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Morphological and Biochemical Responses of Some Promising Tea Genotypes to Aluminum-induced Soil Acidification

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ABSTRACT

The present study aimed to assess both soil and tea plant responses to acidification induced by aluminum (Al). In this way, the effects of four levels of soil acidification by aluminum sulfate were examined (A1=0, A2=500, A3=1000, A4=2000 mg kg^{-1} soil) on five promising tea genotypes (G1=100, G2=440, G3=444, G4=591 and G5=703). The genotypes were originally from Lahijan Tea Research Center and were tested on split plots in a randomized complete block design with three replications. A breakpoint of 250 mg kg-1 of exchangeable Al was identified as critical for the severe release of Al into the soil solution. Both soluble and exchangeable fractions of soil Al showed strong power regression relationships with soil pH measured in water as well as 1M KCl solution. The genotype with the highest yield (G3) experienced a significantly greater decline in fresh yield following treatments with Al, compared with the genotype having low yield (G4) (22% vs. 6%, on average). Acidification adversely affected all morphological parameters but no significant impacts were detected on selected biochemical parameters (i.e. caffeine, total polyphenols, and chlorophyll index). Leaf Al concentration, followed by shoot weight and leaf thickness showed significant relationships ($p<0.01$) with soluble and exchangeable fractions of Al in the soil. The highest and the lowest leaf Al concentrations were obtained in G4 (837 mg kg^{-1} DM, on average) and in G3 (623 mg kg^{-1} DM, on average), respectively. Based on all morphological traits, the most tolerant genotype to soil acidification was G4, which is a low-yield tea with a relatively high Al accumulation affinity.

Introduction

Tea (*Camellia sinensis* L.) is one of the most popular drinks in Iran and in the world. Annual tea production and its consumption rate in Iran are 26.5 and 83.4 thousand tons, respectively (Chang, 2015). Tea shrubs grow in acidic soils with an optimum pH value between 4.5 to 5.5 (Sivapalan, 1988). While 30 percent of the world's soils are acidic (Sumner & Noble, 2003), acidic soils in Iran are limited to temperate and humid regions on the northern slopes of the Alborz mountain range along the Caspian Sea (Roozitalab et al., 2018). Tea orchards cover almost 35,000 hectares, but at present only 18500 hectares are active (FAO, 2020). Over-use of chemical fertilizers (Meng et al., 2013), nitrification process (Zhou et al., 2014; Souri et al., 2018) and proton secretion into rhizospheres during the uptake of ammonium ions by roots are causes of acidification in the rhizospheric region of soils where tea plants exist (Wan et al., 2012). Tea is also a perennial shrub that accumulates aluminum (Al) in its leaves (Mukhopadyay et al., 2012). Soil acidification is usually exacerbated by the fall of leaves and by leaving residues of pruning on the soil (Ruan et al., 2004).

Al is toxic to many plant species. For this reason,

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long-term contact of roots to a few micro molar of Al^{3+} causes limited root growth (Kochian et al., 2005). However, tea plants can absorb enormous amounts of Al from the soil and transfer it to aerial organs such as leaves (Matsumoto et al., 1976). The Al concentration in mature leaves of tea plants was reportedly 30,000 mg/kg DM (Mukhopadyay et al., 2012). Al can promote the activity of antioxidant defense enzymes, improve plant metabolism (Hajiboland et al., 2013), elongate root and pollen tube length (Yokota et al., 2005), and increase vegetative growth of roots and shoots in tea plants (Sae-Lee et al., 2012). However, excessive amounts of Al can harm tea plant growth (Fung et al., 2008). The major mechanisms of Al toxicity are, namely, decreased root elongation and shoot growth, nutrient imbalance and altered physiological and metabolic processes (Shetty et al., 2021). Accumulation of Al in tea plants is affected by plant age, cultivar type (Shu et al., 2003), and application of nitrogen and phosphorus fertilizers (Fung et al., 2009). Older leaves accumulate 10 times more Al than younger leaves (Carr et al., 2003). Most of the accumulated Al is immobilized in cell walls and the rest is stored in vacuoles (Huang et al., 2021). The concentration of Al in tea leaves increased as the soil extractable Al increased (Wong et al., 1998) and the soil pH decreased (Dong et al., 1999). It has been reported that Al concentration in tea leaves was most strongly correlated with Al extracted by 0.02M $CaCl_2$ (Xie et al. 2001). However, the absorbed Al may not remain in the roots and moves upward to the leaves (Fung et al., 2009). Eventually, drinking Al-rich teas can lead to kidney failure in humans (Jackson & Huang, 1983), Alzheimer's disease (Walton, 2006), and hematocrit problems (Marouani et al., 2007). Al accumulation in tea leaves vary between genotypes (Ruan & Wong, 2001; Shu et al., 2003). Therefore, it can be concluded that Al-tolerant tea genotypes can enter substantial amounts of Al into the human body. This emphasizes the importance of finding and characterizing tea genotypes with high-yield and low-accumulation affinity for Al in acidic soils. Ruan and Wang (2001) also suggested that variety selection is a research-proven way to reduce Al concentration in tea products.

The purpose of this study was to acidify the soil to low pH values by adding aluminum sulfate and to compare the responses of five Iranian tea genotypes under field conditions. The findings will be useful to introduce tea genotypes with maximum yield and high quality as well as minimum Al content.

Materials and Methods

The field experiment

This experiment was conducted in an orchard of the Tea Research Center of Iran located in Lahijan city (412868E, 4116655N). During the experiment (March-2017 to September-2018), the amount of annual rainfall and total evapotranspiration were 545 mm and 719 mm, respectively. Also, the maximum and minimum temperatures were recorded at 33.8°C and 8.7°C, respectively. The maximum and minimum relative humidity were 98% and 49%, respectively. Moreover, the average daily sunshine hours were 6.86 hours. Some characteristics of composite soil sample (0-30 cm) were taken from the plots before the experiment. The values were pH=4.78, OC=1.68%, available P=91 mg kg^{-1}, available K=274 mg kg^{-1}, total N=0.194%, ECEC=10.3 cmolc kg^{-1} and the soil texture was sandy-clay loam. Five promising genotypes (15 years old) were selected based on a recent clonal selection project. These genotypes were named with codes, i.e. 100, 440, 444, 591, 703. For convenience, however, they were presented as G1=100, G2=440, G3=444, G4=591 and G5=703. Before the field experiment began, medium pruning was carried out on 13 March 2017, so that the trunk and main branches remained (Fig. 1-a). Thereafter, Al as aluminum sulfate ($Al_2(SO_4)3.18H_2O$) was added at the rates of 0, 500, 1000 and 2000 mg kg^{-1} to acidify the soils (A1, A2, A3 and A4, respectively). For this purpose, any of the Al treatment solutions were introduced to the soil surface around the roots of tea shrubs via a temporary drip irrigation system (Fig. 1-b). The Al concentration and volume of the solutions were determined in preliminary laboratory experiments. The quality of water from wells was measured for irrigation (Table 1). The reason for using aluminum sulfate instead of direct acidifying chemicals was that Al is the main source and protons contribute to only a small part of acidity in acidic soils (Sparks, 2003).

Based on the soil test results, the required fertilizers including urea (300 kg N ha^{-1}), triple superphosphate (25 kg P ha-1) and potassium sulfate (50 kg K ha^{-1}) were applied to the soil on 25 April 2017. Light sprinkler irrigation was performed after addition of Al treatments and required fertilizers. The experiment was performed as a split-plot in a randomized complete block design with genotype as the main plot at five levels (G1, G2, G3, G4 and G5) and acidification as the sub-plot at four levels (A1, A2, A3 and A4) (Fig. 1-c).

Table 1. Groundwater quality for irrigation purposes

	EC	pH	Cl^-	Ca^{2+}	Mg^{2+}	Na^+	HCO_3^-	NO_3^--N
	dS m^{-1}		meq L^{-1}					mg L^{-1}
Early summer	0.496	7.4	0.2	3.7	1.8	1.7	1.5	0.07
Mid-summer	0.503	7.1	0.1	4.2	1.1	0.58	6.0	1.1

EC: Electrical conductivity

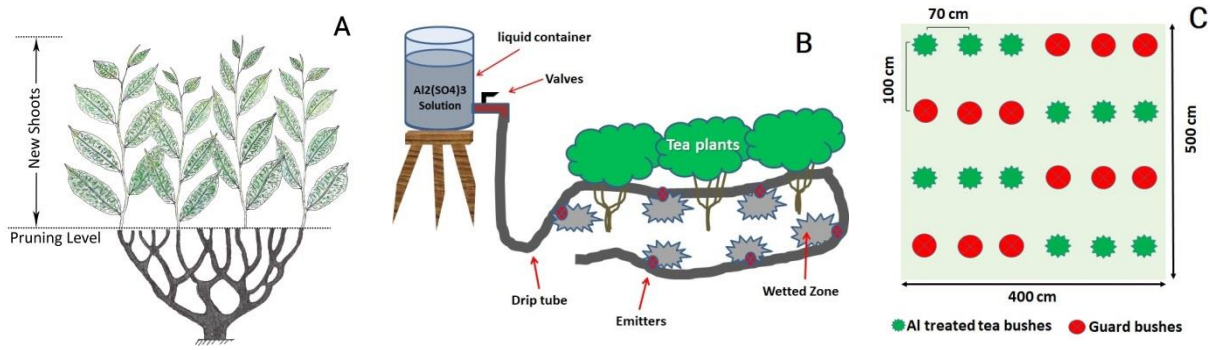

Fig. 1. Medium pruning and harvesting level [A], addition of aluminum sulfate by temporary drip irrigation system [B], dimension of plots [C].

Soil sampling and analysis

Soils (0-30 cm) were sampled from the plots on 10 September 2017. At the same time, the tea leaves were plucked to assess the effect of Al addition on soil chemical properties. Soil Al fractions including soluble and exchangeable forms of Al were extracted by 0.02 M $CaCl_2$ (Dong et al., 2001) and 1M KCl (Bertsch & Bloom, 1996), respectively. The pH_w in 1:1 soil/distilled water and pH_{KCl} in 1:1 soil/1 M KCl were measured (Thomas, 1996).

The soil characteristics which were determined before the start of the experiment included effective cation exchangeable capacity (ECEC) (Grove et al., 1982), available phosphorus (Kuo, 1996), available potassium (Helmke & Sparks, 1996), organic carbon (Nelson and Sommers, 1982), and soil texture (Bouyoucos, 1962).

Growth and morphological responses

On 20 September 2018, shoots were harvested above the pruning level, weighted and considered as fresh yield (Fig. 1-a). They were dried at 75°C to record the dry yield. In addition, the number of shoots produced per plot was counted, and their average weight was reported. From each shoot, 5 leaves (10 leaves in each plot) were randomly selected (IPGRI, 1997) and the leaf area and thickness (mm) were determined (Tsuji, 2000).

Biochemical responses

Tea quality parameters were measured in shoot leaves suitable for beverage use. For this purpose, a bud and two leaves were taken from each experimental plot, dried at 65°C for 48 hours, and the leaf dry matter percentage was calculated (Safaei-Chaeikar et al., 2020). The samples were ground and sieved to measure caffeine (Lakin, 1989) and total polyphenols (ISO, 2005).

Leaf chlorophyll index was measured on 3 leaves using a chlorophyll meter (Opti-Sciences CCM-200) in each plot between 11 to 12 AM, in sunny conditions, and the average values for five leaves were recorded (Liu et al., 2012). From each shoot, 3 leaves were plucked and dried at 65°C for 48 hours to determine leaf Al concentration. Briefly, 0.2 g of each sample was digested in a Kjeldahl tube with 4 ml of concentrated nitric acid and 1 ml of perchloric acid (Erdemoğlu et al., 2000). The Al concentration in the extract was measured by a Perkin Elmer AA800 atomic absorption spectrometer with a nitrous oxide flame.

Statistical analysis

To test the effects of the experimental factors and their interaction on plant response, the data were subjected to ANOVA in a split plot design. Variance analysis and the comparison of means were performed by the SAS software (version 9.4). To compare the means of traits, Tukey's test was used at the 5% probability level. Regression analysis and principal component analysis (PCA) were done using the SPSS and PAST software, respectively.

Results

Soil characteristics

The variance analysis (Table 2) shows a significant effect of Al application (acidification) on measurable Al fractions in the soil ($p<0.01$). However, the main effect of genotype and the relevant interactive effect (acidification×genotype) were insignificant. This means that bulk soils (but not necessarily rhizospheric soils) had similar responses to a given level of soil acidification. The minimum and maximum values of exchangeable Al in the soil were 20 and 617 mg kg^{-1} in Al application levels of A1 and A4, respectively. The corresponding values for soil soluble Al were 3 and 96 mg kg^{-1}, respectively. The observed values are close to those reported for World Tea Orchard Soils. In addition, a four-fold increase in Al addition increased the average exchangeable and soluble Al values from 164 to 581 mg kg^{-1} and from 11.4 to 89.2 mg kg^{-1}, respectively. By increasing the added Al, both Al fractions in the soil showed a significant linear increasing trend (Fig. 2). It can be observed that 27.2% of the added Al was found in the exchangeable form, while only a small fraction of it (4.4%) was present in the form of soluble Al. This means that most of the added Al (68.4%) entered the non-labile Al pool. A significant correlation ($r = 0.971^{**}$) between the exchangeable and soluble fractions of Al was obtained (Table 4). However, Fig. 3 shows that the fitted line can be divided into two segments, including soils with low Al application rates (A1 and A2) and those with high application rates (A3 and A4). The first segment has a steeper slope, however, while the second one shows a stronger relationship. According to the fitted lines (Fig. 3), exchangeable Al was 13.6 and 4.5 times greater than soluble Al in the first and second segments, respectively. An exchangeable Al level of 250 mg kg^{-1} was considered to be a dividing line between the two segments.

The effect of soil acidification on pH_w and pH_{KCl} was statistically significant ($p<0.01$). However, the simple effects of genotype and the interaction effect of acidification and genotype on pH were not significant. It means that pH_w and pH_{KCl} were not affected by genotype. The mean pH_w value for the control treatments (A1) was 4.57. It decreased to 4.19 and 3.82 following Al application at A2 and A3 levels, respectively, and finally reached its minimum value of 3.61 at the A4 level. The results also showed that there was a strong inverse power regression between both exchangeable and soluble fractions of soil Al and pH value (Fig. 4). This finding highlights the importance of low pH in the availability of Al in tea orchards.

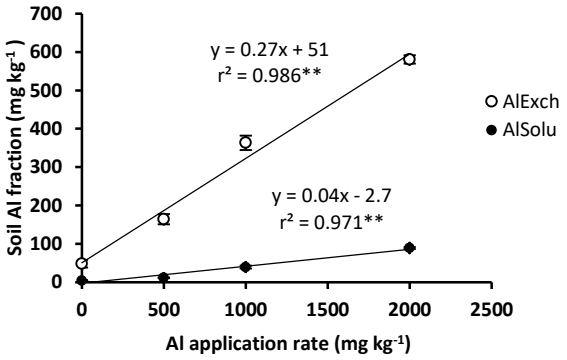

Fig. 2. Soil Al fractions extracted by 0.02M $CaCl_2$ and 1M KCl as a function of Al application rate. AlExch: Exchangeable Al, AlSolu: Soluble Al.

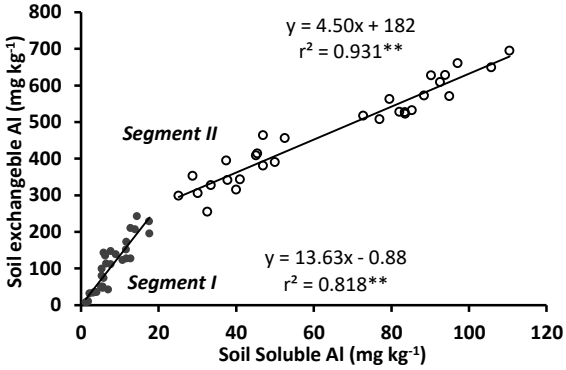

Fig. 3. The relationship between exchangeable and soluble fractions of Al in the soils.

Table 2. The ANOVA of the effects of acidification and genotype on soil and plant parameters

Source of variance	df	Soil exchangeable Al (1M KCl)	Soil Soluble Al (0.02M $CaCl_2$)	pH (1:1) Water	pH (1:1) 1M KCl	Fresh yield	Dry yield	Number of shoots	Shoot weight
Block	2	946	8.7	0.0596	0.0214	0.222 ^{ns}	0.013^{ns}	640.62^{ns}	3.93^{ns}
Genotype	4	3857 ^{ns}	127 ^{ns}	0.0808 ^{ns}	0.0285 ^{ns}	0.90*	0.077**	15395.40**	10^{ns}
$Error_1$	8	4236	76	0.0394	0.0124	0.130	0.011	657.70	4.8
Acidification	3	821410**	22240**	2.6683**	0.8420**	0.384**	0.04**	5318.42**	59.29**
Acidification×Genotype	12	2532 ^{ns}	54 ^{ns}	0.0377 ^{ns}	0.0112 ^{ns}	0.076*	0.014**	671.37*	6.02**
$Error_2$	30	1904	30	0.0198	0.0074	0.036	0.004	278.33	1.53
CV		15.1	15.3	3.5	2.4	17	15	17	12

Source of variance	df	Leaf area	Leaf thickness	Caffeine	Total polyphenols	Chlorophyll index (3rd leaf)	Dry matter percentage	Leaf Al concentration
Block	2	3^{ns}	0.02^{ns}	0.08^{ns}	3.28^{ns}	61^{ns}	9.92^{ns}	3060^{ns}
Genotype	4	333**	0.015^{ns}	0.264^{ns}	28.3**	2999**	26.84**	74712**
$Error_1$	8	11	0.009	0.127	1.47	101	2.73	15001
Acidification	3	17*	0.124**	0.0003^{ns}	1.15^{ns}	157^{ns}	13.35**	484591**
Acidification×Genotype	12	8^{ns}	0.009*	0.037^{ns}	0.57^{ns}	59^{ns}	3.31 ^{ns}	18624^{ns}
$Error_2$	30	5	0.004	0.034	0.62	60	1.49	9544
CV		9.1	16.4	6.2	10	15	5.1	13

** ,*and ns indicate significance levels of 1%, 5% and insignificant, respectively

Table 4. Pearson correlation coefficients between soil and tea plant parameters

parameters	Soil exchangeable Al	Soil soluble Al	pHw	pH $_{KCl}$	Fresh yield	Dry yield	Number of shoots	Shoot weight	Leaf area	Leaf thickness	Caffeine	Total polyphenols	Chlorophyll index	Leaf dry matter percentage
Soil soluble Al	0.971**	1.000												
pH_w	-0.908**	-0.838**	1.000											
pH_{KCl}	-0.888**	-0.805**	0.973**	1.000										
Fresh yield	-0.327*	-0.308*	0.187	0.174	1.000									
Dry yield	-0.308*	-0.315*	0.151	0.136	0.899**	1.000								
Number of shoots	-0.404**	-0.418**	0.299*	0.291*	0.612**	0.650**	1.000							
Shoot weight	-0.665**	-0.638**	0.600**	0.598**	0.448**	0.390**	0.405**	1.000						
Leaf area	0.076	0.037	-0.144	-0.151	0.177	0.033	-0.060	0.096	1.000					
Leaf thickness	-0.631**	-0.602**	0.626**	0.648**	0.241	0.269*	0.476**	0.632**	-0.378**	1.000				
Caffeine	-0.012	-0.007	-0.043	-0.037	0.329*	0.328*	0.498**	0.196	0.164	0.085	1.000			
Total polyphenols	-0.118	-0.106	0.071	0.099	0.364**	0.200	0.225	0.241	0.543**	-0.007	0.306*	1.000		
Chlorophyll index	0.121	0.129	-0.021	-0.042	-0.378**	-0.276*	-0.213	-0.269*	-0.671**	0.035	-0.273*	-0.468**	1.000	
Leaf dry matter percentage	0.386**	0.393**	-0.343**	-0.275*	-0.345**	-0.314*	-0.252	-0.279*	-0.186	-0.139	0.100	0.172	0.380**	1.000
Leaf Al concentration	0.793**	0.753**	-0.788**	-0.791**	-0.448**	-0.405**	-0.491**	-0.597**	0.016	-0.574**	-0.045	-0.160	0.192	0.510**

Growth and morphological responses

Fresh and dry yields

Table 2 shows the significant effect of soil acidification on fresh and dry yields of tea ($p<0.01$). Also, both simple effects of genotype and the relevant interaction effect on fresh yield ($p<0.05$) as well as on dry yield ($p<0.01$) were statistically significant. These results revealed the different yield responses of the genotypes to soil acidification. The comparison of means (Table 3) shows that G1 and G3 genotypes produced a total of 0.6 kg plant-1 (59%) higher fresh yield than the other genotypes (G2, G4 and G5) in non-acidified soil conditions (A1 control treatment). The high-yield genotype (G3) had a significantly greater decline in fresh yield compared to the low-yield genotype (G4) following Al treatments of A2, A3 and A4 (22% vs. 6% on average). The effects of soil acidity on dry yield followed the same trend but with different magnitudes. In brief, an average dry yield reduction of 32% was recorded for the G3 genotype following Al treatments of A2, A3 and A4, but an average increase of 11% for the G4 genotype was detected. Both fresh and dry yields showed significant negative correlations ($p<0.05$) with the soluble and exchangeable Al, but no significant correlation with soil pH was observed (Table 4). Totally, based on fresh yield outcomes, G4 was the most tolerant and G3 was the most sensitive among the genotypes in response to soil acidity stress.

Table 3. Interaction effects (acidification×genotype) on parameters of tea plants

Main plots (Genotypes)	Sub plots: Acidification=Al levels (mg kg^{-1})				Mean
	0 (A1)	500 (A2)	1000 (A3)	2000 (A4)	
			Fresh yield (kg plant^{-1})		
G1	1.58±0.18a	1.7±0.21a	1.13±0.35cd	1.22±0.15bc	1.41A
G2	1.04±0.22cd	1.06±0.17cd	0.93±0.21cde	0.57±0.21f	0.90B
G3	1.69±0.28a	1.53±0.27ab	1.22±0.32bc	1.21±0.14c	1.41A
G4	1.03±0.34cd	0.84±0.36def	0.93±0.38cde	1.13±0.31cd	0.98B
G5	1.02±0.13cde	1.02±0.16cde	0.71±0.26ef	0.71±0.18ef	0.87B
Mean	1.27A	1.23A	0.98B	0.97B	
			Dry yield (kg plant^{-1})		
G1	0.52±0.04bc	0.58±0.09ab	0.40±0.04de	0.4±0.06de	0.47 AB
G2	0.33±0.10def	0.42±0.03cde	0.36±0.05def	0.22±0.06g	0.33C
G3	0.66±0.06a	0.52±0.06bc	0.43±0.15cd	0.40±0.06de	0.50A
G4	0.37±0.09def	0.4±0.10de	0.39±0.12de	0.44±0.09cd	0.40BC
G5	0.37±0.04def	0.34±0.03def	0.27±0.07fg	0.33±0.06ef	0.33C
Mean	0.45A	0.45A	0.37B	0.35B	
			Number of shoots per plant		
G1	144±16c	128±24cd	104±20def	94±12efg	117B
G2	72±3ghi	77±8fgh	64±10hi	46±4i	65C
G3	192±22a	173±22ab	147±25bc	97±14efg	152A
G4	87±16efgh	106±4de	81±16efgh	75±13gh	87C
G5	79±12efgh	86±18efgh	65±13hi	66±11hi	74C
Mean	115A	114A	92B	76B	
			Shoot weight (g plant^{-1})		
G1	12.63 ±1.16ab	10.53 ±0.7cd	8.51 ±0.65d-g	8.04 ±0.28fgh	9.9AB
G2	14.38 ±0.86a	10.15 ±0.76cde	9.19 ±0.19def	8.91 ±0.45def	10.7A
G3	14.46 ±0.54a	11.7 ±0.77bc	9.7 ±1.04c-f	6.81 ±0.71gh	10.7A
G4	8.56 ±1.83deg	8.01 ±0.63fgh	9.19 ±0.54def	8.11 ±1.26e-h	8.5B
G5	11.59 ±0.83bc	11.43 ±1.09bc	8.85 ±0.45d-g	6.32 ±1.12h	9.5AB
Mean	12.3A	10.4B	9.1C	7.6D	
			Leaf thickness (mm)		
G1	0.493 ±0.045bcd	0.336 ±0.038f-i	0.298 ±0.06hi	0.255 ±0.012i	0.345B
G2	0.506 ±0.03bc	0.407 ±0.036c-g	0.36 ±0.023f-i	0.309 ±0.035ghi	0.395AB
G3	0.633 ±0.048a	0.485 ±0.066b-e	0.378 ±0.069fgh	0.272 ±0.024i	0.442A
G4	0.412 ±0.022c-g	0.435 ±0.04b-f	0.401 ±0.016c-h	0.394 ±0.031d-h	0.410AB
G5	0.527 ±0.084b	0.388 ±0.036d-h	0.384 ±0.068e-h	0.256 ±0.026i	0.389AB
Mean	0.514A	0.410B	0.364B	0.297C	

Different letters indicate significant differences between the means at α=5%.
Capital and small letters show the simple and interaction effects, respectively.
Tea genotypes are symbolized by G1-G5.

Number of shoots

Table 2 provides the same results on significance of the treatment effects regarding the number of shoots as those obtained in the case of fresh and dry weights. The comparison of means (Table 3) shows that G3 and G1 genotypes had high numbers

of shoots per plant (192 and 144, respectively) in non-acidified soils. The other three genotypes (G2, G4 and G5) had the same number of shoots (79, on average). With increasing soil acidity, the number of shoots decreased, but the extent of reduction was genotype-dependent. The number of shoots decreased by 35%, 36%, 49%, 14% and 16% in G1, G2, G3, G4 and G5 genotypes with increasing soil acidity from A1 to A4, respectively. Significant linear relationships occurred between the number of shoots which were positive with soil pH values and negative with soil Al fractions (Table 4). However, the strength of relationships was stronger in the case of soil Al fractions than in soil pH values. However, according to Fig. 5-a, only G1 and G3 genotypes participated in strong relationships. This finding shows that lower soil pH, and in particular, a higher Al concentration resulted in a decrease of the number of shoots in tea plants. As mentioned earlier, G1 and G3 genotypes exhibited the same behavior and can be categorized into the first group, and the remaining genotypes were categorized into the second group. In the first group, decreasing one pH unit caused a decline of 94 shoots, while this number was merely 15 in the second group. Therefore, the second group seems to be more tolerant to the lower pH value in the soil, compared to the first group (Fig. 5-a).

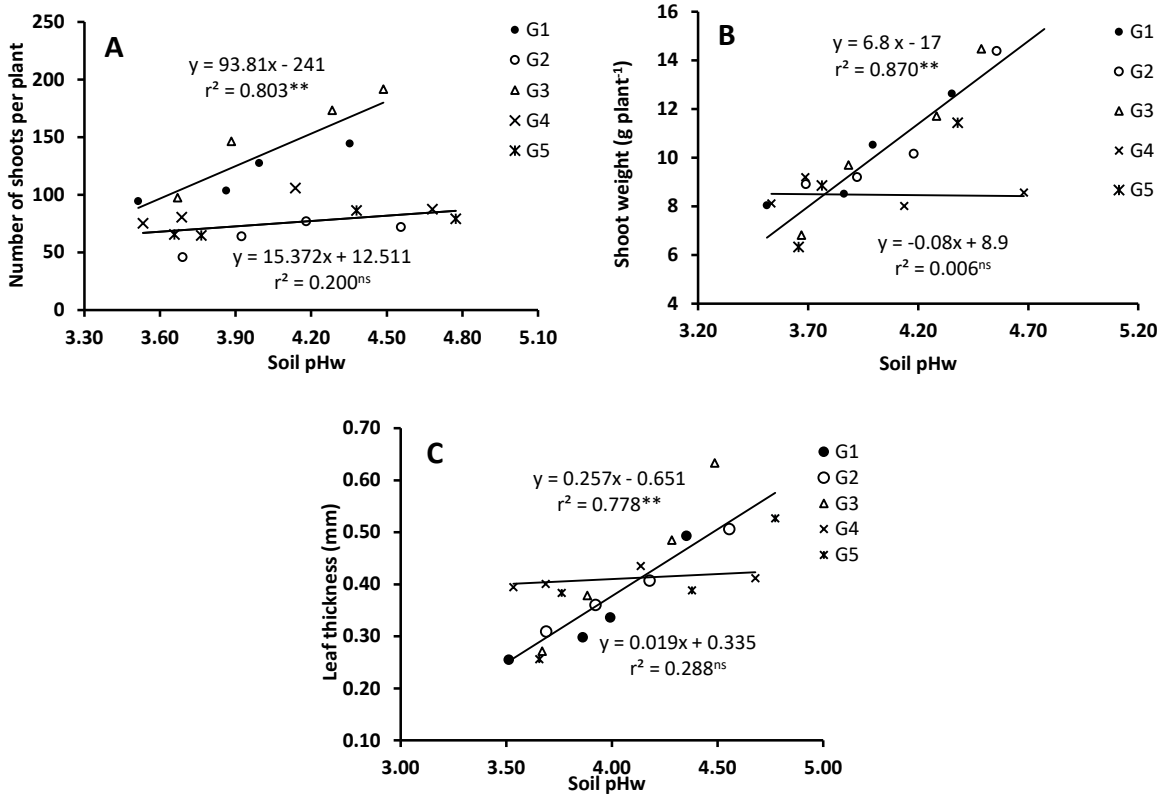

Fig. 3. Linear regressions between number of shoots [A], shoot weight [B] and leaf thicknesses [C] of tea genotypes (symbolized by G1-G5) and soil pH_w.

Shoot weight

According to Table , the simple effect of soil acidification and the interactive effect of acidification × genotype on shoot weight were statistically significant ($p<0.01$). However, the effect of genotype was not significant. The G1, G2 and G3 genotypes had the highest shoot weight in non-acidified soil conditions. However, there was no significant difference in shoot weight between G1 and G5 genotypes. In addition, G4 genotype showed the lowest shoot weight in the control treatment. Table 3 shows that from A1 to A4, the shoot weight decreased by 36%, 38%, 53%, and 45% in G1, G2, G3, and G5 genotypes, respectively, while the decline was not significant in G4 genotype. Significant linear relationships between shoot weight and soil Al fractions as well as soil pH were found at almost similar levels. In addition, soil parameters provided stronger relationships with shoot weight than with the number of shoots. However, plant parameters (fresh and dry yields) produced weaker relationships with shoot weight than with the number of shoots (Table 4). According to Fig. 5-b, for each pH unit of decrease in pH_w in G1, G2, G3 and G5 genotypes, the shoot weight decreased by 7 g per plant. However, this was not significant in G4. Therefore, in terms of shoot

weight, the four aforementioned genotypes were more sensitive to soil acidification than G4.

Leaf area

According to the analysis of variance (Table 2), the main effects of genotype ($p<0.01$) and soil acidification ($p<0.05$) on the 5th leaf area were statistically significant. However, the interactive effect (acidification × genotype) was not significant. According to the comparison of means (Table 3), acidification decreased the leaf area only at A3 and A4 levels of Al application compared to the control, but there was no significant difference between the means of two levels. Also, the G3 genotype was followed by G1 and showed the highest leaf area. However, there were no significant differences between the other genotypes. Leaf area convincingly indicated no significant correlations, neither with soil nor with plant morphological parameters (except leaf thickness) (Table 4).

Leaf thickness

According to the analysis of variance (Table 2), the simple effect of acidification ($p<0.05$) and the interactive effect (acidification × genotype) on leaf thickness ($p<0.01$) were statistically significant. However, the effect of genotype was not significant. According to Table 3 and Fig. 5-c, the four G1, G2, G3, and G5 genotypes had the largest thickness of leaves, but the G4 genotype had the least thickness that decreased in response to higher soil acidity. The comparison of means (Table 3) shows that from A1 to A4, leaf thickness reduced by 48%, 39%, 57%, and 51% in G1, G2, G3, and G5 genotypes, respectively, but the reduction was not significant in G4. Leaf thickness showed significant correlations with all morphological plant parameters (except dry yield) (Table 4).

Biochemical and physiological parameters

Caffeine, total polyphenols and chlorophyll index

According to Table 2, the simple effects of acidification on chlorophyll index and the contents of caffeine and total polyphenols in tea plants were not significant. Also, none of the interactive effects (acidification × genotype) were significant. However, the main effect of genotype on total polyphenols content and chlorophyll index, but not on caffeine content, was significant ($p<0.01$). The G1 and G4 genotypes had the highest and lowest contents of total polyphenols (10 and 5.7 g 100^{-1} g DW, respectively) (Table 3). Total polyphenols had significant positive correlations with fresh yield, leaf area ($p<0.01$) and caffeine ($p<0.05$). Caffeine also showed similar correlations with fresh and dry yields ($p<0.05$) and number of shoots ($p<0.01$) (Table 4).

Leaf dry matter percentage

The analysis of variance (Table) showed that the simple effects of acidification and genotype on leaf dry matter percentage were significant ($p<0.01$). However, the interactive effect (acidification × genotype) was not significant. This means that the two factors were independent of each other. The highest leaf dry matter percentage was in G5 (26.3%, on average), and the lowest was in G3 (22.6%, on average). Acidification increased the leaf dry matter percentage from 23.1% (on average) in A1 and A2 to 24.5% (on average) in A3 and A4, respectively. The softest and the roughest leaves were observed in G3 and G5, respectively. Also, the leaves became rougher at lower soil pH values. The leaf dry matter percentage is one of the important indicators of green tea leaf quality relevant to tea making process. The results showed that G3 had the best quality for making tea, but its quality would decrease in low soil pH values. Leaf dry matter percentage showed significant correlations with the soil and with some morphological parameters in the tea plants (Table 4).

Leaf Al concentration

The analysis of variance (Table 2) showed that the simple effects of acidification and genotype on the concentration of Al in the 3rd leaf were significant ($p<0.01$). However, the interaction effect was not significant. Increasing the Al application rate from control to 2000 mg kg^{-1} increased leaf Al concentration from 546 to 954 mg kg^{-1} DW, on average, in tea plants. In addition, the highest and the lowest leaf Al concentrations were obtained for G4, with an average of 837 mg kg^{-1} DM, and for G3, with an average of 623 mg kg-1 DM, respectively. Similar values of leaf Al concentrations have been reported in several studies. Fig. 6 shows strong logarithmic relationships between leaf Al concentration and values of soil Al fractions. In addition, negative linear relationships between leaf Al concentration and soil pH values were observed. From these equations, the concentration of leaf Al can be estimated from soil Al fractions or pH values. Since the measured parameters showed different behaviors to provide a general and comprehensive judgment about the response of genotypes to soil acidification, the principal component analysis (PCA) diagram and cluster analysis were used for the control treatment (A1) without acidification (Fig. 7-a, b), and for the highest level of acidification (A4) (Fig. 7-c, d). Biplot diagrams for the first two principal components for both control and acidification conditions were plotted (Fig. 7).

Accordingly, genotypes were classified into three groups under control and acidification conditions. In the latter, the first group consisted of genotypes G1 and G3. These genotypes had the highest scores for the first principal component because parameters such as fresh yield, shoot number, polyphenol and chlorophyll had a highly positive coefficient. These genotypes were identified as tolerant genotypes under the acidification condition. The second group included genotypes G4 and G5, and the third group contained genotype G2.

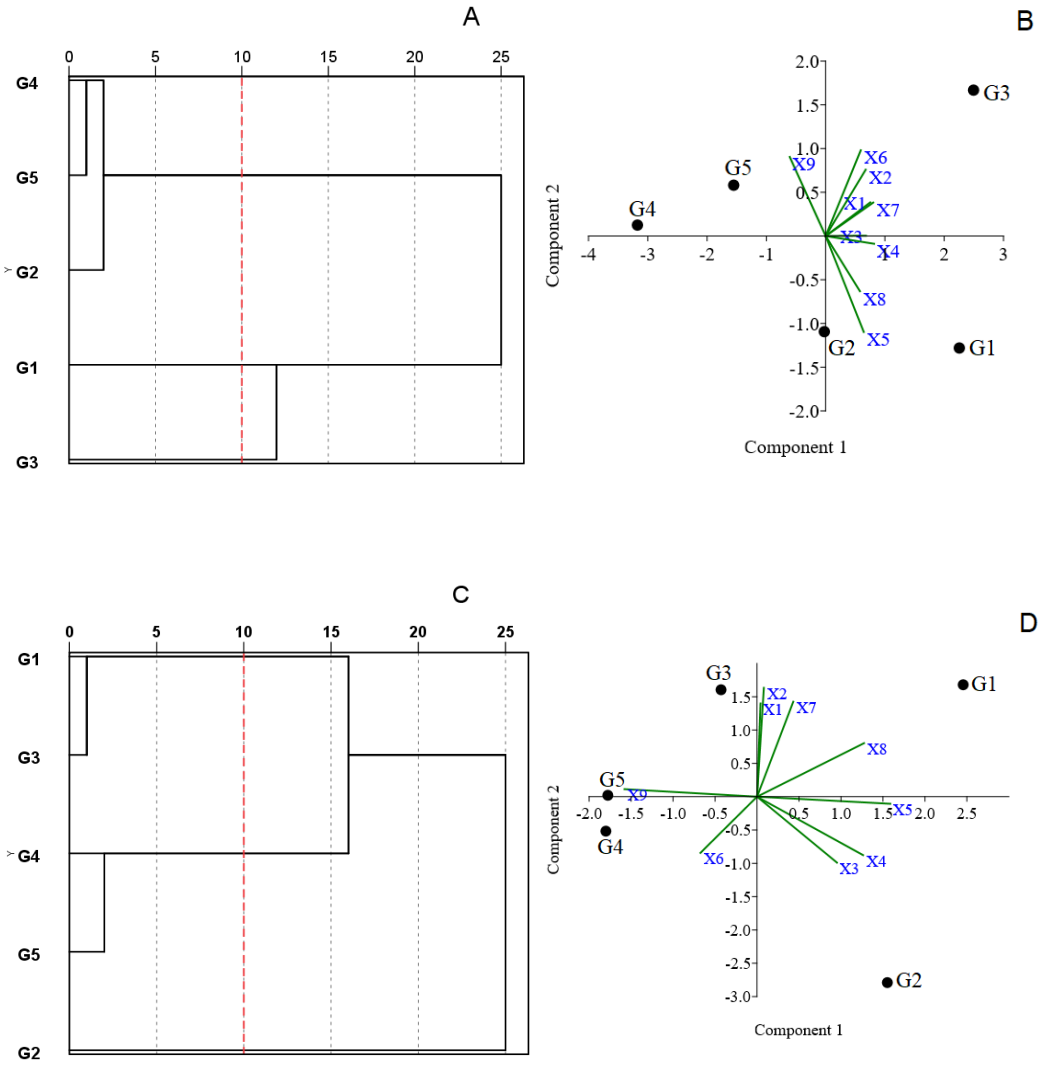

Fig. 7. The results of PCA and cluster analysis. Diagrams of control treatment (A1) [A and B]. Diagrams of the highest acidification level (A4) [C and D]. x1 to x9 represent fresh yield, number of shoots, leaf weight, leaf area, leaf thickness, caffeine content, total polyphenols content and chlorophyll index, respectively. Tea genotypes are symbolized by G1-G5.

Discussion

The observed range of exchangeable Al (20-617 mg kg-1) and soluble Al (3-96 mg kg^{-1}) in the soils of the present research are supported by the available literature. Xie et al. (2007) reported an exchangeable Al concentration of 43-623 mg kg^{-1} and a soluble Al concentration of 0.12-14.3 mg kg^{-1} for tea plantation soils in several central provinces of China. Also, the concentration of soluble Al in the soils of 13 tea orchards was reportedly between 1.58-101 mg kg-1 in eastern provinces of China (Dong et al., 1999). Additionally, the observed increase in Al reservoir (mainly as exchangeable Al) following Al treatments (from 48 to 164, 363 and 518 mg kg-1 soil after the addition of 500, 1000 and 2000 mg Al kg-1 soil on average) were recorded by previous researchers. According to Al-Baquy et al. (2017), Al supplementation increased the concentration of exchangeable Al in some Ultisols in China. The values ranged between 8.1 to 764 mg kg^{-}

[1] in humans and between 5.4 to 448 mg kg^{-1} in soils of the Anhui region. In addition, Ruan et al. (2003) reported that when the application rate of Al was 500 mg kg^{-1}, the Al extracted by 0.2 M $CaCl_2$ increased from 36 mg kg^{-1} in the control soil to 124 mg kg^{-1}. The observed decrease in soil pH_w after application of Al (from 4.57 to 4.19, 3.82 and 3.61 after addition of 500, 1000 and 2000 mg Al kg^{-1} soil on average) was consistent with previous reports. Ruan et al. (2003) observed a significant decrease in soil pH_w from 4.89 to 3.82 by applying 0 to 500 mg Al kg^{-1} in a soil pot experiment in China. Our results also showed a strong inverse power regression between both exchangeable and soluble fractions of soil Al and pH. Similar relationships have already been reported by Manrique (1986) who established quadratic relationships between exchangeable Al and pH in Ultisols in different regions. However, contrary to our results, they observed stronger relationships in the case of pH_{KCl} (R2=0.64**) than in pH_w (R2=0.39**). Our results were also in agreement with the findings of Al-Baquy et al. (2017) and Ruan et al. (2006) who respectively presented exponential and logarithmic equations between exchangeable Al and pH values in soils of southern China.

Previous researchers assessed different responses of tea plants to elevated levels of acidity using hydroponic culture (Sun et al., 2020) or soil pot experiments (Huang et al., 2017), with only a few field-based experiments. According to our field study, excessive Al availability as well as low pH value negatively influenced all yield-related morphological traits including fresh and dry yield, number of shoots, shoot weight, leaf area and leaf thickness in tea plants. Quality-related physiological traits, including caffeine and total polyphenols, remained statistically unchanged. The same result was observed in the case of chlorophyll index, although leaf dry matter (%) was adversely affected by Al stress. As the leaves became rougher, their quality for making tea decreased (Willson and Clifford, 1992). In our study, no change in total polyphenol content occurred in the indictor leaves (i.e. in a bud and two leaves) in response to increasing acidity. This may be attributed to stress adaptation during long-time growth period. However, lower leaves (supporting leaves) in the plant architecture, which are not indicative of tea quality, may be susceptible to being influenced by Al treatments. Contrary to our results, some short-term hydroponic studies on tea plantation systems showed significant decreases in chlorophyll index and total polyphenols content, following Al treatments (Mukhopadyay et al., 2012). Fresh and dry yields significantly decreased by the Al application of 1000 mg kg^{-1} (equivalent to exchangeable and soluble Al values of 365 and 39.6 mg kg^{-1} in the soils, respectively on average). In a soil pot experiment, Sivasubramaniam and Talibudeen (1971) evaluated the effect of Al application rates on the growth of tea plants. They recorded a negative response of tea plants when soil soluble Al increased from 5.4 to 23 mg kg^{-1} and simultaneously soil exchangeable Al increased from 315 to 495 mg kg^{-1}. Our results show that both fresh and dry yields significantly decreased, with the decrease of pH_w from 4.19 (500 mg Al kg^{-1}) to 3.82 (1000 mg Al kg-1). Similarly, the highest shoot dry weight of tea was obtained at pH=4.2 in a hydroponic culture (Yamashita et al., 2020). A statistically significant decrease in leaf thickness with an increase in soil acidity was observed (from 0.514 mm in control to 0.297 mm in the highest Al addition treatment). Leaf thickness reportedly decreased in response to Al stress in eucalyptus trees (Yang et al., 2015). On the whole, the thickness of the tea leaves in this study was greater than that of the value (0.2 mm) reported by Tsuji (2000) in Japan. Leaf thickness is related to the size of the cells, especially to the height of the palisade cylindrical cells. A decrease in the height of these cells usually hinders CO_2 intake (Terashima et al., 2011). In addition, leaf water storage decreases, with the decrease of leaf thickness (Becker, 2007). However, a previous report suggested that some other stressors can thicken plant leaves (Souri and Tohidloo, 2019). Leaf Al concentration significantly increased in response to higher Al values (from 546 mg Al kg-1 DW to 678, 842 and 954 mg Al kg-1 DW in A2, A3 and A4 treatments, respectively). According to Hajiboland et al. (2013), the Al concentration in the third and fourth leaves of an Iranian tea hybrid was in the range of 551 to 694 mg kg-1 DW. The concentration of Al in young tea leaves was reportedly 468-930 mg kg-1 DW (Ruan and Wong, 2001), 1800-2300 mg kg-1 DW (Ruan et al., 2004), 230-454 mg kg^{-1} DW (Ruan et al., 2003), and 270-2681 mg kg^{-1} DW (Xie et al., 2007). These results indicated the easy pathway of added Al from soil to the leaves via the symplast or apoplast (Fung and Wong, 2002). However, the genotypes differed significantly in leaf Al concentration. Accordingly, the highest and lowest leaf Al concentrations were observed in G4 (837 mg Al kg^{-1} DW, on average) and G3 (623 mg Al kg^{-1} DW on average) genotypes, respectively. Different tolerance of tea genotypes to aluminum toxicity was previously reported by Yadav and Mohanpuria (2009). The results showed strong correlations between leaf Al concentration and soil exchangeable Al (r=0.793**), soil soluble Al (r=0.753**), soil pH_w (r=0.788**) and soil pH_{KCl} (r=0.791**) in our experiment. Dong et al. (1999) reported that the correlation coefficients between

0.02M CaCl2 extractable Al in the soil and tea leaf were 0.64** for topsoils and 0.79** for subsoils. Dong et al. (2001) also observed that these relationships were stronger in the case of old leaves ($p<0.01$) than mature leaves ($p<0.05$). Furthermore, Xie et al. (2001) reported such relationships in young and old leaves (r=0.77**) and mature leaves (r=0.66*). Fung and Wong (2002) also reported a strong correlation coefficient of 0.721**** between soil exchangeable Al and leaf Al concentration.

Conclusion

An increase in soil acidity using aluminum sulfate not only decreased soil pH, but also increased the concentration of exchangeable and soluble Al as well as the amount of Al that was available to tea plants. The addition of Al caused a linear increase in the soluble and exchangeable fractions of Al (labile Al pools) in the soils. However, most of the added Al was moved into non-labile pools. This means that large amounts of aluminum sulfate was required to establish a given level of acidity in soils of tea orchards. In addition, a huge increase in the concentration of labile Al resulted in a slight decrease in soil pH. The results revealed that low-yield tea genotypes were more tolerant to soil acidification than high-yield ones. Al-tolerant genotypes are not recommended for cultivation, not only because of their genetically low-yield potential, but also because of their low quality, small polyphenol content and high leaf Al concentration. This suggests that care is needed when selecting tea genotypes. It is advised that such selections be based on tolerance data to ensure maximum yield and quality.

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Conflict of interest

The authors declare that they have no conflict of interest.

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