Microbial Diversity in the Rhizosphere of Field Grown Herbicide-Tolerant Transgenic Canola

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Abstract

In Saskatchewan it is becoming a common agricultural practice to include herbicide tolerant transgenic canola (*Brassica spp.*) varieties in crop rotation. These varieties provide an economic and agronomic benefit to farmers because of their ability to provide superior weed control with the use of a minimal number of herbicides. However, concerns regarding the effects of transgenic plants on soil and rhizosphere microbial communities have been raised. As part of an ongoing three-year field study we assessed the effects of field-grown transgenic canola on soil microbial diversity. Four transgenic and four non-transgenic commercial canola varieties were grown at six field locations across Saskatchewan. The rhizosphere and endophytic microbial communities were characterized through community level physiological profiles (CLPP), fatty acid methyl ester analysis (FAME) and DNA analysis. Results from the first year of our field study indicated that in some cases transgenic canola varieties supported different microbial communities than their non-transgenic counterparts, but in some cases field and soil type significantly influenced these differences. In addition, there were differences among non-transgenic varieties, which implies that microbial communities vary from plant to plant and site to site. Differences between the microbial communities of transgenic and non-transgenic plants may not be due to genetic engineering, but to soil variance.

Introduction

Herbicide-tolerant transgenic canola is commonly grown in Western Canada; however, the impact of these transgenic plants on microbial community dynamics is just beginning to be investigated (Germida et al., 1998; Siciliano et al., 1998). Recently, Siciliano et al. (1998) found that the microbial community associated with the roots of a transgenic canola (*Brassica* spp.) variety, was significantly different than the communities associated with two other tested non-transgenic varieties. However, the above study was limited to two field sites and did not represent a wide range of soil characteristics. Therefore it was necessary to confirm these findings for additional canola varieties at multiple field sites.

The objectives of this study were:

1. To evaluate the effect of plant genetics and soil type on microbial biodiversity and community structure.
2. To characterize the rhizosphere and endophytic microbial communities through community level physiological profiles (CLPP) and fatty acid methyl ester analysis (FAME).
3. To characterize the non-culturable rhizosphere communities through amplified ribosomal DNA restriction analysis (ARDRA).

Materials and Methods

Field studies (set-up and sample analyses)

Nine field sites were established at different geographical locations throughout Saskatchewan. Eight canola cultivars (four transgenic and four non-transgenic – Table 1) were seeded at each site in a replicated (n=4) randomized complete block design (RCBD).

Table 1. Characteristics of canola varieties used for the field study.

Variety name	Variety characteristics
Fairview	*Brassica rapa*
Innovator	*Brassica napus*; glufosinate ammonium-tolerant canola; **transgenic**
Invigor 2153	*Brassica napus*; glufosinate ammonium-tolerant canola; **transgenic**, hybrid canola
Exceed	*Brassica napus*; glufosinate ammonium-tolerant canola; **transgenic**, hybrid canola
AC Excel	*Brassica napus*
Quest RR	*Brassica napus*, gyphosate-tolerant, **transgenic**, hybrid canola
Hyola 401	*Brassica napus*, hybrid canola
45A71	*Brassica napus*, hybrid canola, imidazolonone-tolerant

Fatty acid methyl ester (FAME) analysis

FAME analysis was performed as described by Cavigelli et al. (1995). Fatty acids were extracted from 0.6 g of roots or 5 g of soil and analysed using a Hewlett Packard 5890 Series II Gas Chromatograph.

Community level physiological profiling (CLPP)

CLPP was performed as described in Siciliano and Germida (1999) with Biolog™ GN2 plates. Briefly, 100 µl of the 10^{-3} or 10^{-4} dilutions, for rhizosphere and endophytic samples respectively, was inoculated into each well and incubated at 28°C for 7 days. Colour development was measured as optical density (OD) at 590 nm, using an automated plate reader. The data were collected using the Microlog 3E software (Biolog, Inc.)

Amplified ribosomal DNA restriction analysis (ARDRA)

DNA was directly extracted from soil (5g) according to the method described in Fortin et al. (1998). DNA (50µL) was purified using a Sephracryl-400 Hr MicroSpin column

(Pharmacia Biotech Inc). Polymerase chain reaction on soil DNA extracts was performed with universal bacterial primers F1 and R10 (Dorsch and Stackebrandt, 1992). The F1 primer was end labelled with γ-^{32}ATP. To generate restriction profiles, amplified rDNA will be restricted using Taq 1 and Cfo 1 restriction endonucleases. Digests were separated on 6% polyacrylamide gels and gels were scored visually for the presence or absence of bands.

Results

Principal component analysis separated the rhizosphere microbial communities of the canola cultivars. For some locations, such as Kipling, the rhizosphere communities of transgenic plants are separated from non-transgenic plants, whereas at other field sites, such as Eyebrow, there were no transgenic effects (Fig. 1).

FAME profiles of the root associated microbial communities were altered due to changes in plant genotypes. Principal component analysis of the FAMEs separated the rhizosphere microbial communities of the canola cultivars. However, field site had an effect on microbial community structure. Regardless of changes in the communities associated with soil type and field location, the FAME profiles of the microbial communities associated with the roots of transgenic canola plants were significantly different from the profiles of the communities associated with the roots of non-transgenic plants (Fig. 2).

Amplified rDNA restriction analysis (ARDRA) of rhizosphere soil indicated that canola variety and soil type both affect the genetic structure of the microbial community (Fig. 3). Preliminary work indicated that at Eyebrow and Melfort, plant genetics affected the structure of the microbial community. For example, the community associated with Excel plants grown in Eyebrow was more similar to the community associated with Excel plants grown in Melfort than it was to Quest plants grown in Eyebrow (Fig. 3). However, at Kipling, soil type had more of an influence on the structure of the microbial community, than plant genetics.

Summary

The diversity of the soil microbial community can be affected by plant genetics; however, this is dependent on the soil characteristics. In addition, there were differences among non-transgenic varieties, which implies that microbial communities vary from plant to plant and site to site. Differences between the microbial communities of transgenic and non-transgenic plants may not be due to genetic engineering, but to soil variance.

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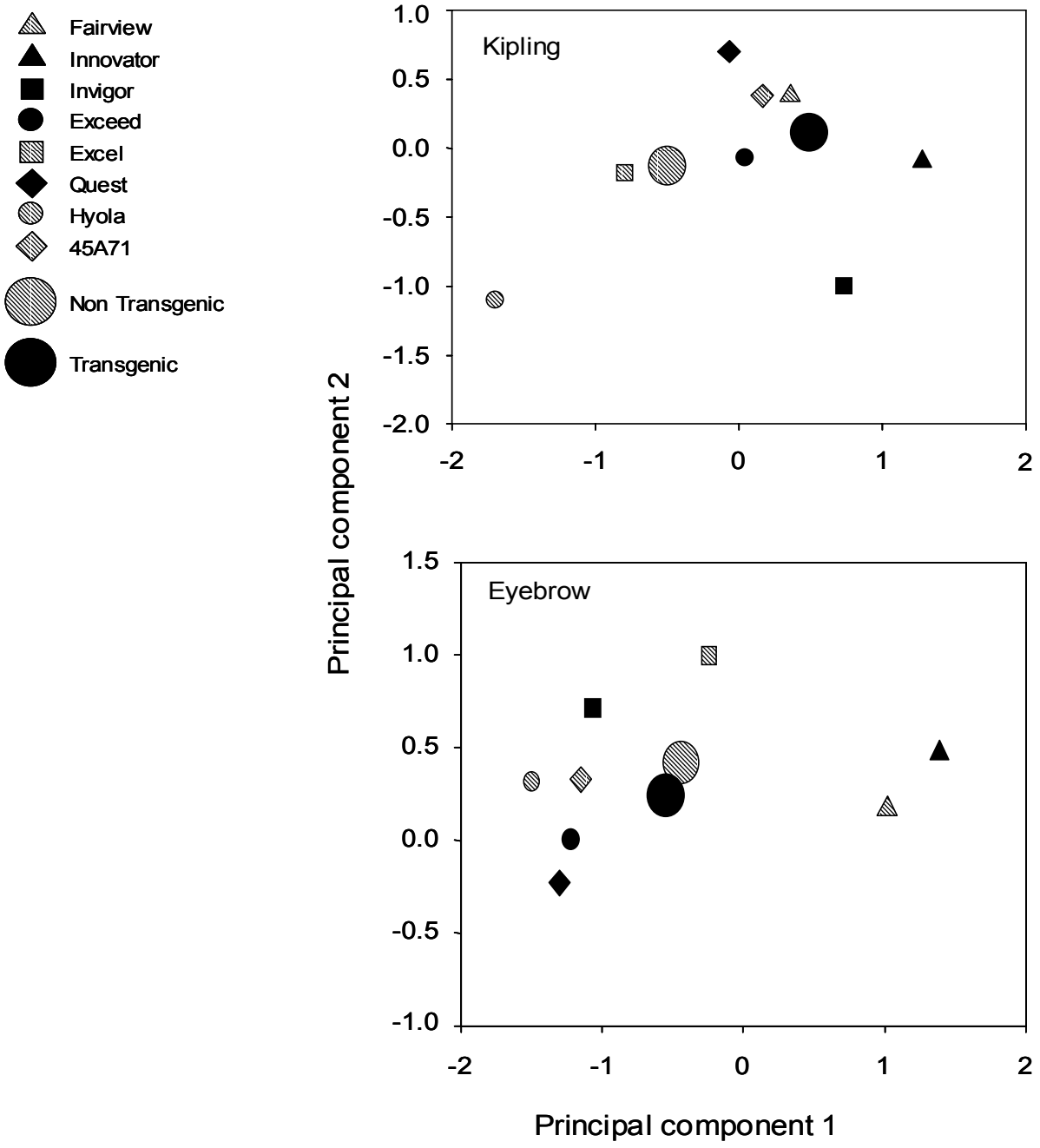

Fig. 1. Principal component analysis of the cumulative Biolog™ scores of the rhizosphere community of the canola cultivars grown at Kipling and Eyebrow, Saskatchewan. Symbols represent the mean of four replicates (n=4).

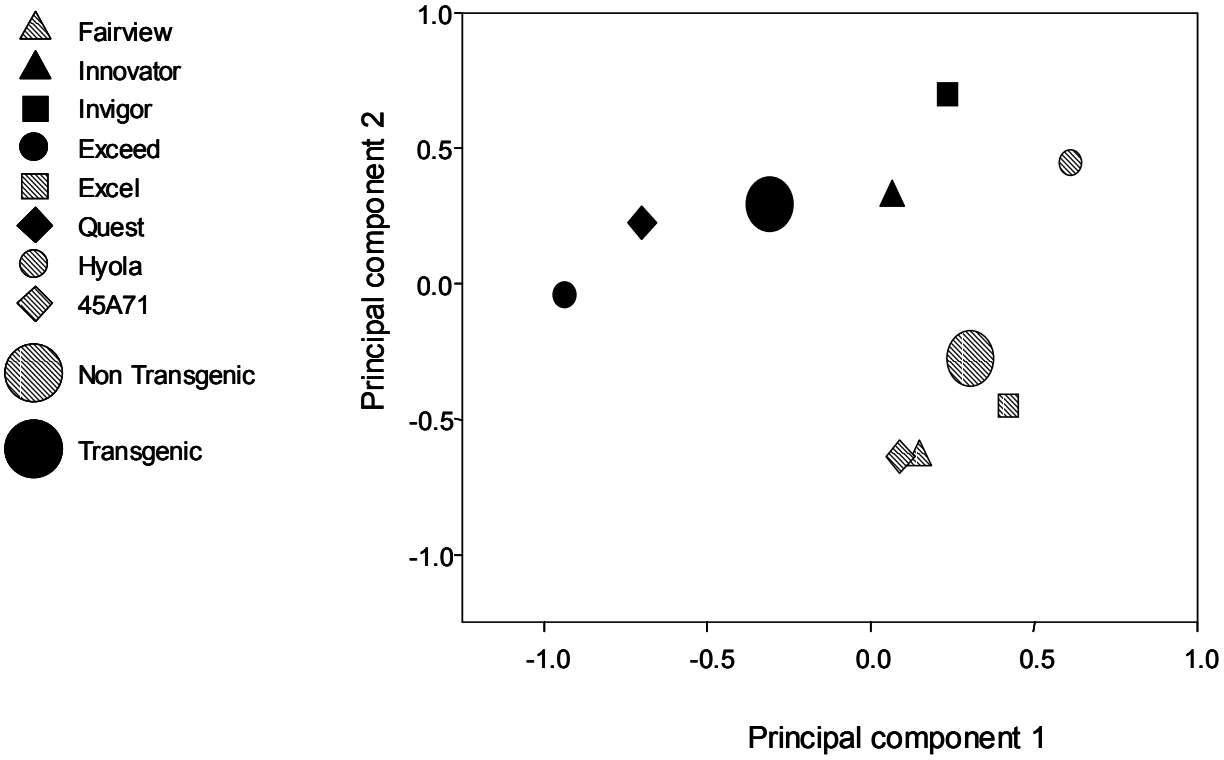

Fig. 2. Principal component analysis from FAMEs obtained from the roots of canola cultivars, at all field locations. Symbols represent the mean of four replicates and six field locations (n=24).

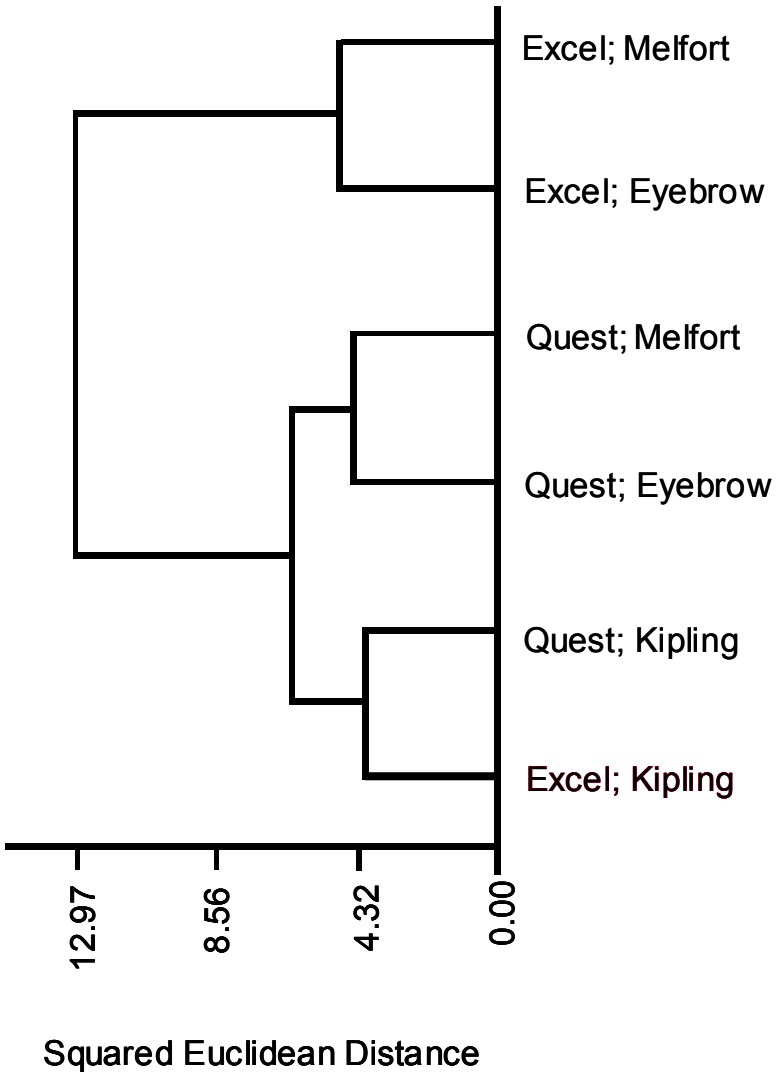

Fig. 3. Dendrogram representing genetic similarity of microbial communities associated with transgenic and non-transgenic canola plants (Quest, Excel) based on ARDRA patterns. Canola was grown in three locations in Saskatchewan, Canada (Eyebrow, Kipling, Melfort).