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Selection of Inbred Lines for Breeding of Maize with High Efficiency in Iron Utilization

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Abstract

Crops are often subjected to iron (Fe)-deficiency due to the limited solubility of this essential element in most neutral or basic soils. Developing cultivars with high efficiency in Fe utilization via breeding programs can provide solutions to this problem as a long term strategy. In the present study, to select inbred lines for breeding of maize with high efficiency in Fe utilization, we screened 123 inbred lines at the seedling stage by analyzing secretion pattern of phytosiderophores, a class of non-protein amino acids released by graminaceous species for Fe utilization, using high-performance liquid chromatography. One hundred and twenty three inbred lines were clustered into nine groups. The low PS secretion rate under Fe-sufficient condition and high PS secretion rate increment after Fe-deficiency treatment type were the ideal inbred lines for breeding of maize with high efficiency in Fe utilization.

Keywords

Maize, Phytosiderophore, Iron-Efficient Utilization

1. Introduction

Iron (Fe) is one of the essential nutrients for plant growth, which plays important roles in many crucial metabolic pathways [1]. Although it is the fourth most common element in the Earth's crust, plants are often subjected to Fe-deficiency due to its low solubility especially in calcareous soils (about 30% of world's cultivated soils) [2] [3]. In world-wide agricultural production, it is an acute contradiction between Fe-abundance in soils and Fe-

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deficiency in crops. Although this issue can be solved through soil improvement and foliage spray of Fe fertilizer, it is a real economic burden. Developing cultivars with high efficiency in Fe utilization via breeding programs can provide solutions to this problem as a long term strategy.

Under Fe-deficiency stress condition, plants have evolved two distinct strategies to solubilize and transport Fe [4]. Strategy I plants, including dicotyledonous and non-graminaceous monocotyledonous species, are characterized by release of proton (H^+) and Fe (III)-chelate reductase to increase Fe-acquisition [5]. Graminaceous species (strategy II plants) secrete phytosiderophores (PSs), a class of non-protein amino acids, into the rhizosphere to solubilize and utilize insoluble Fe (III) in soils [1].

It has been demonstrated that PS secretion rate varies with different graminaceous species, and even with different cultivars within the same species [4] [6]. In addition, Fe-deficiency treatment increases PS secreting rate dramatically [4] [7]. So, it is necessary to characterize PS secreting pattern for developing cultivars of graminaceous species with high efficiency in Fe utilization. At present, no publications on this field are available.

In the present study, to select inbred lines suited to develop maize hybrids with high efficiency in Fe utilization, PS secretion patterns of 123 inbred lines were analyzed. These lines were clustered into nine groups based on PS secretion rates under Fe-sufficient condition and PS secretion rate increment after Fe-deficiency treatment. And then, lines suited to developing cultivars with high efficiency in Fe utilization were discussed. We believe that our work will benefit the Fe-efficient maize breeding.

2. Materials and Methods

2.1. Plant Materials and Growth Condition

One hundred and twenty three maize inbred lines were used in the present study, which were displayed in **Table 1**. Seeds were incubated on moist paperbed at 25˚C for germination. Germinated seeds were transferred to a net floating on a continuously aerated Hoagland's solution (pH 6.0) in the temperature-controlled growth chamber with a 16 h light/8 h dark photoperiod at 25˚C. After 7 d of culture, seedlings were removed endosperm and then transferred into the aerated Hoagland's solution without Fe element for seven days for collection of root exudates under Fe-deficiency stress. Root exudates under Fe-efficient condition were collected from plants cultured in Hoagland's solution with every essential element. All experiments were performed with three replicates.

2.2. Collection of Root Exudates

Firstly, roots of maize seedling were washed clean with deionized water, and then placed in 500 ml of deionized water for collection of root exudates. Root exudates collection was started at 7:00 and terminated at 15:00, lasting eight hours.

2.3. High-Performance Liquid Chromatography (HPLC) Analysis of PS

Root exudates were chromatographed on Amberlite IR120B (H^+ form) followed by freeze-dried in vacuum. The freeze-dried materials were dissolved in 10 ml ultrapure water, and then analyzed by HPLC using C18 column (4.6 mm × 250 mm) with wavelength 209 nm, mobile phase 0.5% $(NH_4)H_2PO_4$ (pH 2.85) and flow rate 0.6 $ml \cdot min^{-1}$.

2.4. Statistical Analysis

DPS 7.05 software was used for data processing [8]. Chi-square distance and sum of squared deviations were adopted in cluster analysis.

3. Results

3.1. PS Secretion Characteristics of the 123 Inbred Lines at Seedling Stage

HPLC analysis was performed to characterize PS secretion of 123 inbred lines at seedling stage. **Table 1** listed the PS secretion data of these lines. The average of PS secretion rate under Fe-sufficient condition was 7.281 mg/(g dry root*8h). The variation range among different genotypes was from 1.786 ± 0.222 mg/(g dry root*8h) [mean ± standard deviation (S.D.), Dan340] to 19.695 ± 0.442 mg/(g dry root*8h) (LL0726-1). The average of

Table 1. Phytosiderophores (PS) secretion rate [mg·g^{-1} dry root (8 h)$^{-1}$] of 123 maize inbred lines under Fe-sufficient condition (+Fe) and after Fe-deficiency.

Inbred lines	PS secretion rate (+Fe) [mg/g dry root*8h]	PS secretion rate (−Fe) [mg/g dry root*8h]	Inbred lines	PS secretion rate (+Fe) [mg/g dry root*8h]	PS secretion rate (−Fe) [mg/g dry root*8h]
196	4.754 ± 0.252	4.958 ± 0.217	04qun0631-1	5.474 ± 0.376	7.386 ± 0.276
502	7.196 ± 0.068	7.653 ± 0.414	04qun-12-2	6.856 ± 0.818	11.427 ± 0.767
543	6.296 ± 0.251	8.084 ± 0.409	04qun-22-1	5.396 ± 0.513	6.134 ± 0.362
933	10.084 ± 0.538	13.796 ± 1.377	04qun-33	3.903 ± 0.205	11.942 ± 0.927
1029	4.515 ± 0.118	7.670 ± 0.991	958-1-9	6.615 ± 0.226	9.112 ± 1.744
2102	13.170 ± 0.528	23.335 ± 0.798	99qun-8-2	4.404 ± 0.112	4.446 ± 0.089
3189	9.753 ± 0.192	10.524 ± 0.530	A150-3-1	2.626 ± 0.232	5.330 ± 0.282
3841	9.393 ± 0.333	10.151 ± 0.908	A210-1	10.583 ± 1.027	12.706 ± 0.486
3904	10.826 ± 0.339	11.017 ± 0.883	A22	5.536 ± 0.484	6.234 ± 0.408
4866	4.768 ± 0.318	6.684 ± 0.263	A348-4-2	6.389 ± 0.031	6.692 ± 0.141
5005	5.079 ± 0.628	7.685 ± 0.765	A369-2-1	3.432 ± 0.370	5.989 ± 0.362
5237	13.143 ± 0.169	14.023 ± 0.186	A6-2-1	11.629 ± 1.571	13.734 ± 0.688
8723	3.474 ± 0.179	3.969 ± 0.073	B jian8	5.552 ± 0.618	8.775 ± 0.167
9418	5.173 ± 0.329	14.945 ± 0.037	B104-1-2	9.297 ± 0.200	13.021 ± 0.256
52106	5.134 ± 0.346	7.799 ± 0.451	B117	3.566 ± 0.581	7.103 ± 0.134
4880-1-4	4.845 ± 0.445	5.320 ± 0.213	B121	4.361 ± 0.276	8.766 ± 1.252
03qun-3-3	10.311 ± 0.524	12.291 ± 0.833	B138-1	9.418 ± 0.568	12.354 ± 1.032
03qun-8-2	7.156 ± 0.129	7.491 ± 0.154	B178-1	5.782 ± 0.374	7.506 ± 0.544
04qun0601-3	5.034 ± 0.294	5.114 ± 0.318	B209	4.108 ± 0.347	4.165 ± 0.456
04qun0603-2	8.072 ± 0.558	10.320 ± 0.599	B217-2-2	18.275 ± 1.066	19.069 ± 0.408
04qun0603-9	7.533 ± 0.272	8.554 ± 0.446	B223-1	6.523 ± 0.727	11.162 ± 1.419
04qun0604-4	6.218 ± 0.774	8.379 ± 0.203	B280-1-1	8.134 ± 0.238	8.771 ± 0.463
04qun0611-3	8.329 ± 0.508	11.877 ± 0.273	B283	19.124 ± 0.203	19.414 ± 0.951
B302	7.109 ± 0.442	7.641 ± 0.507	HN0709-1-1	13.109 ± 0.580	24.526 ± 1.518
B38-1	10.649 ± 0.545	17.866 ± 0.453	HN0713-2-1	4.195 ± 0.592	6.427 ± 0.450
B40-2	6.113 ± 0.184	6.200 ± 0.061	HN0718-2-1	7.357 ± 0.230	9.440 ± 0.464
B50	12.111 ± 1.158	15.440 ± 1.387	Huang C	9.643 ± 0.548	10.080 ± 0.309
B52	3.359 ± 0.157	5.759 ± 0.317	Huangyesi	5.242 ± 0.085	5.349 ± 0.223
B57	5.225 ± 0.422	9.400 ± 0.686	Huangzaosi	6.578 ± 0.600	7.012 ± 0.271
Cai 11-8	5.722 ± 0.395	6.834 ± 0.726	Ji 842	4.668 ± 0.574	7.053 ± 0.419
Chang 7-2	5.750 ± 0.627	6.765 ± 0.792	Ji 853	4.751 ± 0.749	5.816 ± 0.731
D10-2	2.377 ± 0.257	2.414 ± 0.200	K12	5.141 ± 0.416	6.337 ± 0.296
D18-1-1	6.743 ± 0.699	7.889 ± 0.696	LL0706-1-1	14.680 ± 0.403	15.473 ± 0.161
D25-2-1	7.400 ± 0.387	7.953 ± 0.118	LL0726-1	19.695 ± 0.442	22.915 ± 1.277
D26-2	7.198 ± 0.216	7.525 ± 0.251	Longkang 11	9.781 ± 0.433	12.727 ± 0.743
Dan 340	1.786 ± 0.222	3.684 ± 0.783	Lp08-17	6.992 ± 0.127	7.879 ± 0.290
Danhuang 02	4.335 ± 0.234	5.138 ± 0.371	Lp08-19	5.217 ± 0.596	7.149 ± 0.958
Danhuang 212	3.191 ± 0.205	7.858 ± 0.411	M54	3.223 ± 0.891	7.341 ± 0.852
Dasui-1-3	3.786 ± 0.438	9.071 ± 0.550	NH07001-3-1	6.017 ± 0.652	11.029 ± 0.232
E2-1-1	6.426 ± 0.376	6.920 ± 0.492	P138	3.451 ± 0.653	4.710 ± 0.083
E3-1-2	7.915 ± 0.505	8.541 ± 0.704	PH4CV	12.159 ± 0.541	14.875 ± 0.572

Continued

E4-2	3.357 ± 0.622	4.773 ± 0.900	Pr07001-1	12.234 ± 0.043	14.600 ± 1.625
F22-1	5.571 ± 0.279	5.641 ± 0.056	Pr07142	8.890 ± 1.295	11.431 ± 0.515
Fangxi	7.833 ± 0.584	8.495 ± 0.161	pr07148	12.176 ± 0.932	12.758 ± 1.550
H21	4.967 ± 0.574	10.059 ± 0.790	Pr07169	11.974 ± 0.743	14.400 ± 0.790
HB08F28-1	8.801 ± 0.235	10.491 ± 0.882	Pr07357	2.937 ± 0.111	9.835 ± 0.147
HB08II-37	14.343 ± 0.092	16.086 ± 0.798	Pr07483	3.397 ± 0.382	14.380 ± 0.971
HN0701-1-1	9.902 ± 0.424	10.722 ± 0.264	Pr07504	2.531 ± 0.241	9.524 ± 1.562
Pr7404	5.543 ± 0.464	9.485 ± 0.748	Y36-1	9.511 ± 1.456	10.866 ± 0.618
Qi310	7.715 ± 0.646	9.757 ± 0.604	Y4-2	10.628 ± 0.279	15.264 ± 0.547
Qichang-3-1	10.026 ± 0.297	10.564 ± 0.233	Y5-2	7.849 ± 0.290	8.487 ± 0.469
Ren-4-1	13.079 ± 0.742	17.807 ± 0.206	Y8-1-2	10.029 ± 0.580	14.358 ± 1.352
Shen118	9.854 ± 0.465	12.000 ± 0.093	Yang3-2-1	6.055 ± 0.279	9.534 ± 0.522
Shen137	6.759 ± 0.077	14.061 ± 1.500	Ye 478	4.104 ± 0.119	6.659 ± 0.635
Sizisi	4.195 ± 0.563	4.957 ± 0.185	Ye 488	2.702 ± 0.156	3.010 ± 0.197
VT187	2.241 ± 0.982	2.423 ± 0.383	Ye 8001	5.015 ± 0.371	5.144 ± 0.190
W618	4.411 ± 0.356	5.579 ± 0.968	ZH-2-1	6.033 ± 0.292	6.294 ± 0.392
x178-1	12.431 ± 0.672	12.684 ± 0.515	05ZH-3-1	13.831 ± 0.746	14.819 ± 0.779
Y17-2	3.869 ± 0.449	4.074 ± 0.368	Zheng 58	5.914 ± 0.301	7.634 ± 0.798
Y3-2	5.083 ± 0.258	5.794 ± 1.203	Zheng 653	7.471 ± 0.481	9.914 ± 0.511
Y32-1-1	7.031 ± 1.057	9.928 ± 0.366	Zhonghuang 204	7.204 ± 0.126	10.104 ± 0.342
Y30-1-1	11.755 ±1.057	12.510 ± 0.481			

PS secretion rate after Fe-deficiency stress was 9.560 mg/(g dry root*8h), and the variation range was from 2.414 ± 0.200 mg/(g dry root*8h) (D10-2) to 24.526 ± 1.518 mg/(g dry root*8h) (HN0709-1-1). These data strongly suggested that PS secrete rates were clearly different among these inbred lines at the seedling stage under Fe-sufficient condition and under Fe-deficiency treatment. In addition, Fe-deficiency treatment increased PS secretion rate among all 123 inbred lines. And the increment was from 0.037 mg/(g dry root*8h) (D10-2) to 11.417 mg/(g dry root*8h) (HN0709-1-1). These data revealed that sensibility to Fe-deficiency treatment was obviously different among these inbred lines.

3.2. Clustering Analysis of the 123 Inbred Lines Based on PS Secretion Characteristics at the Seedling Stage

Figure 1 listed the data gained from Chi-square distance and sum of squared deviations analysis of the 123 inbred lines. These lines were grouped based on their PS secretion rates under Fe-sufficient condition (**Figure 1(a)**) and PS secretion rate increment after Fe-deficiency treatment (**Figure 1(b)**).

With the threshold value 15.20, these inbred lines can be categorized into three groups based on the PS secretion rate under Fe-sufficient condition: low (L), intermediate (M) and high (H) (**Figure 1(a)**). The average secretion rates of the three groups were 4.307, 7.001 and 11.792 mg/(g dry root*8h) respectively, raging from 1.786 to 5.782 mg/(g dry root*8h), from 5.914 to 8.329 mg/(g dry root*8h), and from 5.914 to 19.695 mg/(g dry root·8h) respectively. With the threshold value 12.82, these lines were also categorized into three groups based on PS secretion rate increment after Fe-deficiency treatment: low (l), intermediate (m) and high (h) (**Figure 1(b)**). The average secretion rate increment of the three groups were 0.595, 2.382 and 5.792 mg/(g dry root*8h) respectively, raging from 0.037 to 1.416 mg/(g dry root*8h), from 1.690 to 3.329 mg/(g dry root*8h), and from 3.479 to 11.417 mg/(g dry root*8h) respectively.

To cluster the 123 inbred lines more accurately, these lines were divided into nine groups based on both the two parameters described above (**Table 2**). The nine groups were: 1) the high PS secretion rate under Fe-sufficient condition and high PS secretion rate increment after Fe-deficiency treatment (Hh); 2) the high PS secretion rate and intermediate PS secretion rate increment (Hm); 3) the high PS secretion rate and low PS secretion rate

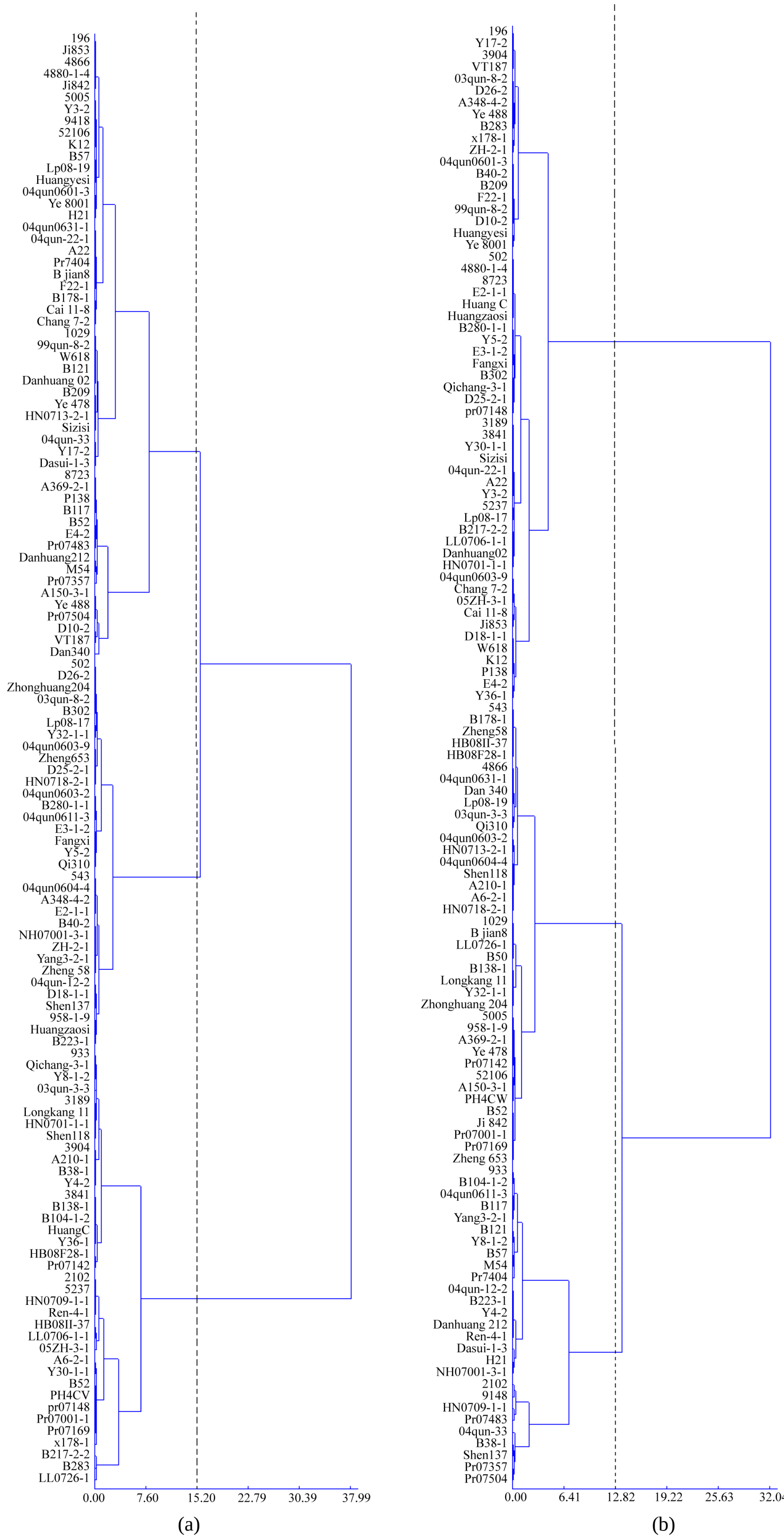

Figure 1. Clustering of 123 maize inbred lines based on PS secretion rate under Fe-sufficient condition (a) and PS secretion rate increment after Fe-deficiency treatment (b).

Table 2. Grouping of 123 maize inbred lines based on PS secretion rate [mg·g^{-1} dry root (8 h)$^{-1}$] under Fe-sufficient condition (+Fe) and PS secretion rate increment after Fe-deficiency treatment (−Fe).

+Fe \ −Fe	high PS secretion rate increment [h, 3.479 - 11.417 mg/(g dry root*8h)]	intermediate PS secretion rate increment [m, 1.690 - 3.329 mg/(g dry root*8h)]	low PS secretion rate increment [l, 0.037 - 1.416 mg/(g dry root*8h)]
High PS secretion rate [H, 8.801 - 19.695 mg/(g dry root*8h)]	933, 2102, B104-1-2, B38-1, HN0709-1-1, Ren-4-1, Y4-2, Y8-1-2	03qun-3-3, A210-1, A6-2-1, B138-1, B50, HB08F28-1, HB08II-37, LL0726-1, Longkang 11, PH4CV, Pr07001-1, Pr07142, Pr07169, Shen118	3189, 3841, 3904, 5237, B217-2-2, B283, HN0701-1-1, Huang C, LL0706-1-1, pr07148, Qichang-3-1, x178-1, Y30-1-1, Y36-1, 05ZH-3-1
Intermediate PS secretion rate [M, 5.914 - 8.329 mg/(g dry root*8h)]	04qun0611-3, 04qun-12-2, B223-1, NH07001-3-1, Shen137, Yang 3-2-1	543, 04qun0603-2, 04qun0604-4, 958-1-9, HN0718-2-1, Qi310, Y32-1-1, Zheng 58, Zheng 653, Zhonghuang 204	502, 03qun-8-2, 04qun0603-9, A348-4-2, B280-1-1, B302, B40-2, D18-1-1, D25-2-1, D26-2, E2-1-1, E3-1-2, Fangxi, Huangzaosi, Lp08-17, Y5-2, ZH-2-1
low PS secretion rate [L, 1.786 - 5.782 mg/(g dry root*8h)]	9418, 04qun-33, B117, B121, B57, Danhuang 212, Dasui-1-3, H21, M54, Pr07357, Pr07483, Pr07504, Pr7404	1029, 4866, 5005, 52106, 04qun0631-1, A150-3-1, A369-2-1, B jian8, B178-1, B52, Dan 340, HN0713-2-1, Ji 842, Lp08-19, Ye 478	196, 8723, 4880-1-4, 04qun0601-3, 04qun-22-1, 99qun-8-2, A22, B209, Cai 11-8, Chang 7-2, D10-2, Danhuang 02, E4-2, F22-1, Huangyesi, Ji 853, K12, P138, Sizisi, VT187, W618, Y17-2, Y3-2, Ye 488, Ye 8001

increment (Hl); 4) the intermediate PSs secretion rate and high PSs secretion rate increment (Mh); 5) the intermediate PS secretion rate and intermediate PS secretion rate increment (Mm); 6) the intermediate PS secretion rate and low PS secretion rate increment (Ml); 7) the low PS secretion rate and high PS secretion rate increment (Lh); 8) the low PS secretion rate and intermediate PS secretion rate increment (Lm); 9) the low PS secretion rate and low PS secretion rate increment (Ll) (**Table 2**).

4. Discussion

Fe is one of the essential nutrients for microorganisms, plants, animals and human beings. Fe in plants is an important source of this element for human beings, especially in developing countries [9]. However, plants are often subjected to Fe-deficiency due to the limited solubility of Fe in most neutral or basic soils [3]. In graminaceous species, PSs are the ones which are secreted into the rhizosphere to solubilize and utilize insoluble Fe (III) [5]. In the present study, to select inbred lines suited to developing maize hybrids with high efficiency in Fe utilization, we characterize PS secretion pattern of 123 lines.

In this study, PS secretion rate ranged from 1.786 ± 0.222 to 19.695 ± 0.442 mg/(g dry root*8h) under Fe-sufficient condition, and PS secretion rate increment ranged from 0.037 to 11.417 mg/(g dry root*8h) under Fe-deficiency stress, suggesting that PS secretion pattern differed with genotypes.

Based on PS secretion rates under Fe-sufficient condition and PS secretion rate increment after Fe-deficiency treatment, 123 maize inbred lines were divided into nine groups, Hh, Hm, Hl, Mh, Mm, Ml, Lh, Lm and Ll (**Table 2**). PSs are metal chelators that play a major role in Fe and Zn acquisition [10] [11]. So it is unnecessary for plants cultured in Hoagland's solution to secrete large amounts of PSs. Under Fe-deficient stress, however, more increase of PS secretion rate contributes evidently to quickly adapt the stress circumstance. Therefore, the Lh type was the ideal inbred lines for breeding of maize with high efficiency in Fe utilization.

5. Conclusion

In the present study, to select inbred lines suited to develop maize hybrids with high efficiency in Fe utilization, PS secretion patterns of 123 inbred lines were analyzed. These lines were clustered into nine groups based on PS secretion rates under Fe-sufficient condition and PS secretion rate increment after Fe-deficiency treatment. The Lh type was the ideal inbred lines for breeding of maize with high efficiency in Fe utilization.

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