Comprehensive screening of low nitrogen tolerant maize based on multiple traits at the seedling stage

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Background. The efforts to screen stress-tolerant plants are of immense importance to increase crops productivity. However, the different parameters were used for stress-tolerant plant screening in the various researches. Therefore, a method that can integrate different parameters to evaluate stress tolerance is urgently needed. **Methods.** Six maize genotypes were subject to low nitrogen stress for twenty days. Then seventeen traits of the six maize genotypes related to nitrogen were investigated. Nitrogen tolerance coefficient (NTC) was calculated as low nitrogen traits to high nitrogen trait. Then principal component analysis was conducted based on the NTC. Based on fuzzy mathematics theory, a D value was introduced to evaluate maize tolerant to low nitrogen. **Results.** The higher D value the greater tolerance of maize to low nitrogen stress. On the contrary, maize with lowest D value was consider as the potential nitrogen inefficient maize that sensitive to low nitrogen. **Conclusions.** The present study introduced D value to evaluate stress tolerance. This method may reduce the complexity of the investigated traits and enhance the accuracy of stress tolerant evaluation. In addition, this method not only can screen potentially tolerant germplasm for low-nitrogen tolerance quickly, but also can comprise the correlated traits as many as possible to avoid the one-sidedness of single parameter.

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Abstract

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Conclusions. The present study introduced D value to evaluate stress tolerance. This method may reduce the complexity of the investigated traits and enhance the accuracy of stress tolerant evaluation. In addition, this method not only can screen potentially tolerant germplasm for low-nitrogen tolerance quickly, but also can comprise the correlated traits as many as possible to avoid the one-sidedness of single parameter.

Introduction

Nitrogen is essential nutrient for plants growth and development, and it also a major driving force for crop productivity improvement. Screening and developing varieties with nitrogen efficient crop plays the pivotal role in agriculture sustainable development. Nitrogen uptake and utilization efficiency for grain production depends on those processes associated with absorption, translocation, assimilation and redistribution of nitrogen operate effectively. Plants uptake the nitrate through the low- and high-affinity nitrate transporters (Fan et al., 2017). While the ammonium uptake was mediated by the saturable high-affinity (ammonium transporters) and the nonsaturable low-affinity (aquaporins or cation channels) uptake system (Tegeder & Masclaux-Daubresse, 2018). The nitrate reductase (NR), glutamine synthetase (GS) and glutamate synthase (GOGAT) were the key enzymes for nitrogen assimilation that indirectly affect the metabolism, allocation and remobilization of nitrogen in plants (Lea et al., 2006; Martin et al., 2006).

Maize (*Zea mays* L.) is not only the important world's food and feed crop, but also an important energy crop (Yin et al., 2014). Moreover, maize is the crop with highest production among all crops and is also the crop with the greatest demands for nitrogen (Sivasankar et al., 2012). Due to the differences in nitrogen absorption and utilization among maize genotypes (Harvey, 1939), more focus was paid on screening and improving the nitrogen efficiency (Hirel et al., 2007). The large differences in growth and yield among the maize lines and hybrids were associated with both the nitrogen uptake and utilization efficiency in response to low nitrogen stress (Hirel & Gallais, 2011). The root architecture of maize is a key factor affect the nitrogen absorption, and more photosynthate will distribute to the root to enhance the root surface of the nitrogen efficient maize under nitrogen limitation (Sinclair & Vadez, 2002), The absorption of nitrogen in roots requires the involvement of the high-affinity nitrogen transporter (NRT2 and AMT1), especially

under the nitrogen limitation (Dechorgnat et al., 2019). Among the four *ZmNRT2* which identified in the maize genome, only *ZmNRT2;1* and *ZmNRT2;2* have proven to be correlated with nitrate (NO_3^-) uptake capacity (Plett et al., 2010; Garnett et al., 2013). Furthermore, *ZmAMT1;1a* and *ZmAMT1;3* have been identified to encode functional ammonium transporters for high-affinity ammonium uptake in maize roots (Gu et al., 2013).

Nitrogen is significantly influenced the productivity and characteristics of maize (Teixeira et al., 2014). However, the higher nitrogen fertilizer application led to negative effects on the ecological environment because of lower nitrogen uptake and utilization efficiencies of plants. Development of nitrogen-tolerant maize requires a series of complex breeding research. Hence, selection of maize germplasm tolerant to low nitrogen stress is more important and feasible. Plants tolerant to low nitrogen is a quantitative trait affected by many factors. Therefore, a fast method for effective selection of nitrogen efficiency maize genotype is urgently needed, which can reduce time and cost when developing nitrogen-tolerant maize. Principal component analysis is a quantitatively rigorous method for multivariate datasets simplification. It can transform more original indicators into several new relatively independent comprehensive indicators. Absolute subordination of elements to sets was broke in the theory of fuzzy mathematics. Subordinate function analysis was one of effective ways used in comprehensive evaluation of abiotic stresses (Shi et al., 2010). In the present study, a D value was calculated after the principal component analysis and subordinate function analysis. Our study would provide a comprehensive and dependable method for evaluating low tolerance in maize.

Materials & Methods

Plant material, growth and treatment conditions

The six maize, GEMS42-I, Ji846, SY998, CML223, CML114 and GEMS42-II, with significant difference in grain yield and nitrogen tolerance were used in the present study. The surface-sterilized seeds were germinated on wet sand in the culture room. Then, the 4-day old seedlings were transferred into the nutrient solution for continuing growth. The complete basal nutrient solution contained 0.24 g/L NH_4NO_3, 0.50 g/L $MgSO_4$, 0.15 g/L KCl, 0.36 g/L $CaCl_2$, 0.05 mM EDTA-Fe and a microelement solution (Hoagland & Arnon, 1950). The nutrient solution contain 1/10 N of the complete nutrient solution was used for low nitrogen treatment (-N), and the seedlings growing under the complete nutrient were used as control (+N). Keep the culture room parameter as follow: a cycle of 16 h/24 °C day and 8 h/22 °C night, light intensity of 300-320 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and relative humidity of 65-80%. Roots and leaves of all the six maize were harvested separately on the 20^{th} day after transplanting. Each treatment was replicated three times.

Biomass and phenotypic characteristics of the root system

Root was floated in water and scanned with scanner (Epson Expression 11000XL) using the WinRHIZO Pro software (Version 2.0, 2005, Regent Instrument Inc., Quebec, Canada). The root total length, root volume, root surface area and root average diameter were calculated with Tennant's statistical method in WinRHIZO. The seedlings were washed with distilled water, and the fresh weight (FW) were measured after drying with bibulous paper.

Measurement the NO_3^- and NH_4^+ content of the seedlings

For nitrates (NO_3^-) determination, roots and shoots (approximately 0.5 g FW) were cut into pieces and suspended in 5 mL boiling water for 10 min (Tang et al., 2013). Then the supernatant was diluted to 25 mL. The assay mixture containing 0.1 mL samples and 0.4 mL 5% salicylic

acid-sulfuric acid, was incubated at 20 ℃ for 20 min, then mixed with 9.5 mL 8% NaOH (w/v). Its absorbance was measured at 410 nm wavelength.

The ammonium (NH_4^+) of root and shoot were extracted by homogenizing in 0.3 mM H_2SO_4 (pH 3.5). After centrifugation at 3900 g for 10 min, the supernatant was collected using for determination of ammonium (NH_4^+) content as previously described (Lin & Kao, 1996).

After NO_3^- the and NH_4^+ determination, the root nitrogen accumulation, shoot nitrogen accumulation and total plant nitrogen accumulation were calculated.

Enzyme activity assays

Approximately 0.5 g fresh roots were homogenized with 10 mM Tris-HCl buffer (pH 7.6) containing 1 mM $MgCl_2$, 1 mM EDTA and 1 mM β-mercaptoethanol in chilled pestle and mortar. After centrifugation at 15 000 g for 30 min (4℃), the supernatant was used as enzyme extract (Ren et al., 2017).

The whole extraction procedure was carried at 4℃.

For glutamine synthetase (GS, EC6.3.1.2) activity assayed, a 1.0 mL reaction mixture (pH 8.0) contained 80 μmol Tris-HCl buffer, 40 μmol L-glutamic acid, 8.0 μmol ATP, 24 μmol $MgSO_4$, and 16 μmol NH_2OH and enzyme extract. The enzyme extract was added to initiate the reaction. After incubation for 30 min at 30℃, the reaction was stopped by adding 2 mL 2.5% (w/v) $FeCl_3$ and 5% (w/v) trichloroacetic acid in 1.5 M HCl. After centrifugation at 3000 g for 10 min, the absorbance of the supernatant was measured at 540 nm. GS activity was expressed as 1.0 μM L-glutamate γ-monohydroxamate (GHA) formed g^{-1} FW h^{-1}, with μmol GHA·g^{-1} FW·h^{-1}.

For glutamate synthase (GOGAT, EC1.4.7.1) activity assayed, a 3 mL reaction solution was prepared with 25 mM Tris-HCl buffer (pH 7.6), which contained 0.5 mL enzyme extract, 0.05 mL 0.1 M 2-oxoglutarate, 0.1 mL 10 mM KCl, 0.2 mL 3 mM NADH and 0.4 mL 20 mM L-

glutamine. The reaction was initiated by adding L-glutamine immediately following the enzyme preparation. The decreased in absorbance was recorded for 3 min at 340 nm. The GOGAT activity was expressed as μmol NADH·g^{-1} FW·h^{-1}.

Nitrate reductase (NR, EC1.7.1.1) activity was determined according to Wojciechowska et al with minor modifications (Wojciechowska et al., 2016). The NR activity was expressed as μg NO_2^-·g^{-1} FW·h^{-1}.

Quantitative RT-PCR analysis

Total RNA was isolated using TRIzol reagent (Invitrogen, CA, USA) and then first-strand cDNA was synthesized using the M-MLV Reverse Transcriptase (Promega, WI, USA) according to the manufacturer's instructions. For quantitative real-time PCR (qRT-PCR) experiment, 20 μL reaction components were prepared according to the manufacturer's protocol for SYBR Green Real Master Mix (TIANGEN, Beijing). Using *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) as the endogenous control. Real-time PCR was conducted on the CFX96™ Real-Time PCR Detection System (Bio-Rad, CA, USA), and the primer pairs used for quantitative RT-PCR were shown in supplemental Table S1.

Data analysis and D value calculation

Sample variability was expressed as the standard error of the mean. All analyses of significance were conducted at the $p < 0.05$ level. Consider that the biological differences among the different maize genotypes, evaluation of the low nitrogen tolerance of maize by nitrogen tolerance coefficient (NTC) may more reasonable. The NTC was calculated as: $\mathrm{NTC} = \frac{low\ nitrogen\ trait}{high\ nitrogen\ trait}$.

Then principal component analysis was conducted based on the NTC in SPSS (Statistical Product and Service Solutions) software (version 18.0). The principal component (PCi, i = 1, 2 … n) with eigenvalue (λ_i, i = 1, 2 … n) > 1 was selected as new index. PCi is the i-th principal

147 component. λ_i is the eigenvalue of the i-th principal component. The eigenvalue (λ_i, i = 1, 2 …
148 n) and factor score (FACi, i = 1, 2 … n) was also present in the results of principal component
149 analysis. The principal component value Xi was calculated as: $Xi = \mathrm{FAC}i \times \sqrt[2]{\lambda_i}$ (i = 1, 2 … n).
150 Then the subordinate function value was calculated as: $U(Xi) = \frac{Xi - Xmin}{Xmax - Xmin}$ (i = 1, 2 … n). $Xmax$
151 and $Xmin$ represent the maximum and minimum value of the i-th principal component,
152 respectively. The weight coefficient was calculated as: $W(i) = \frac{\mathrm{P}i}{\sum_{i=1}^{n}\mathrm{P}i}$ (i = 1, 2 … n). Pi represent
153 the proportion of variance explained of the i-th principal component. Finally, the D value was
154 calculated as: $D = \sum_{i=1}^{n}[\mathrm{U}(Xi) \times \mathrm{W}(i)]$ (i = 1, 2 … n).

155 ***Statistical analysis***

156 Sample variability was expressed as the standard error of the mean. Significance of differences
157 were conducted using SAS 9.2 (SAS Institute, Cary, NC, USA). Data were subjected to ANOVA
158 using PROC LSD (p< 0.05) in SAS.

159 ## Results

160 ***Plant physiological changes in response to nitrogen stress***

161 Low nitrogen (ca. 0.3 mM N) significantly inhibited the growth of Ji846 but not of the other five
162 genotypes maize (Fig. 1), and even increased the root biomass of SY998 and GEMS42-II (Fig.
163 1c). Consider that root is the primary organ for water and nutrients capturing, the morphology of
164 root is investigated by WinRHIZO Pro software. Root diameter of the six maize were decreased
165 in response to low nitrogen stress (Fig. 2b). All of the root total length, root surface and root
166 volume of Ji846 were significantly decreased in response to low nitrogen stress (Fig. 2). On the
167 contrary, the root total length, root surface and root volume of SY998 and GEMS42-II were
168 prominently increased under low nitrogen (Fig. 2b, 2d, 2e). Low nitrogen not affected the root

total length, root surface and root volume of GEMS42-I, CML223 and CML114 (Fig. 2b, 2d, 2e). These results indicated that Ji846 was sensitive to low nitrogen stress.

NO_3^- and NH_4^+ content in maize

Nitrate ion (NO_3^-) and ammonium ion (NH_4^+) are the main form of nitrogen for plant absorption. Maize can uptake both nitrate and ammonium. Both the NO_3^- and NH_4^+ contents were significantly decreased in all maize genotypes under low nitrogen stress (Fig. 3). However, only the root NO_3^- content of SY998 was significantly higher than the other genotype under low nitrogen (Fig. 3a). Under low nitrogen stress, Ji846 has the lowest NH_4^+ content both in root and shoot, while the GEMS42-I and SY998 has the highest NH_4^+ content both in root and shoot (Fig. 3c and 3d). The lowest nitrogen accumulation was observed in Ji 846 under nitrogen limitation, no matter in root, shoot or total plant (Table 3). While the highest nitrogen accumulation was observed in SY998 under nitrogen limitation (Table 3). Interestingly, the NO_3^- content was higher than the NH_4^+ content of all the six maize, irrespective of the nitrogen nutritional status of the plants. In the high nitrogen condition, the NO_3^- of root and shoot was 12.3 and 10.4 times of the NH_4^+ in Ji846, respectively. While under the low nitrogen condition, the NO_3^- of root and shoot was 7.1 and 6.6 times of the NH_4^+ in Ji846, respectively (Fig. 3).

Expression of the nitrate and ammonium transporter genes

The expression of *ZmNRT2;1* and *ZmNRT2;2* in Ji846 and SY998 were significantly increased under low nitrogen condition (Fig. 4a and 4b). The expression of *ZmNRT2;1* under low nitrogen was 9 and 3 times of the high nitrogen condition in Ji846 and SY998, respectively. The expression of *ZmNRT2;2* under low nitrogen was 41 and 11 times of the high nitrogen condition in Ji846 and SY998, respectively (Fig. 4a and 4b). In addition, the expression of *ZmNRT2;2* in CML223 was also significantly increased (5 times) under nitrogen limitation (Fig. 4b). For the

ammonium transporters genes, the expression of *ZmAMT1;1a* was significantly increased under nitrogen limitation in GEMS42-I, Ji846, SY998 and GEMS42-II, which increased 16, 10, 20 and 3 times, respectively (Fig. 4c). The expression of *ZmAMT1;3* was significantly increased under nitrogen limitation in Ji846, CML223, CML114 and GEMS42-II, which varied from 1.4 to 4.3 times (Fig. 4).

Enzyme activity of the key enzymes referring to nitrogen metabolism

Low nitrogen significantly decreased the activities of key nitrogen metabolism enzymes in some of maize. The greatest reduction in the activities of NR (approximately 82%) in Ji846 (Fig. 5a), GS (approximately 88%) in Ji846 (Fig. 5b), (GOGAT approximately 56%) in CML223 (Fig. 5c). The NR activities of GEMS42-I, CML223 and CML114 were decreased 60.3%, 68.6% and 48.6%, respectively (Fig. 5a). In addition, the lower activities of NR and GS were observed in SY998, CML223 and GEMS42-II, irrespective of the nitrogen condition (Fig. 5a and 5b). Nitrogen limitation affected the activity of GOGAT less than that of NR and GS. The GOGAT activities only decreased in CML223 and GEMS42-II in response to nitrogen limitation (Fig. 5c).

Principal component analysis based on the nitrogen tolerance coefficient (NTC)

The first four principal components jointly explain the major part of total variance (96.8%), being PC1 responsible for 47.4%, PC2 for 21.6%, PC3 for 15.5% and PC4 for 12.3% of total variance, respectively (Table 1). The eigenvalue of PC1, PC2, PC3 and PC4 were 8.054, 3.676, 2.627 and 2.091, respectively (Table 1). The factor score of the four principal components of each maize were directly extracted from the principal component analysis results (Table 2). Then, the principal component value and subordinate function value was calculated. Finally, each maize has a D value (Table 2). The SY998, GEMS42-I and GEMS42-II has the higher D

value that can define as low nitrogen tolerant maize, while the Ji846 with the lower D value was defined as low nitrogen sensitive maize (Table 2).

Discussion

Different maize performs quite differently in the complex physiological and development of root and shoot in response to nitrogen limitation (Hirel et al., 2001; Giehl & Gruber, 2014). The root morphology changes affect the nitrogen efficiency through the alteration of nitrogen absorption. Since the root architecture and function that contribute to nitrogen absorption efficiency (Trachsel et al., 2011). The morphology of roots was closely associated with the acquisition of nitrogen and the development of plant shoots (Mi et al., 2010; Lynch, 2013; Li et al., 2017). The root and shoot biomass of Ji846 was significantly decreased under the nitrogen limitation (Fig. 1b and 1c). In addition, the root total length, root surface and root volume were significantly decreased in Ji846 under low nitrogen condition (Fig. 2b, 2d and 2e). These results indicated that Ji846 was potential nitrogen inefficient maize. A lower D value of Ji846 was observed (Table 2). The shoot and root fresh weight of SY998, GEMS42-I and GEMS42-II have not significantly inhibited by nitrogen limitation, which exhibited high nitrogen efficiency to maintain plant growth (Fig.1). the root total length, root surface and root volume were increased in SY998 and GEMS42-II in response to nitrogen limitation (Fig. 2b, 2d and 2e). This consist with the previous study that plant shoot could be associated with nitrogen efficiency in selecting for improving grain yield under low nitrogen conditions (Chen et al., 2016). In addition, the D value of the three maize is higher than 0.7 in the present study (Table 2). Therefore, SY998, GEMS42-I and GEMS42-II were the potential nitrogen efficient maize.

Interestingly, the higher expression of *ZmNRT2;1*, *ZmNRT2;2*, *ZmAMT1;1a* and *ZmAMT1;3* in Ji846 not increased its nitrate and ammonium content under nitrogen limitation (Fig. 3 and Fig.

4). Ji846 even has the lowest nitrogen accumulation under nitrogen limitation (Table 3). Among the three higher D value maize, the expression of *ZmNRT2;1* and *ZmNRT2;2* only significantly increased in SY998 not in GEMS42-I and GEMS42-II (Fig. 4 and Table 2). All of the three maize have higher nitrogen accumulation under nitrogen limitation, especially SY998 has the highest accumulation (Table 3). The expression of *ZmNRT2;1*, *ZmNRT2;2*, *ZmAMT1;1a* and *ZmAMT1;3* not correlative with nitrogen content in maize suggested that evaluation of nitrogen efficiency by these genes was inappropriate, at least in maize seedlings. In other hand, some other uptake systems might exist in maize for nitrogen absorption.

Nitrate (NO_3^-) and ammonium (NH_4^+) taken up by plants must first be assimilated into amino acids before it can be used for proteins synthesis for plant growth. Hence the nitrogen-assimilation enzyme is a feasible strategy for improve nitrogen efficiency. NR is the first enzyme to reduce the NO_3^- to NO_2^-, and further reduce to NH_4^+ by nitrite reductase (Lea et al., 2006; Takahashi et al., 2001). The NH_4^+ is assimilated into amino acid by the GS-GOGAT cycle, which is a crucial step for converting inorganic nitrogen into organic nitrogen in plants (Martin et al., 2006). The NR and GS activities of JI846 were decreased over 80%, while the NR activities decreased 20% and GS activities decreased 30% both in SY998 and GEMS42-II under low nitrogen condition (Fig. 5). The nitrogen assimilation was directly associated with the activities of these enzymes, so low nitrogen has great impact on the nitrogen utilization efficiency of Ji846 than the potential low nitrogen tolerant maize, SY998 and GEMS42-II.

Conclusions

Seventeen traits of six maize genotype related to nitrogen was investigated and a D value was introduced to screen potential low nitrogen-tolerant maize in the present study. Using D value can comprehensively evaluate low nitrogen tolerant based on the multiple nitrogen related traits,

260 which can avoid the one-sidedness of single parameter. Since the D value was calculated based
261 on the the theory of fuzzy mathematics. This method may also provide the benefit of
262 development techniques to screen other potentially stress-tolerant traits. In summary, using the D
263 value to evaluate stress tolerance of plants can integrate all the correlated traits to a single index,
264 which may reduce the complexity of the investigated traits and enhance the accuracy of
265 evaluation.

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361

362 **Table 1** Eigenvalue, proportion and cumulative of the first four principal components based on
363 the nitrogen-tolerant index of maize
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367 **Table 3** The nitrogen accumulation of each maize
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Fig. 3 Effects of low nitrogen stress on nitrate (NO_3^-) and ammonium (NH_4^+) content of maize. (a) root nitrate (NO_3^-) content, (b) shoot nitrate (NO_3^-) content, (c) root ammonium (NH_4^+) content, (d) shoot ammonium (NH_4^+) content. The +N and –N represent the seedling under high nitrogen (3 mM N) and low nitrogen (0.3 mM N), respectively. Values represent the mean ± SD of of three independent replicates, where each replicate involve ten seedlings. Bars with different letters show significant differences at $p<0.05$ (ANOVA, LSD).

Fig. 4 Effects of low nitrogen stress on nitrogen transporter genes of maize roots. (a) *ZmNRT2;1*, (b) *ZmNRT2;2*, (c) *ZmAMT1;1a*, (c) *ZmAMT1;3*. The +N and –N represent the seedling under high nitrogen (3 mM N) and low nitrogen (0.3 mM N), respectively. Values represent the mean ± SD of three independent replicates. Bars with different letters show significant differences at $p<0.05$ (ANOVA, LSD).

Fig. 5 Effects of low nitrogen stress on nitrogen metabolism enzymes of maize roots. (a) Nitrate reductase (NR) activity, (b) Glutamine synthetase (GS) activity, (c) Glutamate synthase (GOGAT) activity. The +N and –N represent the seedling under high nitrogen (3 mM N) and low nitrogen (0.3 mM N), respectively. Values represent the mean ± SD of three independent replicates. Bars with different letters show significant differences at $p<0.05$ (ANOVA, LSD).

Supplemental Table

Table S1 Sequence of the primers used for real-time PCR.

Table 1(on next page)

Table 1 Eigenvalue, proportion and cumulative of the first four principal components based on the nitrogen-tolerant index of maize

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Index	Principal component			
	1	2	3	4
Eigenvalue	8.054	3.676	2.627	2.091
Proportion of variance explained (%)	47.376	21.622	15.455	12.299
Cumulative variance explained (%)	47.376	68.997	84.453	96.751
weight coefficient $W(i)$	0.49	0.22	0.16	0.13

Table 2(on next page)

Table 2 The D value of each maize

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Maize	Factor score (FACi)				Principal component value (Xi)				Subordinate function value U(Xi)				Weight coefficient W(i)				D Value
	FAC1	FAC2	FAC3	FAC4	X1	X2	X3	X4	U1	U2	U3	U4	W1	W2	W3	W4	
GEMS42-I	0.17	1.72	-0.30	0.40	0.48	3.29	-0.49	0.58	0.68	1.00	0.47	0.79	0.49	0.22	0.16	0.13	0.74
Ji846	-1.88	-0.42	0.56	0.12	-5.34	-0.81	0.91	0.18	0.00	0.25	0.78	0.70					0.27
SY998	0.42	0.50	1.17	0.18	1.18	0.96	1.90	0.26	0.77	0.57	1.00	0.72					0.75
CML223	0.07	-0.61	-1.52	1.01	0.19	-1.16	-2.64	1.46	0.65	0.18	0.00	1.00					0.49
CML114	0.12	-0.07	-0.63	-1.93	0.33	-0.14	-1.02	-2.79	0.67	0.37	0.36	0.00					0.47
GEMS42-II	1.11	-1.12	0.72	0.22	3.16	-2.15	1.16	0.32	1.00	0.00	0.84	0.73					0.72

Table 3(on next page)

Table 3 The nitrogen accumulation of each maize

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Maize Genotype	Root [μg N·plant^{-1}]		Shoot [μg N·plant^{-1}]		Total plant [μg N·plant^{-1}]	
	CK	-N	CK	-N	CK	-N
GEMS42-I	29.74±0.77e	16.85±0.75g	65.22±0.18de	30.15±1.39f	94.96±0.95f	47.00±0.64g
Ji846	34.96±0.24d	3.25±0.70h	68.68±6.60d	5.52±0.01g	103.64±6.35ef	8.78±0.70h
SY998	86.72±1.04a	56.33±0.46b	206.48±23.85a	97.54±1.68c	293.20±22.80a	153.87±1.22d
CML223	44.07±3.05c	18.47±0.28fg	79.82±0.93cd	33.28±0.06f	123.89±2.12e	51.75±0.34g
CML114	37.45±0.22d	20.91±2.76f	143.35±0.21b	42.96±3.36ef	180.80±0.01c	63.87±0.61g
GEMS42-II	42.40±0.53c	36.39±1.79d	185.37±24.67a	76.15±15.44cd	227.77±24.14b	112.55±17.24ef

Figure 1

Morphological appearance and biomass

Fig. 1 The morphological appearance (**a**), roots biomass (**b**) and shoots biomass (**c**) of the different genotype maize in response to low nitrogen stress. The different genotype maize subjected to nitrogen stress for three weeks. The +N and –N represent the seedling under high nitrogen (3 mM N) and low nitrogen (0.3 mM N), respectively. Values represent the mean ± SD, bars with different letters show significant differences (ANOVA, LSD, $P<0.05$).

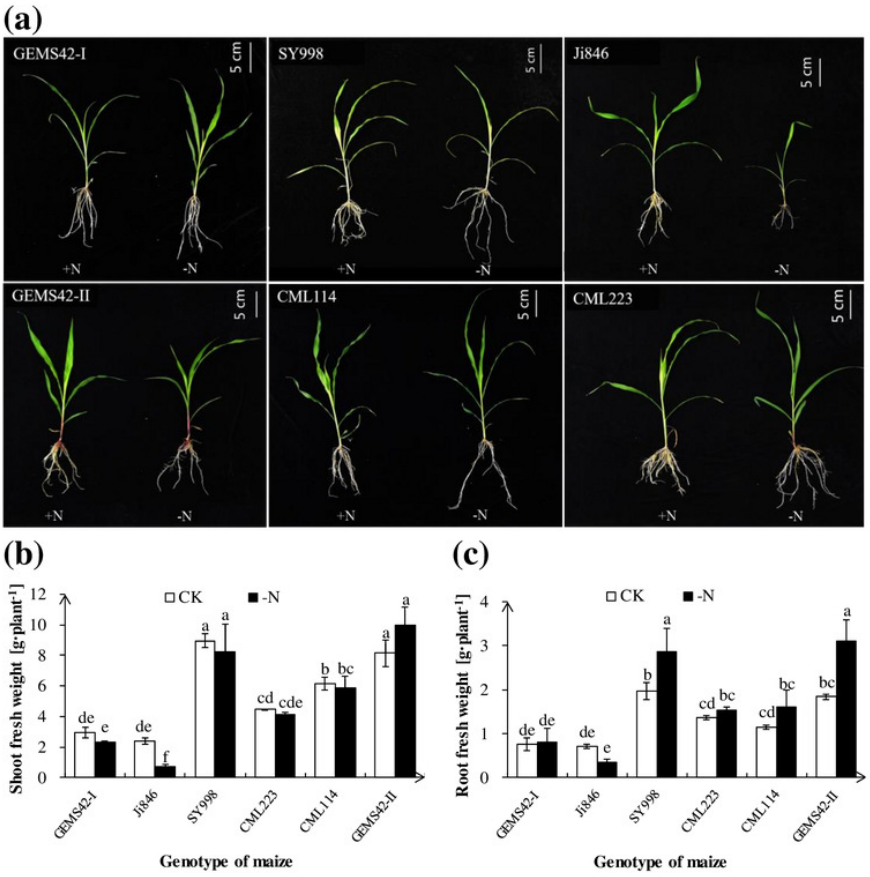

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Figure 2

Root morphology and root paramenters

Fig. 2 Effects of low nitrogen stress on root morphology. (a) Morphological appearance of roots, (b) root total length, (c) root average diameter, (d) root surface area and (e) root volume. The +N and –N represent the seedling under high nitrogen (3 mM N) and low nitrogen (0.3 mM N), respectively. Values represent the mean ± SD of ten seedlings in each treatment. Bars with different letters show significant differences at $p<0.05$.

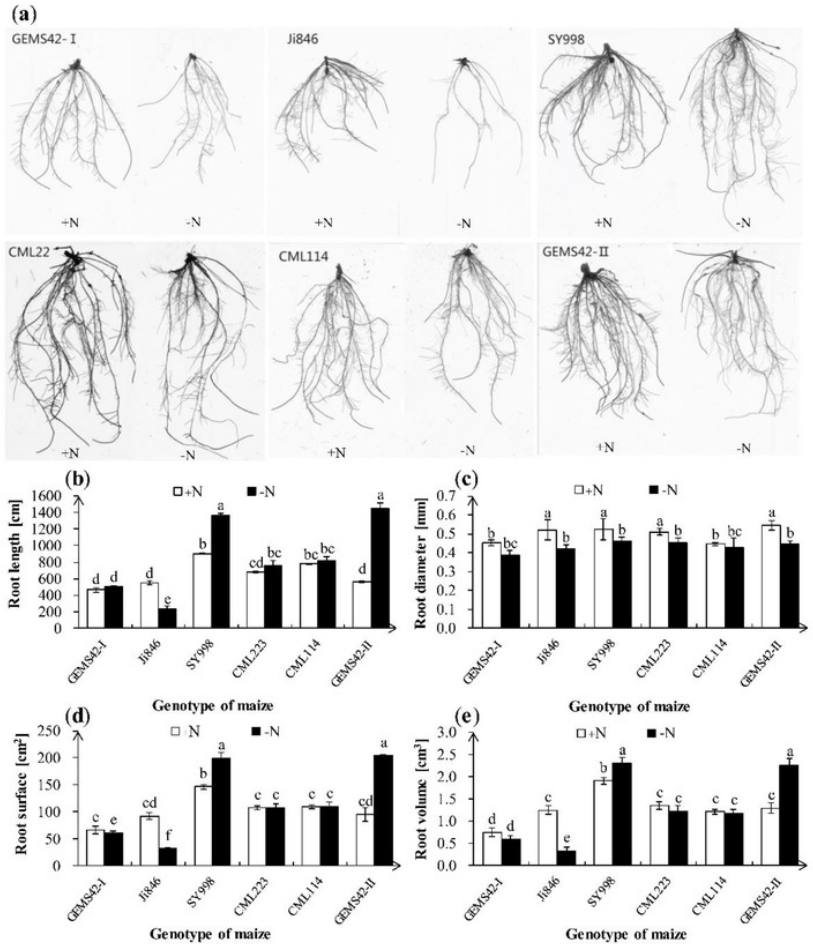

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Figure 3

Nitrate and ammonium content of maize seedling

Fig. 3 Effects of low nitrogen stress on nitrate (NO_3^-) and ammonium (NH_4^+) content of maize. (a) root nitrate (NO_3^-) content, (b) shoot nitrate (NO_3^-) content, (c) root ammonium (NH_4^+) content, (d) shoot ammonium (NH_4^+) content. The +N and –N represent the seedling under high nitrogen (3 mM N) and low nitrogen (0.3 mM N), respectively. Values represent the mean ± SD of ten seedlings in each treatment. Bars with different letters show significant differences at $p<0.05$.

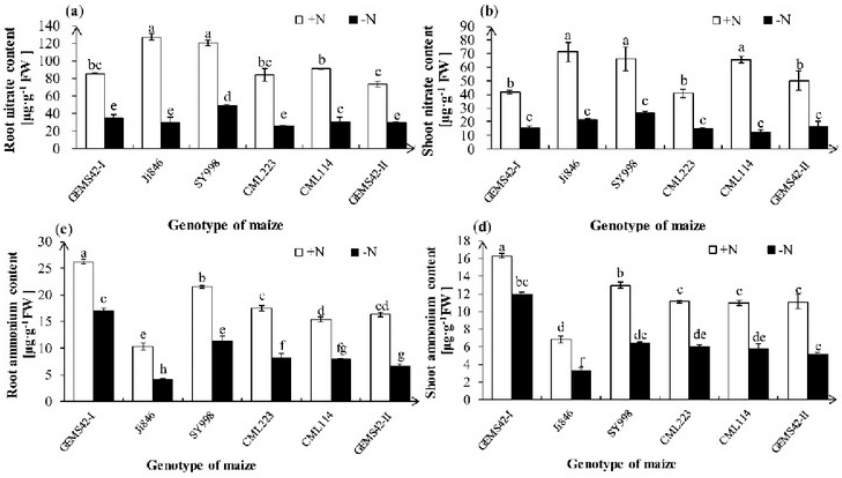

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Figure 4

Relative expression level of *ZmNRT2;1*, *ZmNRT2;2*, *ZmAMT1;1a*, *ZmAMT1;3*

Fig. 4 Effects of low nitrogen stress on nitrogen transporter genes of maize roots. (a) *ZmNRT2;1*, (b) *ZmNRT2;2*, (c) *ZmAMT1;1a*, (c) *ZmAMT1;3* . The +N and –N represent the seedling under high nitrogen (3 mM N) and low nitrogen (0.3 mM N), respectively. Values represent the mean ± SD of ten seedlings in each treatment. Bars with different letters show significant differences at $p<0.05$.

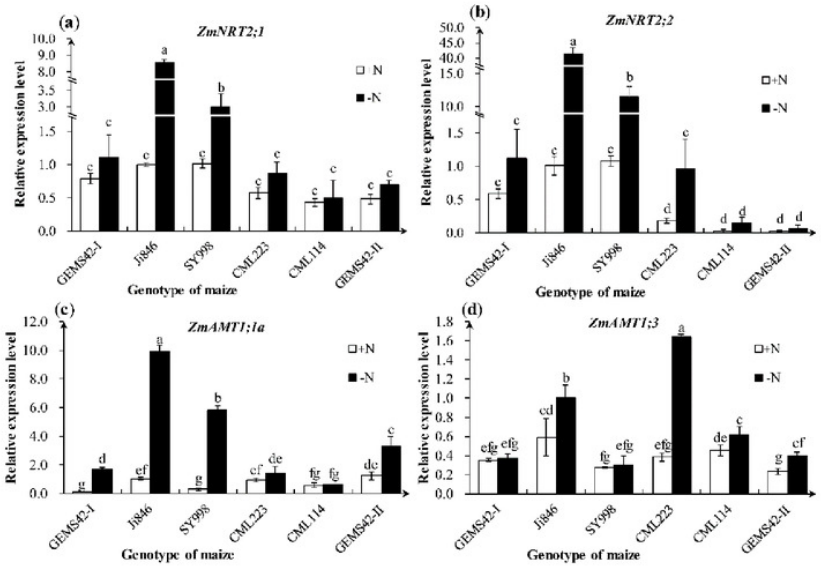

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Figure 5

Effects of low nitrogen stress on nitrogen metabolism enzymes of maize roots

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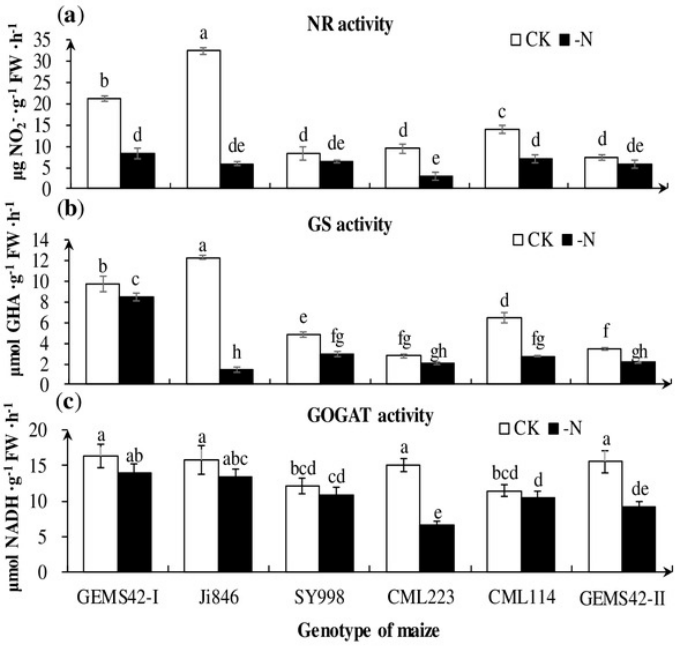

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