Effect of different genotypes of flue-cured tobaccos and different culture methods on k nutrition in rhizospheric and non-rhizospheric soils

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Abstract
The experiment was carried out to investigate the effect of Cation Exchange Capacity (CEC) on the variation of readily available K content and non-exchangeable K content in rhizospheric and non-rhizospheric soils with three different genotypes (i.e. Nongda 202, K326 and NC89) of flue-cured tobaccos (Nicotiana tabacum L.) in soil and sand culture. The activating effects of root exudates and population of rhizospheric microbes on K content of soil was also investigated. Results show that the readily available K content in both rhizospheric and non-rhizospheric soils varies for difference genotypes, i.e. Nongda202＞K326 ＞NC89. Root exudates activate K content in the soil to different levels with maximum activation observed for Nongda202. The difference is significant among all three genotypes. The fungi population in the rhizospheric soils is larger compared to that in non-rhizospheric soils for all three genotypes. There were significantly higher fungi populatiosn in Nongda202 rhizospheric soils than that of K326 and NC89. The difference between latter two was not significant. The difference in population was significant in rhizospheric bacteria but not in actinomycete. Nongda 202 shows better CEC than NC89 and K326 with insignificant difference between latter two. Therefore, the physiological properties of the roots should be taking into account when breeding K-enriching tobacco varieties.

Key Words
Flue-cured tobacco, potassium, root exudates, soil microbe, cation exchange capacity (CEC).

Introduction

Potassium content of tobacco leaves is closely related to the maturity, aroma and taste of tobacco leaves, as well as safety of cigarette products, has been treated as one of the most important indicators of tobacco quality for many years. In most areas of China, especially the northern part, average potassium content of tobacco leaves can hardly exceed 1.5%, which is the major problem for improving quality of tobacco leaves. Previous studies show that to significantly increase the potassium content of tobacco leaves, two to three times more potassium fertilizer compared to the optimum amount is required. However, this approach is limited due to the shortage of potassium fertilizer resources in mainland China. Previous investigations conducted on crops such as wheat, corn, and Amaranthus hypochondriacus demonstrate that genotype with high potassium efficiency have enormous potential at improving potassium content (plant-available K)of soil and the utilization efficiency of potassium fertilizer. The soil in northern China (to the north of Yangze River) contains a considerably amount of readily available K and is rich in Potassium content, which can potentially provide large amount of Potassium. However, due to the calcareous nature of soil, especially the strong basicity and high Calcium content, the availability of Potassium content is poor. Moreover, potassium fertilizer tends to be immobilized by the particle agrotype and frequent change of humidity, which also affects the Potassium transmission into of tobacco leaves. Therefore, breeding and selection of genotype that can efficiently utilize the potassium content in the soil is not only important in improving the Potassium content in tobacco leaves, but also beneficial in reducing the production cost and resources used.

The rhizospheric Potassium concentration of a plant mainly depends on its root characteristics, especially the Cation Exchange Capacity (CEC), root exudates, and population and species of rhizosphe microbes. Chui et al. demonstrated that the Potassium efficient genotype of ramee exhibits comparably high CEC. Potassium efficient genotype of Amaranthus hypochondriacus can produce a local Potassium concentration in a region surrounding the rhizospheric area (Chui and Li 2000). A larger population of rhizospheric microbes and more active root exudation is observed compared to other genotypes. Currently, the studies of plant Potassium nutrition mainly focus on the aspects of physiological property and genetic improvement. The effect of the root characteristics for difference types of Potassium efficient tobacco leaves, root exudates, population and species of rhizospheric microbes are not investigated. To facilitate the selection and breeding of high Potassium content tobacco leaves, this study looks into the difference of root exudates, rhizospheric microbes and root characteristics between the Potassium efficient and general genotypes.

Methods

Experiment materials

The soil sample used is light-loamy Chao soil, which was collected at depth of 0-20cm from surface soil at Science Park in Henan Agricultural University. The soil has been tested: organic matter 12.20 g/kg, N 0.84 g/kg, P_2O_5 0.12 g/kg, readily available K 125 mg/kg, slowly available K 681 g/kg, pH 7.47. The flue-cured tobacco samples are Nongda 202 (ND202), K326 and NC89, in which ND202 is the product which is potassium rich. The inheritance of this product is stable after directional selection for over 10 generations and the rest are comparison products. All cultivars were raised seedling in plates filled with growing media and watered in a daily basis to keep certain humidity. The tobacco plants are transplanted to soil culture and sand culture when 6 leaves have grown.

Experiment design

Soil culture experiment was carried out by a pot method. Pots are 27 cm in radius, 30 cm in height. Every pot was filled with 75 g compound fertilize with 15% of N, P_2O_5 and K_2O by randomized block design with 3 replications. Root-bags were used to raise tobacco plants. The plants with 6 visible leaves and good growth consistency were transplanted into root bags made by 300 mesh polyamide net in the size of 14 cm x 14 cm x 14 cm (~500 g soil). After 40 days, tobacco plants were fast growing stage and filled root bags with roots. This is the best time to compare the variation of different genotypes for element uptake due to active metabolism and a large amount of element uptake. A 2 cm upper layer of soil from the root bags was removed. The remaining soil inside bags is rhizospheric soil; the soil outside of bags is non-rhizospheric soil. Contents of available K and non-exchangeable K, as well as the populations of bacteria, fungus and actinomycete were measured in the two kinds of soils.

A sand culture experiment was carried out by the pot method, in which the pots were filled with 1.5 kg quartz sand and watered by Hoagland solution once a day. After 40 days, tobacco plants with good growth consistency were collected for different genotypes. Samples are cultured in 500 ml de-ionized water replaced every 24 hours. The replaced de-ionized water was collected for 3 days, fitted with a SAD-4 resin bar at the flow rate of 5ml/min and then resin was washed by de-ionized water with a volume of 10 times that of the bars, in order to eliminate the nutrient content. Methanol was used to wash off exudate from roots, cold-dried and mixed with high purity water (30 ml). 0.1 mol/L HCL solution was used to alter the pH value to 2.0, get rid of water phase after extraction 3 times by ether. 0.1 mol/L NaOH was used to alter the pH value to 8.0, and then extracted 3 times. Ether was evaporated off, mixed with a small amount of methanol that add de-ionized water added to 5 ml. Concentrated root exudates were thus acquired.

The soil microbes were measured by means of a diluting plate. Beet extract, peptone and agar were used as medium to culture bacteria. The actinomycete and fungi were cultured by Gauss's synthetic agar and Martin medium respectively. Cation exchange capacity (CEC) was measured (Chui and Li 2000).
SPSS 12.0 was used for analysing the data by ANOVA and comparative analysis.

Results

Effect of different genotypes on different types of K content at rhizospheric soil

Table 1 shows available potassium and non-exchangeable potassium in rhizospheric soil from the 3 genotype tobaccos are lower than that of non-rhizospheric soil. ND202 has the highest available K content with the 18.47% and 13.63% exceeding to NC89 and K326 respectively. Meanwhile, ND202 contains the much lower amount of non-exchangeable K than the other two varieties. The K content in non-rhizospheric soil shows significant difference with ND202 containing highest amounts of available K (156.5 mg/kg).

Table 1. Contents of available and non-exchangeable potassium in rhizospheric soils for flue-cured tobaccos of different (K_2O mg/kg).

Genotypes	Rhizospheric soil		Non-rhizospheric	
	Available potassium	Non-exchangeable potassium	Available potassium	Non-exchangeable potassium
ND202	95.4 aA	750.1 bB	156.5 aA	766.5 aA
NC89	84.3 bA	855.2 aA	138.7 bB	708.7 Bb
K326	84.0 bA	845.5 aA	132.1 bB	789.8 aA

Notes: Within column, means followed by the same small or capital letters are not significantly different at 5% or 1% levels by LSD test, respectively. The same as below.

Effect of different genotypes on soil K activation of root exudates of flue-cured tobaccos
Table 2 shows all 3 genotypes of tobaccos have higher amount of available potassium extracted from root exudates than the comparison sample (pure water). The differences are 45.6%, 35.8% and 24.4% respectively. This indicates that the root exudates from tobacco plant play a role on enhancing the available potassium content of soils.

Table 2. Soil K activation of root exudates of flue-cured tobaccos different in genotype (K_2O mg/kg).

Genotype	Available potassium
ND202	239.8 aA
K326	223.8 bB
NC89	205.2 cC
Deionized water	164.7 dD

Effect of different genotypes on soil microbse in rhizospheric soils for flue-cured tobaccos
Table 3 shows the number of fungi in rhizospheric soil are higher than that in non-rhizospheric soils of ND202, K326 and NC89, with increments of 26.5%, 6.5% and 8.6% respectively.The fungi population in the rhizospheric soils is larger compared to that in non-rhizospheric soils for all three genotypes. Nongda202 has significantly higher fungi population than K326 and NC89. The difference between latter two was not significant. The difference in population was significant for rhizospheric bacteria but not for actinomycete.

Table 3. Numbers of soil microbes in rhizospheric soils for flue-cured tobaccos different in genotype (number/g dry soil).

Genotypes	Rhizospheric soil		
	Fungi number	Bacteria number	Actinomyces number
ND202	4.728E+04 aA	1.224E+06 bB	9.574E+06 aA
NC89	4.053E+04 bB	1.6144E+06 aA	1.012E+07 aA
K326	3.971E+04 bB	1.0695E+06 cC	1.027E+07 aA

Effect of different genotypes on cation exchange capacity of the roots and potassium content for organs of flue-cured tobaccos
Table 4 illustrates the CEC of the roots and potassium content in root, stalk and leaf of tobaccos. Nongda 202 shows better CEC than NC89 and K326 with insignificant difference between latter two.

Table 4. Cation exchange capacity of the roots and potassium content in organs of flue-cured tobaccos different in genotypes.

Genotypes	CEC(me100/g)	Content of potassium (K_2O g/kg)		
		Root	Stalk	Leaf
ND202	51.0 aA	5.4 aA	11.1 aA	33.6 aA
NC89	43.7 bB	4.9 bA	11.2 aA	30.2 bB
K326	43.4 bB	4.9 bA	10.8 aA	29.8 bB

Conclusion
The highest amount of available potassium content were in rhizospheric soil of ND202 but there was relatively low non-exchangeable potassium. The root extraction from the ND202 root system shows the largest activation energy for potassium content in soil, resulting in excess non-exchangeable potassium a and shifted dynamic balance of these two kinds of potassium in soils. On the other hand, the highest concentration of available potassium was found in rhizospheric soil of ND202. This may due to the root exudates which help transport available potassium from non-rhizospheric to rhizospheric soil. Extracts from roots of tobacco plants provide a medium for multiplication of fungi. The higher activation ability of potassium is related to the large amount of root exudates. It is also proved that cation exchange capacity affects potassium absorption by plants directly. The generation of potassium ions in free space enhances the indirect absorption of potassium and promotes the transport of potassium to upper parts of plants.

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