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Full Length Article

Mosaic Disease is Positively Correlated with Soil Sand Content and Negatively Correlated with Nutrient Availability for Tobacco Production

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Abstract

Tobacco is an important economic crop that faces severe threat from mosaic virus. To understand the relationship between tobacco mosaic disease and soil physicochemical properties, we conducted a field experiment on 76 plots with 50 plants each in plot in the largest tobacco producing area in Yunnan province, China. The degree of tobacco mosaic disease of 3800 individual tobacco plant was investigated at the rosette and topping stages in situ. Tobacco yield and agronomic traits, soil physiochemical properties, and nutrient concentration for nine elements in soil and plant were measured. The results revealed that leaf dry weight and main agronomic traits were negatively correlated with the incidence and index of tobacco mosaic disease. Stepwise linear regression analysis showed that the disease incidence and index were significantly positively correlated with the increase in soil sand content but negatively with silt content. Soil nitrogen and manganese concentrations were significant negatively correlated with tobacco mosaic disease incidence and index. It concludes that tobacco mosaic disease became increasingly serious with the increase in soil sand content and the decrease in soil nutrient availability of nitrogen and manganese.

Keywords: Disease resistance; Mosaic disease; Nutrient availability; Soil texture; Tobacco production

Introduction

As an important economic crop, the global planting area for tobacco is approximately 4 million ha with more than 120 countries and regions worldwide (FAOSTAT 2016). China has the largest tobacco industry in the world, accounts for 40% of the global tobacco production with the cultivation area of 1.27 million ha in 2016 (China Statistical Yearbook 2017). However, the tobacco industry is facing severe challenges due to the frequent occurrence of tobacco mosaic disease. Tobacco mosaic disease is an infection by *Tobacco mosaic virus* (TMV), which produces mosaic-like mottling discoloration symptoms on tobacco leaves (Islam *et al.* 2018). TMV is the major and the most devastating plant viruses in many regions of the world, especially in tobacco-growing areas, and can cause up to 80% mortality in tobacco crops endangered serious infectious diseases (Liu *et al.* 2010). As the main tobacco producing area in China, Yunnan province has an area of 440,000 ha of tobacco, thereby accounting for 33% of the total planting area (China Statistical Yearbook 2017). With the rapid increase in the planting area for vegetables as rotation crop, an increase was also observed for the vectors of TMV, such as aphid, and the risk of tobacco mosaic disease during the last decade (Li *et al.* 2014). The incidence was as high as 90 to 100%, and the tobacco yield can be reduced to 50 to 70% or even to 0% (Wang 2012), thereby causing huge economic losses. Although the impact of this disease can be reduced by pesticide application, the use of pesticides that aimed to inhibit viral replication also harms the host plant (Zhao *et al.* 2017). However, pesticides have also severe adverse effects on humans and ecosystems (Islam *et al.* 2018).

Nutrients are not only important for plant growth and but also affect the disease tolerance or resistance to the pathogens (Agrios 2005; Dordas 2008). The deficiency of most essential nutrients increases disease severity (Huber and Haneklaus 2007). Improved mineral nutrition helps plants escape diseases by the following two mechanisms: the formation of physical barrier that reduces infection by pathogens or virus (Eraslan *et al.* 2007) and stimulation of natural defense compounds, such as antioxidants (Dordas 2008). For a healthy agricultural system, nutrient manipulation through fertilization or modification of soil properties to influence nutrient availability is necessary to

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control plant disease (Barbetti *et al.* 2007; Huber and Jones 2013). As an important property, soil texture controls many processes in soils and regulates nutrient dynamics, soil organic matter and water retention and infiltration (Rabot *et al.* 2018). Considering that clay fraction can transport adsorbed nutrients, soils with high sand content generally have low fertility (Huang 2000; Calero *et al.* 2008). Thus, soil texture can potentially serve as an indicator for macro- and micronutrient availability (Mahnkopp *et al.* 2018). The relationship between nutrient availability and disease resistance of plants has been a research hotspot (Lacroix *et al.* 2014).

Nutrient availability contains many elements and as a whole affects the occurrence and development of disease (Huber and Jones 2013). Considering the complexity of factors affecting plant disease resistance, a reasonable statistical analysis model is very important. Multiple linear regression assesses the effects of the explanatory variables as predictor of response variables through correlation coefficient (Parisi-Kern *et al.* 2015). The stepwise linear regression is a widely used method for multivariate linear regression and quick way to identify the key impact factors from large amounts of candidate factors (Shuai *et al.* 2018). A large number of studies focused on the relationship between single element and disease (Amtmann *et al.* 2008; Whitaker *et al.* 2015). However, the relationship of tobacco mosaic disease with soil texture and availability of mineral nutrients has been rarely reported at large scaling. Our hypothesis was that high soil sand content leads to serious tobacco mosaic disease, thereby decreasing tobacco yield and nutrition uptake, and improved soil nutrient availability would increase the resistance for mosaic virus infection and reduce the severity of tobacco mosaic disease. This study aimed to improve the understanding of the relationship between tobacco mosaic disease and the environmental factors of plant growth and to provide theoretical basis for improving fertilizer management, controlling the occurrence of disease, and reducing the dependence of production system on pesticides in the future.

Materials and Methods

Selection of experiment region and sites

The study area was situated in the Yi Ethnic Township (24°10′N, 102°46′E, 2230 m a.s.l) of Lishan, Tonghai Country, Yunnan province, China. The mean annual temperature and total average annual rainfall were 15.2°C and 1000 mm, respectively.

Site selection was performed by experienced staff members from extension office of Tonghai Tobacco Company, local agricultural technicians, and individual farmers at the villages. The incidence of tobacco mosaic disease, land use history, fertilization and irrigation in the target sampling sites were greatly considered. According to the information above, 76 plots were finally identified in the Yi Ethnic Township of Lishan. Every plot had an area of 30 m^2 (5 m × 6 m) and contained 50 tobacco plants. The distance between the individual plots in the most cases was < 500 m. The geographical coordinates and altitudes of the plots were recorded by GPS (Garmin Colorado 300, USA). After the survey of tobacco mosaic disease degree of 3800 individual tobacco plants *in situ*, plant and soil samples were obtained for agronomical and chemical analyses.

Survey of tobacco mosaic disease incidence and disease index

The survey of tobacco mosaic disease degree was conducted in the selected 76 plots at the resettling (June 21 to 23, 2016) and topping stages (July 19 to 21, 2016). Tobacco mosaic disease classification was according to the National Standard of tobacco pest classification survey method of China (GBT-23222-2008). The degree of the disease in 3800 tobaccos plants was recorded in 76 plots. To avoid the personal variation of classification, we investigated each plot by two independent and experienced personnel. Two investigators obtained the average of the number of disease tobaccos and degree of disease to calculate the tobacco mosaic disease incidence and disease index. The tobacco mosaic disease incidence and index were calculated as follows:

$$\text{Disease infection (\%)} = \frac{\text{No. of tabacco plants with disease}}{\text{No. of tabacco plants investigated}} \times 100\%$$

$$\text{Disease index} = \frac{\sum\left(\text{No. of a degree}' \text{s tobacco plants} \times \text{disease degree}\right)}{\text{No. of tabacco plants investigataed} \times \text{the highest disease degree}} \times 100$$

Plant sampling and analysis

After surveying the degree of tobacco mosaic disease, one representative plant of each disease degree was immediately selected in the plot. The chosen tobacco plants were cut off at a distance of 0.5 cm from the ground and brought the samples back to the laboratory for agronomic trait and elemental determination.

Leaf SPAD (Soil Plant Analysis Development) values were measured the top 4^{th} to 6^{th} completely expanded leaves for each tobacco using a chlorophyll meter (SPAD-502, Konika Minolta Sensing Inc., Japan). Leaf number was counted from the base to the top, excluding the top 1 to 2 not fully expanded leaves. Plant height was measured from the base of stem to the growing point. Stem diameter was measured at 5 cm from the base of the stem by using a Vernier caliper. Leaf area was obtained by measuring the length and width of two largest leaves for its calculation. The fresh weight of leaves and stems were recorded, and the samples were put into the oven pre-heated to 105°C for half an hour to avoid the potential transformation of C and N in plant by killing the microbial activity. Afterwards, the samples were dried at 75°C for 48 h (Merchant *et al.* 2010).

The dried plant samples were pulverized coarsely

using a grinder (FZ102, Zhongxing Instrument Co., Ltd., China). A part of these samples were pulverized again with a ball mill (MM2000, Retsch, Haan, Germany). The N concentrations were determined using a Costech Elemental Analyzer (Costech ECH 4024 CHNSO, Costech, Italy). Leaf $\delta^{13}C$ was measured using aC isotope analyzer (Picarro CM-CRDS, Picarro, USA). Another 0.5 ± 0.05 g of coarse powder sample was weighed and transferred into the microwave digestion tube. HNO_3-H_2O_2 digestion liquid was then added using microwave digestion (BHW-09A, Shanghai Broadcom Chemical Technology Co., Ltd.) for digestion and boiling. P, K, Ca, Mg, Fe, Mn, Cu and Zn concentrations in the digestion liquor were determined by ICP-AES (Optima 3300DV, Perkin Elmer, USA). The leaf element concentration was calculated as follows:

$$\text{Leaf element concentration (mg kg}^{-1}) = \frac{\sum(\text{element concentration} \times \text{No. of this disease degree tobacco})}{\text{No. of totalrepresentation tobacco}}$$

Soil sampling and analysis

Soil sampling of all 76 plots was undertaken with the aid of a soil auger (3.5 cm diameter) at the 0 to 20 cm depth at the topping stage (July 19 to 21, 2016). Three individual soil samples in each plot were mixed. Soil samples were air dried for 5 days and grounded by hand to pass a 2.0 mm sieve for future laboratory analysis. Soil texture was determined using laser particle size analyzer (Malvern Mastersizer 2000, Malvern, Worcestershire, United Kingdom). Soil pH was measured in 1:2.5 soil: water solution using a combined electrode pH meter (IS 126, Shanghai instrument sales Instrument Technology Co., Ltd., China). Subsamples were powdered in a ball mill (MM200, Retsch, Haan, Germany). Soil total N concentration was determined using a Costech Elemental Analyzer (CostechECH 4024 CHNSO, Costech, Italy). Extractable soil Olsen-P was estimated from 1:5 soil: HCl (0.05M)-1/2H_2SO_4 (0.025M) extracts using an UV–vis Spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan). Extractable soil K was estimated from 1:10 soil-NH_4OAc (1M) extracts using a flame photometer (Flame photometer 410, Sherwood scientific, United Kingdom). The soil Ca, Mg, Fe, Mn, Cu, and Zn concentrations were determined from Mehlich 3 extracts by ICP-AES (Optima 3300DV, Perkin Elmer, USA).

Statistical analysis

Statistical analysis and calculations were performed using S.A.S. (version 9.2; S.A.S. Inc., Cary, North Carolina, USA) with the general linear model. One-way ANOVA was applied to determine the significance among the groups classified by soil sand content. Multiple comparisons of the mean values were corrected using Duncan's and least significant difference tests at 0.05 probability level. Correlation analysis was performed to examine relationships among the incidence, disease index, yield components and elemental concentration of soil using Pearson's correlation coefficients. Stepwise linear regressions among disease index, incidence and soil physical and chemical properties (*i.e.*, soil texture and elements concentration) were applied to identify the key impact factors from large amounts of candidate factors. Multiple linear regressions were implemented in S.A.S. 9.2. Identifying correlations between dependent and independent factors is important before regression analysis (Chen and Lu 2017). Pearson's correlation coefficient was adopted to examine the correlation between dependent and each independent factor before conducting the SLRs. The results were expressed as arithmetic means ± standard error of the means. The levels of significance at 0.05, 0.01, and 0.001 were denoted by *, **, and ***, respectively, and insignificant results were denoted by ns.

Results

Pooled over all 76 plots, the average incidence and index of tobacco mosaic disease were 22% and 15 at the resettling stage and 45% and 28 at the topping stage, respectively (Fig. 1). The average leaf dry weight was 0.83 t ha^{-1} at the resettling stage and 2.20 t ha^{-1} at the topping stage (Fig. 1). Leaf dry weight was negatively correlated with tobacco mosaic disease incidence and disease index (Fig. 2).

Stepwise linear regressions were performed to explore the key parameters that influence the incidence and index of tobacco mosaic disease. Independent factors including sand and silt contents, soil pH, soil element content (N, P, K, Ca, Mg, Fe, Mn, Cu and Zn) and tobacco leaf $\delta^{13}C$ were selected by Pearson's correlation coefficients (data not shown). The coefficient of determination (R^2-adjusted) was used to assess the goodness of fit of the models. The results showed that soil sand content was first selected from the model and had the largest contribution to the incidence (R^2-adjusted=0.284, $P < 0.001$, Table 1, model 1) and index of tobacco mosaic disease (R^2-adjusted=0.323, $P < 0.001$, Table 1, model 1). Soil total N and P contents were selected sequentially and considerably increased the R^2 value of the models. Tobacco mosaic disease incidence and index were significantly and positively correlated with soil sand content but negatively correlated with soil silt content (Fig. 3).

All 76 plots were divided into the following four groups according to the soil sand content with significant differences: soil sand content of ≤ 45%, 46–60%, 61–75%, and > 75%. Meanwhile, the silt and clay content showed opposite results (Table 3). Soil pH and leaf $\delta^{13}C$ were significantly lower in the group with ≤45% soil sand content than that in other three groups. When soil sand content was > 60%, the tobacco mosaic disease incidence and index were significantly higher than that when the sand content was < 60% (Fig. 4). By contrast, the tobacco leaf dry weight was significantly lower. Similar tendency was found for the maximum leaf area, stem dry weight, SPAD value, plant height, and stem diameter (Fig. 5). No difference was

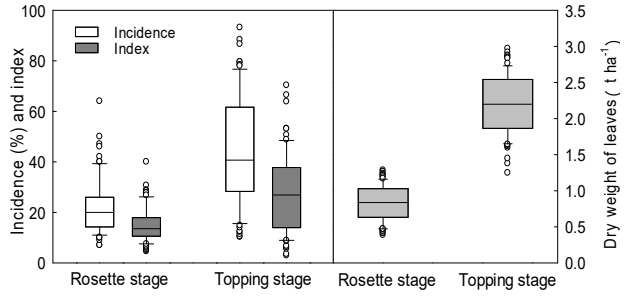

Fig. 1: Average values of incidence and index of tobacco mosaic disease (left) and dry weight of leaves (right) at rosette and topping stages (n=76)

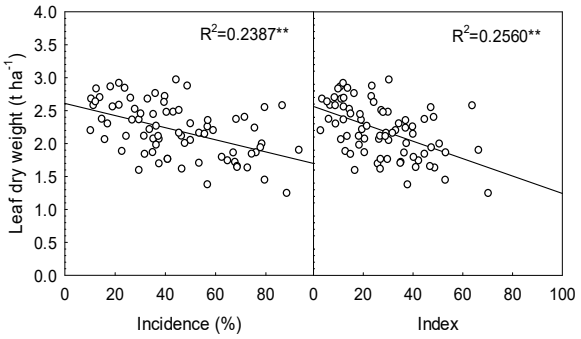

Fig. 2: Correlation between incidence and index of tobacco mosaic disease and dry weight of tobacco leaves at topping stage (n=76). **Significant at 0.01probability level

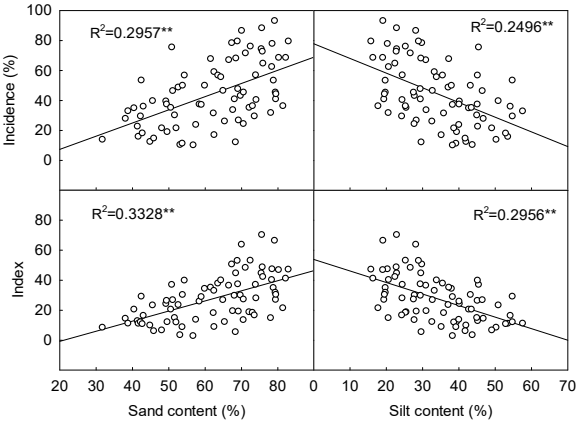

Fig. 3: Correlation between incidence and index of tobacco mosaic disease with soil texture at topping stage (n=76). **Significant at 0.01probability level

detected on leaf number among the groups of soil sand content (Fig. 5).

The tobacco leaf of the group with low soil sand content had significantly higher Mg concentration than that of the group with high soil sand content. Otherwise, no difference in other nutrients' concentrations was observed (Table 2). However, the concentrations of most soil nutrients were significantly higher in the group with low

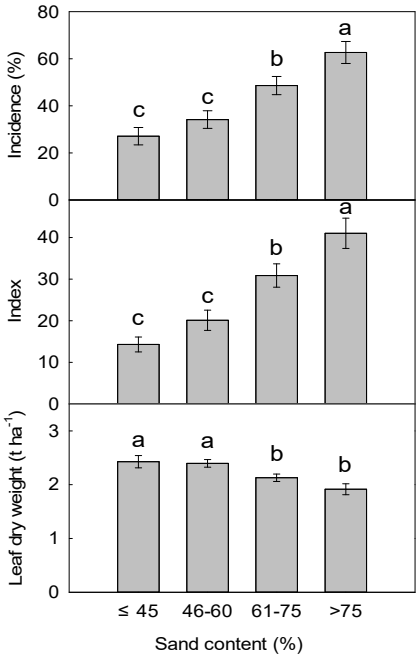

Fig. 4: Incidence and index of tobacco mosaic disease and dry weight of leaves at different groups classified by soil sand content at topping stage. Soil sand content: ≤ 45, n=11; 46–60, n=21; 61–75, n=28; > 75, n=16. Different lowercase letters indicated statistically significant difference among groups ($P < 0.05$)

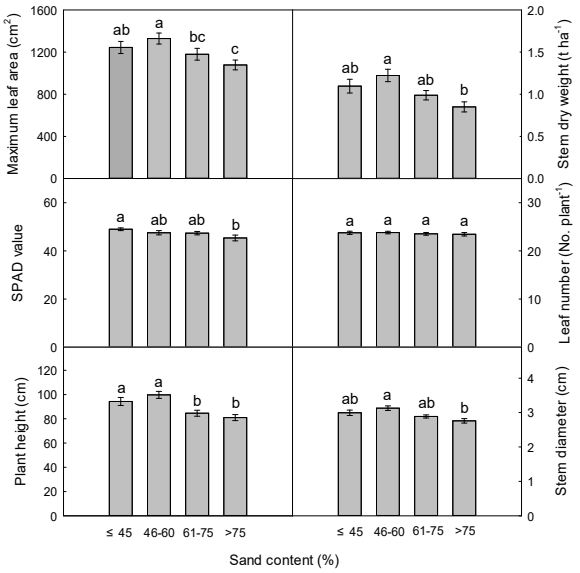

Fig. 5: Maximum leaf area, stem dry weight, SPAD value of leaf, leaf number, plant height, and stem diameter of tobacco plants at topping stage in different groups classified by soil sand content at topping stage. Soil sand content: ≤ 45, n=11; 46–60, n=21; 61–75, n=28; > 75, n=16. Different lowercase letters indicated statistically significant difference among groups ($P < 0.05$)

sand content than that with high sand content, whereas P and Fe showed opposite results (Fig. 6). According to the analysis of significant correlation (data not shown), multiple

Table 1: Linear models explaining tobacco mosaic disease incidence and disease index on the basis of soil physical chemical properties data set after stepwise regression selection (n = 76)

Variables	Parameter estimate	*β*-Value	*P*-Value	R^2	R^2-adjusted	*P*-Value
		Mosaic incidence				
1	Sand content	0.541	0.000	0.293	0.284	0.000
2	Sand content	0.424	0.000	0.353	0.335	0.000
	TN	−0.271	0.011			
		Mosaic index				
1	Sand content	0.576	0.000	0.332	0.323	0.000
2	Sand content	0.473	0.000	0.378	0.361	0.000
	TN	−0.238	0.023			

The model results from a stepwise selection procedure using nine parameters (*i.e.*, $\delta^{13}C$, soil sand content, soil silt content, soil N concentration, soil P concentration, soil Ca concentration, soil Mg concentration, soil Mn concentration, and soil Cu concentration) selected by Pearson's correlation coefficients

Table 2: Average concentration of macro- and micro-elements of tobacco leaves at topping stage at different groups classified by soil sand content (SSC)

SSC	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
(%)	(g/kg)	(g/kg)	(g/kg)	(g/kg)	(g/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
≤45	28a	2.4a	36a	22a	6.3a	366a	96a	7.9a	41a
46–60	33a	2.4a	36a	24a	6.4a	365a	124a	10.6a	40a
61–75	33a	2.5a	38a	23a	5.8ab	351a	122a	10.1a	42a
75	30a	2.6a	38a	21a	5.3b	320a	100a	9.1a	38a

Soil sand content: ≤ 45, n=11; 46–60, n=21; 61–75, n=28; > 75, n=16. Within each column, different lowercase letters indicate statistically significant difference among groups (P <0.05)

Table 3: Contents of sand, silt and clay, soil pH and tobacco leaf $\delta^{13}C$ at topping stage in different groups classified by soil sand content

SSC	Sand content	Silt content	Clay content	pH	$\delta^{13}C$
%	%	%	%		
≤ 45	40.7d	50.4d	8.9a	6.3a	-26.2a
46–60	52.5c	42.3c	5.2b	5.2b	-26.4b
61–75	68.3b	29.7b	2.1bc	5.1b	-26.6b
> 75	78.8a	19.9a	1.3c	5.3b	-26.9b

Soil sand content: ≤ 45, n=11; 46–60, n=21; 61–75, n=28; > 75, n=16. Within each column, different lowercase letters indicate statistically significant difference among groups (P<0.05)

Table 4: Linear models explaining tobacco mosaic disease incidence and index on the basis of soil physical chemical properties data set after multiple linear regression analysis (n=76)

Variables	*β*-Value	*P*-Value	R^2	*P*-Value
Mosaic incidence				
Mn	-0.350	0.023**	0.360	0.000***
N	-0.300	0.055*		
P	0.130	0.248ns		
$\delta^{13}C$	0.088	0.459 ns		
Ca	0.063	0.717 ns		
Mg	0.062	0.757 ns		
Cu	-0.028	0.877 ns		
Mosaic index				
Mn	−0.355	0.020**	0.368	0.000***
N	−0.294	0.059*		
P	0.156	0.165 ns		
Mg	0.102	0.610 ns		
$\delta^{13}C$	−0.093	0.431 ns		
Ca	−0.035	0.841 ns		
Cu	−0.030	0.866 ns		

*Significant at 0.05 probability level. **Significant at 0.01 probability level. ***Significant at 0.001 probability level. ns, not significant

linear regression was performed with tobacco leaf $\delta^{13}C$ and six nutrients as explanatory variables and disease incidence or index as response variable. The R^2 values were 0.360 and 0.368, and the p-value of regression were < 0.001 (Table 4), which indicated the regression was advisable. In the regression analysis with disease index as the response variable, the standardized regressive coefficient (β-value) of Mn and N were −0.355 and −0.294, with $p<0.05$, respectively (Table 4). The similar results were found in the regression with disease incidence as the dependent variable (Table 4), which indicated significant negatively impact on disease incidence and index. The other factors did not have an impact (Table 4).

Discussion

The TMV can rapidly accumulate in the host plants (Scholthof 2004) and its replication may be integrated with the metabolism of infected tobacco plants (Zhao *et al.* 2017). Meanwhile, the increasing TMV coat proteins destroys plant nutrient transport tissues, thereby causing the growth of tobacco plants to be slow, dwarfed, deformed,

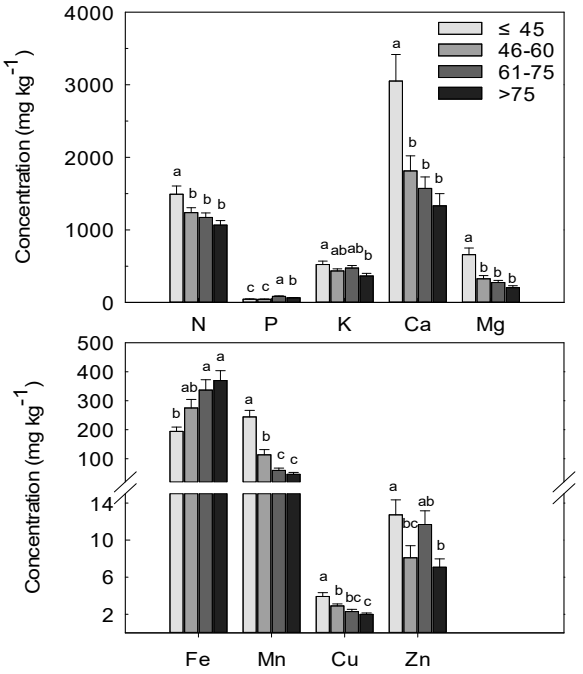

Fig. 6: Macro- and microelement contents in soil in different groups classified by soil sand content at topping stage. Soil sand content: ≤ 45, n=11; 46–60, n=21; 61–75, n=28; > 75, n=16. Different lowercase letters indicated statistically significant difference among groups ($P < 0.05$)

and even lead to death (Zhu and Francki 1992; Scholthof 2004). Given the tobacco strain does not have a perfect immune metabolic system, once the virus invades the body, it is difficult to remove and will re-infect other plants with low resistance to disease (Ma and He 2005). This phenomenon can be clearly confirmed by our results, wherein the incidence and index of tobacco mosaic disease were significantly higher at the topping stages than at rosette stage. Meanwhile, leaf dry weight was negatively correlated with tobacco mosaic disease incidence and index (Islam *et al.* 2018). Although the plant's resistance and tolerance to tobacco mosaic disease are genetically controlled (Agrios 2005), they are affected by the air temperature, humidity, and especially soil fertility, including nutrition availability (Huber and Jones 2013; Bittner *et al.* 2016).

To identify the key impact factors from large candidate parameters, stepwise linear regressions were used after identifying correlations between dependent and independent variables (Chen and Lu 2017). Our results demonstrated that soil sand content made the largest contribution to the incidence and index of tobacco mosaic disease. Meanwhile, tobacco mosaic disease incidence and index were significantly positively correlated with the increase in soil sand content while negatively with the increase in soil silt content. According to American soil texture classification standard, increasing 10 to 20% sand content would change the soil property (Whiteside *et al.* 1967). With the 15% increase in soil sand content, the incidence and index were significantly increased, while the leaf dry weight and agronomic traits significantly decreased. These results confirmed that soil sand content was one of the most important key factors determine the severity of tobacco mosaic disease. This result agreed with the findings of a similar study in which a negatively correlation between soil clay content and apple replant disease (Mahnkopp *et al.* 2018) and a positively correlation with the biomass production (Hamoud *et al.* 2019) were reported. Soil with high clay content generally has high soil organic matter content, which can not only improve soil structure but also increase the availability of soil nutrients, especially the immobile micro elements that is closely related to plant disease (Tian *et al.* 2018; Rabot *et al.* 2018). Our results prove that the higher the soil sand content is, the lower the soil total N and available K, Ca, Mg, Mn, Cu, and Zn concentrations are, and the higher the incidence and index of tobacco mosaic disease will be.

Soil nutrition level and plant defense mechanism are highly correlated (Dordas 2008). Nutrient availability could directly limit the production of viral nucleic acids and proteins (Whitaker *et al.* 2015), thus decreased tobacco mosaic disease index. Virus infection can induce the accumulation of reactive oxygen in plant tissues (Xi *et al.* 2010). To prevent damage by reactive oxygen, plant has a set of antioxidant enzyme defense systems, including superoxide dismutase, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (Gholi-Tolouie *et al.* 2018). Several mineral nutrients have beneficial effects on health and natural defense in response to pathogens (Garcia-Mina 2012). However, the results are inconsistent and contradict each other (Walters and Bingham 2007). According to results of multiple linear regression, soil N and Mn concentrations have a positive impact against tobacco mosaic disease. This result can be confirmed by a previous study, in which plant was cultivated in water culture solution with different Mn concentration and artificial inoculated with TMV (Welkie and Pound 1958). However, direct evidence from field investigations verifying the relationship between the effectiveness of soil Mn and the incidence of tobacco mosaic disease is rare. The effect of nutrients on reducing the severity of diseases can be attributed to the involvement in physiology and biochemistry of the plant because many of the essential nutrients are involved in many processes that can affect the insistence response of plants to pathogens (Dordas 2008).

Generally, the soil N fertilizer additions above the recommended rate (90 kg N ha^{-1}) can increase disease severity caused by the obligate parasites, such as mosaic virus (Marchetti *et al.* 2006; Whitaker *et al.* 2015). By contrast, our results showed that tobacco mosaic disease index decreased with the increase in soil N concentration (Fig. 6). With the increase of N application rates, the activity of polyphenol oxidase and phenylalanine ammonia lyase of leaves tended upwards (Wang *et al.* 2005). In

addition, N limitation severely compromises the ability of *Arabidopsis thaliana* to express induced resistance to pathogen infection (Dietrich *et al.* 2004, 2005). The N-limited plants would express a delay in defense enzyme expression and a decrease in enzyme levels (Dietrich *et al.* 2004), thereby reducing host investment in N-containing defenses under N limitation. These results suggested that N-limited plants should be more susceptible to tobacco mosaic disease infection. Meanwhile, N forms *e.g.*, ammonium and nitrate may have opposite effects on disease (Huber and Watson 1974; Gupta *et al.* 2013). It has been proved that NO_3^--fed tobacco own higher resistance than NH_4^+-fed tobacco (Gupta *et al.* 2013). Therefore, on one hand, results show that with the increase of soil N concentration, the resistance of tobacco plant increased and the disease index decreased. On the other hand, nitrate contained fertilizer should be used for tobacco plant in order to increase the disease resistance (Wang *et al.* 2009). Mn directly increases host resistance by enhancing lignification and increasing the soluble phenolic compound concentration (Eskandari *et al.* 2018). Another function of Mn is to induce protective mechanisms and increase host resistance (Simoglou and Dordas 2006). Mn can activate plant antioxidant enzymes in the exome of leaves (Kalim *et al.* 2003; Millaleo *et al.* 2010; Heine *et al.* 2011), which may lead to disease resistance. Mn also acts as a cofactor for key enzymes in plant defense, such as phenylalanine ammonia-lyase and inhibits exogenous enzymes produced by some fungi such as pectinase, to degrade host cell walls (Monteiro *et al.* 2016). Given that Mn plays such an important role in plant disease resistance, combined with our results, we suggested the application of Mn fertilizer, especially for sandy soil, to reduce the incidence of tobacco mosaic disease.

Conclusion

Our results demonstrated that high sand content and low nutrient availability in mountainous soil were the important factors responding for the decline of tobacco plant resistance and the occurrence of tobacco mosaic disease. Therefore, it is one of the effective ways to prevent and reduce tobacco mosaic disease by increasing soil fertility and applying micronutrient fertilizer.

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Author Contributions

ML WZ and SL conceived the study and contributed to the design and interpretation of the research. LW, MF, ML, WZ and XX carried out the experiments. XG, JL and LZ contributed to the field experiments and collected samples. MF analyzed the data, prepared figures and wrote the manuscript. SL modified the manuscript.

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