Association of Chickpea Root with Soil Fungi: A Comparison of Cultivars

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Abstract

Field crops influence soil microbiota, impacting the health status and productivity of cropping systems. We conducted a two year field experiment using thirteen genotypes of chickpea and applied deep amplicon pyrosequencing to verify whether plant genetics control the fungal community of the root endosphere. We obtained 63796 sequences of ITS1F/ITS2 and 52129 of 18S rDNA gene clustered into 127 non-mycorrhizal and 89 mycorrhizal operational taxonomic units (OTUs), respectively. Plant genotype and year (soil and weather) had significant effects on the fungal community of chickpea root endosphere. The desi genotypes had higher levels of mycorrhizal and non-mycorrhizal fungal richness and diversity than kabuli genotypes. This study reveals a "genotype effect" of chickpea on the soil microbiota and indicates the possibility to improve the performance of this crop through the selection of genotypes with improved root fungal communities.

Introduction

Plants coexist with a wide variety of beneficial and pathogenic microorganisms at all stages of their life. They employ several genome specific mechanisms to shape the structure and function of their microbial environment (Berg, and Smalla, 2009; Lau, 2011). Fungi represent a diverse group of soil microbiota functioning as decomposers, pathogens and mutualists (Buee, 2009). The arbuscular mycorrhizal fungi develop a symbiosis with the majority of crop plant providing them with improved nutrition and health status, and tolerance to abiotic stresses such as drought and metal toxicity (Finlay, 2008). Dark septate fungal endophytes colonize root tissues causing various responses in the host plant from negative to neutral and positive (Andrade-Linares et al., 2011). Furthermore fungal pathogens are the main factor of root disease and yield decline in agricultural fields. Hence the community of soil fungi associated with roots can impact the health status and productivity of cropping systems. Chickpea is a high value pulse crop, well adapted to the semiarid climate of southern Saskatchewan. We hypothesized that chickpea cultivars develop different fungal communities in the root endosphere. We conducted Field experiments and used

deep amplicon pyrosequencing to compare the fungal communities of root endosphere in thirteen cultivars of chickpea.

Material and methods

Field experiments

Thirteen cultivars of kabuli and desi chickpeas were grown in the randomized complete blocks in four replicates (13 genotype × 4 blocks = 52 plots) and repeated over two years (2010 and 2011). Seeding rate was targeted 40 plants per square meter and the plots were 2 x 6 m. A granular *Mezorhizobium ciceri* inoculant kept inoculum level constant.

Sampling

10 plants were randomly collected from 10 locations in each plot at the stage of mid-flowering. Roots and aboveground materials were separated and sampled. Roots are washed and cut to 4 cm fragments and mixed. A representative sub-sample was and stored at -20°C for DNA (fungal diversity) analysis.

Identification of the AMF and non-AMF fungal communities

Samples of 50 mg root were freeze dried and bead milled in micro-centrifuge tubes for three minutes. The genomic DNA was extracted using the DNeasy plant mini kit (QIAGEN Inc.), according to the manufacturer's instructions. The SSU region of AMF and ITS region of non-AMF fungi were amplified using AMV4.5NF & AMDGR (Lumini, 2009) and ITS1F and ITS2 primer sets respectively (Buee et al, 2009). The PCR products were purified using the AM Pure DNA purification kit, analyzed on an Agilent Bioanalyzer for quality control and sequenced using 454 GL FLX amplicon pyrosequencing (Roche Diagnostics Corporation). The sequences were cleaned and clustered into operational taxonomic units (OTU) based on 97% of similarity using Mothur software V.1.28.

Data Analysis

Permutation based MANOVA and principle component analysis (PCA) were applied to analyze the fungal communities associated with root of the cultivars (PC-ORD software). Chao1 richness and Shannon diversity indices were calculated using Mothur software V.1.28. Analysis of variance (ANOVA) was performed for mean comparisons using Tukey test in R.

Results

Results indicated that AMF and non-AMF fungal communities of root endosphere were significantly different in the cultivars of chickpea. As well the fungal communities of root endosphere were significantly different in 2010 and 2011 suggesting that environmental conditions including soil and weather can have important effects on endosphere fungal

communities (Tables 1 and 2). Each of the chickpea cultivars was more associated with specific operational taxonomic units (OTUs) of AMF and non-AMF communities (Fig.1 and 2).
The Chao1 richness and Shannon diversity indices were significantly different in the cultivars. The indices were also higher in desi than kabuli chickpeas. CDC Corrine had the highest Chao1 and Shannon indices of both AMF and non-AMF communities. CDC Alma and CDC Xena had the lowest Chao1 and Shannon indices of AMF and non-AMF communities respectively (Tables 3 to 6).

Table 1. Per-MANOVA Analysis. The effects of genotype and year on AMF fungal communities of root endosphere.

Source	DF	SS	MS	F	P
Year	1	3.8225	3.3825	17.283	0.00020***
Genotype	12	3.1890	0.26575	1.3579	0.04760*
Interaction	12	2.8061	0.23384	1.1948	0.16980 ns
Residual	52	10.177	0.19571		
Total	77	19.555			

Table 2. Per-MANOVA Analysis. The effects of genotype and year on non-AMF fungal communities of root endosphere.

Source	DF	SS	MS	F	P
Year	1	1.3722	1.3722	4.6438	0.00040***
Genotype	12	4.8973	0.4081	1.3811	0.02020*
Interaction	12	3.6960	0.3080	1.0423	0.38500ns
Residual	78	23.049	0.2954		
Total	103	33.014			

Table 3 – Chao1 richness and Shannon diversity indices of AMF community of chickpea types.

Type	Chao1 richness	Shannon diversity
Kabuli	532.61 a	4.26 a
Desi	616.93 a	4.29 a

Table 4 - Chao1 richness and Shannon diversity indices of non-AMF community of chickpea types.

Type	Chao1 richness	Shannon diversity
Kabuli	2242 b	6.18 b
Desi	2676 a	6.50 a

Table 5 – Chao1 richness and Shannon diversity indices of AMF community of chickpea genotypes.

Genotype	Chao1 richness	Shannon diversity
CDC Orion	660.45 bc	4.27 ab
CDC Leader	513.26 e	4.21 ab
Amit	515.38 e	4.12 ab
CDC Anna	615.09 cd	4.29 ab
CDC Cabri	587.50 d	4.21 ab
CDC Corrine	736.33 a	4.48 a
CDC Cory	686.91 ab	4.33 ab
CDC Alma	396.04 f	3.92 b
CDC Frontier	649.25 bc	4.40 a
CDC Luna	664.20 bc	4.43 a
CDC Nika	430.94 f	4.15 ab
CDC Vanguard	644.82 bc	4.37 a
CDC Xena	615.44 cd	4.40 a

Table 6 – Chao1 richness and Shannon diversity indices of non-AMF community of chickpea genotypes.

Genotype	Chao1 richness	Shannon diversity
CDC Orion	2031.94 de	6.01 bc
CDC Leader	2852.25 bc	6.33 abc
Amit	2189.46 de	6.23 abc
CDC Anna	2300.54 d	6.52ab
CDC Cabri	2622.05 c	6.55 ab
CDC Corrine	3365.76 a	6.69 a
CDC Cory	3018.12 b	6.63 a
CDC Alma	2686.19 c	6.50 ab
CDC Frontier	2314.63 d	6.31 abc
CDC Luna	1934.88 ef	6.01 bc
CDC Nika	1705.05 f	6.01 bc
CDC Vanguard	3084.02 ab	6.58 a
CDC Xena	1666.47 f	5.88 c

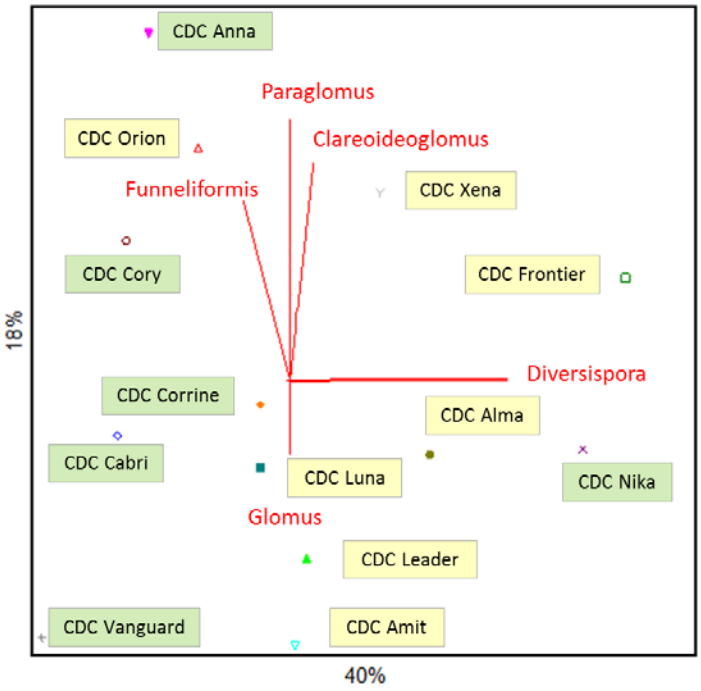

Figure 1 – Principle component analysis - the association of chickpea cultivars to the main operational taxonomic units (OTUs) of AMF community.

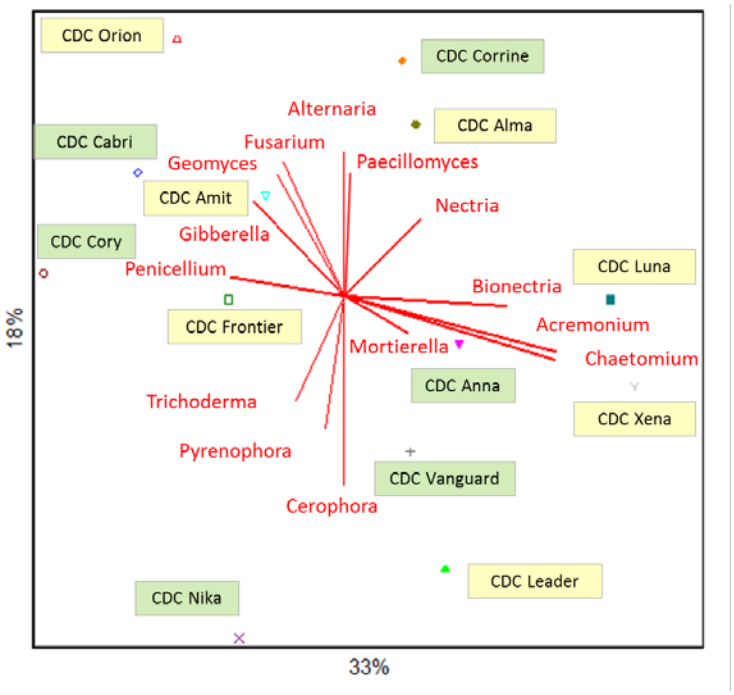

Figure 2 – Principle component analysis - the association of chickpea cultivars to the main operational taxonomic units (OTUs) of non-AMF community.

Conclusions

Chickpea genotypes develop different fungal communities in the root endosphere. *Glomus* and *Diversispora* are the most abundant AMF genera in the chickpea root. *Fusarium, Mortierella, Trichoderma, Gibberella, and Bionectria* are the most abundant non-AMF genera in the chickpea root. Chickpea genotypes have different richness and diversity indices of fungal communities. Desi chickpeas are associated with more diverse non-AMF fungi than Kabuli.

This study reveals a "genotype effect" of chickpea on the soil fungi, suggesting the possibility to the selection of genotypes with improved association with root fungal communities.

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