then placed on distilled water for 30 min and moved for 15 min to a container with 0.2% hematoxylin solution.

The different number of staining pattern of ten seedling roots from each of  $F_2$ ,  $F_3$  and parent plant determined Al tolerance of sorghum plant. Sorghum seedlings with two and maximum of four stained roots had a score of 1 (very tolerant) and 2 (tolerant), respectively. Sorghum seedling which the root stained more than eight had a score of 5 (very vulnerable), and sorghum was classified as susceptible (4 score) if eight colored roots were observed and as intermediate (3 score) is if six stained roots were found.

### Statistical analysis

Based on the performance of the individual plant in the field and hematoxylin-staining screening, the sorghum population grouped into three categories for the elaboration of the effect of early maturity (DF < 50 days) and short PH < 140 cm on the gain of Al tolerance and other agronomic traits. These groups then referred to Al tolerance, early maturity and short PH BS groups after this.

The realized GG for Al tolerance, DF, short PH and GW were calculated according to St. Martin and Futi (2000) and estimated for each sorghum population.

$$\text{Genetic gain} = (X_s - X_{ps}) - (X - X_{po})$$

where  $X_s$  is a mean of selected population in successor population and  $X_{ps}$  is a mean of parents in successor population;  $X$  is a mean of the original population;  $X_{po}$  is a mean of parents in the original population.

Selection differential (sd) calculated the changing of the selection for each character at any population as follows:

$$\text{sd} = (X_s - X)$$

where  $X_s$  is a mean of selected plants,  $X$  is an overall mean of the original population.

Data for regression and path analysis used the complete data of plant record for agronomic characters and Al tolerance from  $F_2$  until  $F_3$  generation. The linear inter-relationship between Al tolerance and other agronomic

characters is illustrated in the regression model as follows:

$$\text{Al-F}_3 = \beta_0 + \beta_1(\text{DW}) + \beta_2(\text{LH}) + \beta_3(\text{DF}) + \beta_4(\text{PH}) + \beta_5(\text{HI}) + \beta_6(\text{GW}) + \beta_7(\text{Al-F}_2)$$

Collinearity diagnostic was performed to increase the accuracy of the regression model and to screen the unstable variable's regression coefficients. Pearson correlation, multiple linear regression analysis and data validation were conducted using SPSS 10.1 for Windows software program (SPSS for Windows, 1999).

A path coefficient is defined as the portion of the standard deviation of the dependent variable that is due to the variation of an independent variable and is simply a standardized partial-regression coefficient (Dewey & Lu, 1959; Li, 1956) as follows:

$$P_{xy} = B\sigma_x/\sigma_y$$

where  $P_{xy}$  is the path coefficient for the direct path from  $X$  (independent variables – seven agronomic characters) to  $Y$  (dependent variable – Al-F<sub>3</sub>);  $B$  is regression coefficient of  $Y$  on  $X$ ;  $\sigma_x$  and  $\sigma_y$  are the standard deviation of  $X$  and  $Y$ , respectively.

The correlation coefficient ( $r$ ) of the compound path between an agronomic character and Al tolerance in an  $F_3$  generation is the sum of the values of the two paths connecting them as follows:

$$r_{x,y} = P_{x,y} + r_{x1x2}P_{x2y} + r_{x1x3}P_{x3y} + r_{x1x4}P_{x4y} + r_{x1x5}P_{x5y} + r_{x1x6}P_{x6y} + r_{x1x7}P_{x7y}$$

$r_{x,y}$  is a correlation coefficient of an independent variable ( $X$ ) to the dependent variable ( $Y$ ). The  $P_{x,y}$  is path coefficient for the direct path from an  $X$  (independent variable) to  $Y$  (dependent variable). The  $r_{x,x}P_{x,y}$  is an indirect effect via an  $X$  independent variable that is calculated by multiplying the correlation between a corresponding independent variable ( $r_{x,x}$ ) with its direct effect ( $P_{x,y}$ ).

## Results

### The GG of Al tolerance

The GG of characters for three sorghum populations referred to Table 1. The GG of Al tolerance in the Al-tolerant sorghum population was moderately low (0.558), although sd of Al tolerance was high in the  $F_2$  generation. The GG of Al tolerance in early maturity and short PH of sorghum population was also meager which was only GG of 1.108 and 0.906, respectively.

Improvement in a GG of the early maturity was shown only in the early-maturity BS sorghum population. It was demonstrated by the decrease of DF in the early-maturity BS sorghum population. However, the reduction of DF was

**Table 1.** Genetic gains (GG) of AI tolerance, days to flowering, plant height and grain weight in three breeding selection (BS) sorghum populations.

Breeding selection	Genetic gain <sup>a</sup>				Selection differential <sup>b</sup>
	AI tolerance	Days to flowering (d)	Plant height (cm)	Grain weight (g plant <sup>-1</sup> )	
AI-tolerant population	0.558	0.103	-45.770	6.028	-1.089
Early-maturity population	1.108	-1.710	-54.233	-6.375	-2.942
Short plant height population	0.906	2.544	-67.311	-1.301	-38.165

<sup>a</sup>Genetic gain was calculated from the average of GS1 and GS2 sorghum population.

<sup>b</sup>Selection differential was only calculated for AI tolerance in AI-tolerant population, early maturity in early-maturity population and plant height in short plant height population. Positive values of AI-tolerant genetic gain showed low improvement in AI tolerance. Scores 1 and 5 are very tolerant and very susceptible, respectively. The negative genetic gain of early maturity and short plant height population showed a decrease of days to flowering or plant height. The positive genetic gain of grain weight showed improvement of grain number of the sorghum plant.

still low (-1.710) in magnitude compared to the sd (sd = -2.942). The result also showed that there was a little improvement of AI tolerance (GG = 1.108) in this population (Table 1).

The GG of PH was negative in all BS sorghum population. This data indicated that there was a significant reduction of PH in the successor population in all BS sorghum populations. The GG of AI tolerance in short PH BS sorghum population was only 0.906. However, the GG of AI tolerance in the early maturity BS sorghum population was 1.108 (Table 1). These results suggest that the improvement of AI tolerance would be faster in short PH BS population than in the early-maturity BS sorghum population.

GG for GW was moderately low in all BS sorghum population. However, improvement of GW in AI tolerance BS sorghum population was higher than the other BS sorghum population. The GW slightly increased in AI tolerance BS sorghum population during the selection of AI tolerance implemented to AI tolerance BS sorghum population. On the other hand, early-maturity and short PH BS sorghum population showed the negative GG of GW. These findings indicate that intensifying selection for early-maturity and short PH has a consequence on a decrease in GW.

### The contribution of agronomic characters to AI tolerance

The collinearity test confirmed that there was severe multicollinearity with the GW character in the regression model (Table 2). The tolerance value was small,

**Table 2.** The collinearity test of grain weight character (GW) in the regression model of the contribution of seven agronomic characters on the AI tolerance in an F<sub>3</sub> generation (AI-F<sub>3</sub>).

Sorghum population	Tolerance	Variance of inflated (VIF)
C9/H11//ICR3	0.007	152.026
C9/H13//ICR3	0.007	144.073

Note independent variables: dry weight (DW), grain weight (GW), length of head (LH), days to flowering (DF), plant height (PH), harvest index (HI) and AI tolerance in F<sub>2</sub> (AI-F<sub>2</sub>). Dependent variable: AI tolerance in the F<sub>3</sub> generation (AI-F<sub>3</sub>).

indicating that the GW is highly intercorrelated with other agronomic characters. It showed that the other agronomic characters could explain 70–90% of the variance in GW. When the tolerance values were close to 0, there was high multicollinearity, and the standard error of the regression coefficients will be inflated. A variance inflation factor (VIF) was very high in this character (Table 2). It is not surprising if GW had highly significant correlations with the almost other agronomic characters in both GS populations. The GW showed no significant correlation only with the DF in GS2 population.

Therefore, the GW was excluded from the linear regression model because it showed a linear combination to the other independent variables. The ANOVA of multiple linear regression coefficients that excluded the GW characters as predictor variable showed a significant difference ( $F \leq 0.05$ ) for both GS sorghum populations (Table 3). There was a direct relationship between six independent variables and the dependent variable in the regression model (Figure 1). The multiple linear regression models of GS1 and GS2 population explained the variation in AI tolerance with the R<sup>2</sup> value of 0.501 and 0.555, respectively (Table 3). These models mean that six agronomic traits included in these models revealed more than 50% variability of AI tolerance in the F<sub>3</sub> generation (Figure 1).

### The population of grain sorghum-1 (GS1)

The DW, LH, HI and AI-tolerant F<sub>2</sub> significantly influenced the improvement of AI tolerance of sorghum. The excellent performance of DW indicated the highest direct influence on AI tolerance in this population (Table 4). The direct and indirect contribution of DW was more dominant than the other agronomic traits for the improvement of AI tolerance in GS1 population.

AI tolerance in the F<sub>2</sub> generation had affected the improvement of AI tolerance in the F<sub>3</sub> generation (Table 4). However, the direct effect of AI tolerance in F<sub>2</sub> on the progression of AI tolerance in F<sub>3</sub> was relatively small (0.195). The DW character indirectly contributed (0.113) to the enhancement of AI tolerance in the F<sub>2</sub>



**Table 3.** The coefficient of determination and significance of the regression model for both C9/H11//ICR3 and C9/H13//ICR3 sorghum population.

Population	R	R <sup>2</sup>	Adjusted R <sup>2</sup>	Std. error of the estimate	Significance of the model
C9/H11//ICR3	0.707 <sup>a</sup>	0.501	0.317	1.019	0.045
C9/H13//ICR3	0.744 <sup>a</sup>	0.555	0.247	0.908	0.049

<sup>a</sup>Six independent variables: DW, LH, DF, PH, HI and Al-F<sub>2</sub>. Dependent variable: Al-F<sub>3</sub>.

generation. Meanwhile, the remaining indirect effects of agronomic traits on Al tolerance in the F<sub>2</sub> population contributed equally to the improvement of Al tolerance in the F<sub>3</sub> generation.

The HI and length of the head showed a small direct effect on improvement of Al tolerance in the F<sub>3</sub> generation. HI and LH affected Al tolerance mainly through their indirect effect via high DW, which showed an excellent influence by the improvement of Al tolerance in the F<sub>3</sub> generation. The DF and PH had no significant correlation with Al tolerance in this population.

#### The population of grain sorghum-2 (GS2)

All agronomic traits except HI significantly influenced the Al tolerance in the F<sub>3</sub> generation (Table 5). The PH showed the highest contribution ( $r = -0.535$ ) to the improvement of Al tolerance of F<sub>3</sub> generation, which it was mainly contributed by the direct effect ( $r = .80.888$ ) of PH on Al tolerance.. The direct and indirect contribution of PH was more dominant to the

improvement of Al tolerance in GS2 population compared to the other agronomic traits (Table 5).

DF and Al tolerance showed a significant correlation in this population, although early DF had no direct contribution to the improvement of Al tolerance. The indirect contribution of PH ( $r = -0.422$ ) compensated the absence of contribution of early DF on the Al tolerance.

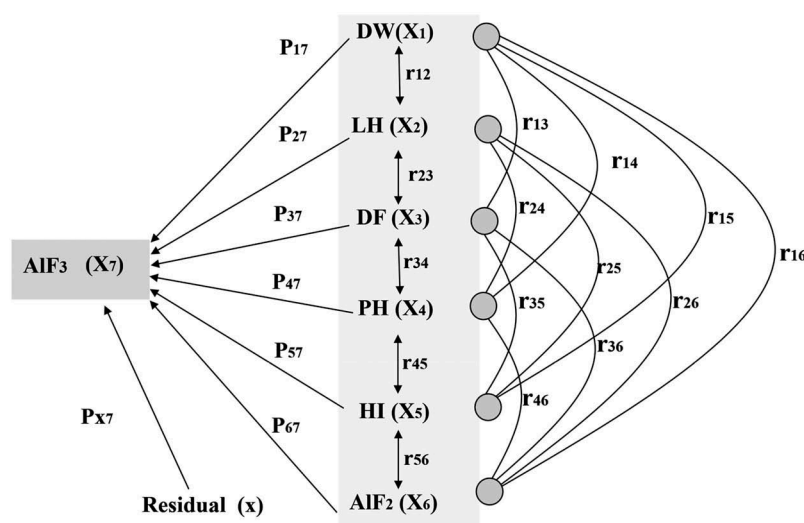
The effect of Al tolerance in the F<sub>2</sub> generation to F<sub>3</sub> generation of the GS2 population was higher in magnitude compared to the GS1 population. However, the indirect effect of the PH mainly contributed to the Al tolerance trait in this F<sub>2</sub> population.

The LH and DW mainly affected Al tolerance through the indirect effect of PH that was higher in magnitude than the direct effect of these traits on Al tolerance. The LH and PH both contributed to the improvement of Al tolerance.

#### Discussion

The GG of Al tolerance in three BS sorghum populations was relatively low. It seems that the high segregation of Al tolerance occurred in the F<sub>3</sub> sorghum generation. In fact, there was a just little improvement of Al tolerance in the F<sub>3</sub> sorghum population (Table 1). Anas and Yoshida (2004) have reported that low realize heritability (0.35–0.43%) of Al tolerance was observed in sorghum populations.

The low GG of Al tolerance suggested that several genes might have controlled Al tolerance in sorghum. Caniato et al. (2007) reported multiple alleles at the *Alt<sub>5B</sub>* locus with



**Figure 1.** Path diagram shows a causal relationship between the independent variable, Al tolerance in F<sub>3</sub> (AIF<sub>3</sub>-X<sub>7</sub>), and the six component variables, dry weight (DW-X<sub>1</sub>), length of head (LH-X<sub>2</sub>), days to flowering (DF-X<sub>3</sub>), plant height (PH-X<sub>4</sub>), harvest index (HI-X<sub>5</sub>) and Al tolerance in F<sub>2</sub> (AIF<sub>2</sub>-X<sub>6</sub>). Path coefficients (direct effect on Al tolerance in F<sub>3</sub>) are represented by P<sub>17</sub>, P<sub>27</sub>, P<sub>37</sub>, P<sub>47</sub>, P<sub>57</sub> and P<sub>67</sub>.  $r_{x,x}$  show correlations among independent variables.

**Table 4.** Direct effect ( $P_{x7}$ ) and indirect effect ( $r$ ) of dry weight (DW), length of head (LH), days to flowering (DF), plant height (PH) and Al tolerance in  $F_2$  (Al- $F_2$ ) on Al tolerance in the  $F_3$  generation (Al- $F_3$ ) for GS1 sorghum population.

	DW	LH	DF	PH	HI	Al- $F_2$	$r$
DW	<u><b>-0.764</b></u>	-0.004	0.166	0.238	-0.093	-0.029	-0.486**
LH	-0.334	<u><b>-0.010</b></u>	0.046	0.140	-0.082	-0.026	-0.265*
DF	-0.492	-0.002	<u><b>0.258</b></u>	0.169	-0.069	-0.049	-0.185
PH	-0.385	-0.003	<u><b>0.093</b></u>	<u><b>0.471</b></u>	-0.069	0.012	0.119
HI	-0.387	-0.004	0.098	<u><b>0.178</b></u>	<u><b>-0.183</b></u>	-0.030	-0.329*
Al- $F_2$	0.113	0.001	-0.065	0.030	<u><b>0.029</b></u>	<u><b>0.195</b></u>	0.303*

Direct effect ( $P_{17}$ ) and indirect effects of a particular agronomic character are read within a row. The direct effect is underlined (bold) and indirect effect is plain characters. Scores 1 and 5 are very tolerant and very susceptible, respectively.  $r$  = correlation of corresponding agronomic character to Al tolerance of the  $F_3$  generation in the compound path. \*\*, \* = significant at the 1% and 5% levels, respectively. The number of samples is 57.

**Table 5.** Direct effect ( $P_{x7}$ ) and indirect effect ( $r$ ) of dry weight (DW), length of head (LH), days to flowering (DF), plant height (PH) and Al tolerance in  $F_2$  (Al- $F_2$ ) on Al tolerance in the  $F_3$  generation (Al- $F_3$ ) for GS2 sorghum population.

	DW	LH	DF	PH	HI	Al- $F_2$	$r$
DW	<u><b>0.091</b></u>	-0.195	0.109	-0.688	0.451	-0.141	-0.372**
LH	<u><b>0.079</b></u>	<u><b>-0.224</b></u>	0.074	-0.549	0.372	-0.116	-0.364**
DF	0.026	-0.044	<u><b>0.375</b></u>	-0.422	-0.093	-0.238	-0.395**
PH	0.070	-0.138	<u><b>0.178</b></u>	<u><b>-0.888</b></u>	0.440	-0.196	-0.535**
HI	0.068	-0.138	-0.058	-0.645	<u><b>0.606</b></u>	-0.094	-0.261
Al- $F_2$	-0.024	0.048	-0.164	0.321	-0.104	<u><b>0.544</b></u>	0.621**

Direct effect ( $P_{17}$ ) and indirect effects of a particular agronomic character are read within a row. The direct effect is underlined (bold) and indirect effect is plain characters. Scores 1 and 5 are very tolerant and very susceptible, respectively.  $r$  = correlation of corresponding agronomic character to Al tolerance of the  $F_3$  generation in the compound path. \*\*, \* = significant at the 1% and 5% level, respectively. The number of samples is 57.

the wide range of Al tolerance in sorghum. The co-dominant and additive genetic effects with some degree of dominance were reported to control Al-tolerant sorghum (Caniato et al., 2007; Gourley, Rogers, Ruiz-Gomez & Clark, 1990). There were at least four gene families including ALMT family, MATE family, ATP-binding cassette (ABC) transporter family, and Nramp family that were associated with Al tolerance in some plant species (Delhaize, Ma & Ryan, 2012; Simões et al., 2012).

These results confirmed the previous research that Al tolerance was a complex trait with a low estimation of heritability value. By using near-isogenic line population, Caniato et al. (2007) reported that the high variability of highly Al-tolerant sorghum tended to segregate in the transgressive pattern. They even proposed that the addition of new sorghum Al tolerance genes contributed to Al-tolerant character.

There was continuous segregation of Al tolerance rather than discontinuous segregation in sorghum. High allocation of genetic resources in the early stage of the sorghum breeding program and late selection of Al tolerance trait is more proper in Al-tolerance breeding program of sorghum to accelerate the improvement of Al tolerance. However, the direct effect of Al tolerance of the  $F_2$  population on the advancement of Al tolerance was low in magnitude. The excellent performance of plant biomass must be accompanied by high sd of Al tolerance to obtain the high GG of Al tolerance from one generation to the next generation (Tables 4 and 5). Anas and Yoshida (2004) have

reported a significant phenotypic correlation of DW and PH with Al tolerance in sorghum.

Selection pressure of early-maturity and short PH reduced the GG of Al tolerance. Selection of short PH became slightly tricky for the improvement of Al tolerance of the next generation (Table 1). There might be a lot of Al-tolerant plants which will not be passed along the selection for short PH and early maturity. Consequently, improvement of Al tolerance should be in simultaneous selection rather than the single selection of agronomic character (Table 4).

Partition of their effect on Al tolerance revealed that the direct effect of high DW more contributed to the improvement of Al tolerance in GS1 population, while the direct and indirect effects of PH most contributed to the Al tolerance in GS2 population. There was a slight difference in the contribution of agronomic traits on the improvement of Al tolerance for these two sorghum populations.. Determining whether this phenomenon is unique to these genotypes used in the current study or it is related to the environmental effect and genetic control of Al tolerance will take additional studies. However, the excellent performances of DW and PH were two crucial agronomic traits for the improvement of Al tolerance in sorghum.

The early-maturity and short PH showed a genetic relationship to each other and also exhibited a genotypic relationship with Al tolerance (Anas & Yoshida, 2004). These two characters might link to each other. Some genes of short PH in sorghum showed homolog

genes with DL gene in Arabidopsis which promotes early maturity, while mutation delays maturity, affecting PH (Upadhyaya, Wang, Gowda & Sharma, 2013).

Significant reduction of DF might also cause a slow improvement of AI tolerance in sorghum (Table 1). Genotypic correlation of DF with AI tolerance was harmful in some sorghum populations (Anas & Yoshida, 2004). This result was in agreement with the negative correlation of DF on AI tolerance in path analysis. This effect was mainly caused by the indirect influence of DW reduction. Consequently, there was no influence from this character on the improvement of AI tolerance (Tables 4 and 5).

It seems that the reduction of plant vigor mainly caused the slow improvement of AI tolerance in the early-maturity sorghum population. Commonly, AI prohibited root growth in some plant species that caused decreased uptake of water and nutrient (Kochian, Piñeros & Hoekenga, 2005). AI inhibited cell division, cell extension and transport of nutrient in the plant (Mossor-Pietraszewska, 2001). Researchers have reported that AI disrupted the cellular function of the soybean plant by binding to phosphate and carbonyl components in the symplast and apoplast (Miransari, 2016). This report suggested that breeding of early-maturity sorghum might be implemented to improve AI tolerance trait where we should maintain a significant amount of plant biomass judging from this evidence.

GW in both BS sorghum populations showed low improvement in GG (Table 1). The active selection for earliness in the F<sub>3</sub> generation of soybean breeding program resulted in a detrimental increase in yield (St. Martin & Futi, 2000). The decrease of plant biomass and PH will reduce the vegetative phase of the plant, giving a shorter time for flowering. Fortunately, the higher GG for GW observed in AI-tolerant BS provides the possibility to breed AI tolerance plant with high GW.

## Conclusion

Based on the GG information, much allocation of progenies in the early generations of AI-tolerant sorghum should apply to obtain improvement of AI tolerance character. Furthermore, the development of GG of AI tolerance in sorghum should employ a strong sd of AI tolerance. Selection pressure for early maturity and short PH resulted in the small advancement of GG for AI tolerance. Partition of effect on AI tolerance showed that high DW and tall PH more contributed to the improvement of AI tolerance. Based on the overall path analysis data, the increase of plant biomass, the significant number of progenies in the early stage of

breeding and strong sd of AI tolerance would be more appropriate to improve the AI tolerance in sorghum.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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