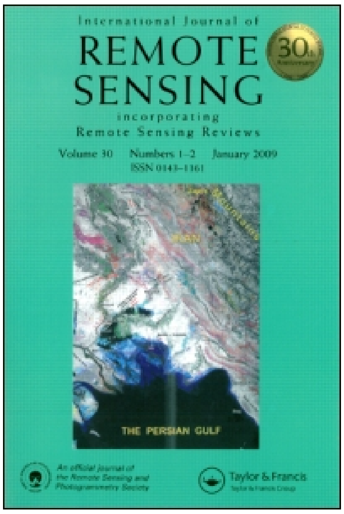

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Technical Note

Hyperspectral studies of transgenic oilseed rape

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One risk of planting transgenic crops is the escape of transgenes to conspecifics and sexually compatible wild relatives. Detecting transgene escape is thus a crucial biosafety issue world-wide, but most current detection methods are expensive and laborious, as well as being unfeasible for large-scale use in commercial cultivation. We undertook field spectral reflectance studies of non-transgenic oilseed rape (*B. napus* cv. Westar), a transgenic oilseed rape, Wild Indian mustard (*B. juncea* var. *gracilis*) and a hybrid Wild Indian mustard. Simulated reflectances for the spectral bands of several different satellite-flown hyperspectral and multi-spectral scanners were generated. The differences obtained between the simulated reflectances of the different plants leads to the possibility that these differences could be used to detect transgene escape and genomic effects among related taxa from the Moderate Resolution Imaging Spectroradiometer (MODIS) hyperspectral scanner and from the Landsat Thematic Mapper (TM), Satellite Pour l'Observation de la Terre (SPOT) High Resolution Visible (HRV) and IKONOS multi-spectral scanners.

1. Introduction

Transgenic techniques have become increasingly important in the production of new agricultural crop varieties since the 1990s (Greene and Allison 1994, Hancock *et al.* 1996, Halfhill *et al.* 2001). Transgenic organisms, a subset of genetically modified organisms, are organisms in which DNA that originated in a different species has been inserted (Daniell 1999, James 2008). Currently, more than 20 species of transgenic crops have been commercially planted in more than 125 Mha of crop land world-wide (James 2008). Despite the widely recognized benefits of this approach, the escape of transgenes is an important regulatory and consumer concern. The escape of transgenes

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from cultivated crops in agricultural fields and their transfer to non-transgenic crops or wild-type relatives can occur via pollen or seed flow. The potential consequence of this escape is transgene introgression that might alter the fitness or life-history characteristics of the recipient plants (Stewart *et al.* 1996, 2003, Halfhill *et al.* 2005).

Transgenic oilseed rape (*Brassica napus*) is widely grown around the world, and ranks fourth among transgenic crops in terms of its cultivated area (5.9 Mha) (James 2008). *B. napus* can outcross with wild-type relatives and its pollen can be dispersed over long distances by wind and insects (Rieger *et al.* 2002). There is a plethora of data showing that oilseed rape easily hybridizes with wild-type relatives, including *Brassica rapa* (Stewart *et al.* 2003, Wilkinson *et al.* 2003). As a widely distributed weed in Chinese cropland, wild Indian mustard (*Brassica juncea*) hybridizes with oilseed rape, and its ability to form hybrid progeny when crossed with *B. napus* ranks second after that of *B. rapa* (Scheffler and Dale 1994; figure 1). The rate of spontaneous hybridization between *B. napus* and *B. juncea* was reported to range from 0.3 to 2.3%, depending on the relative proportions of the two parental plants, in previous experiments (Jørgensen *et al.* 1998). In China, with equivalent proportions of parental plants, a 0.9% hybridization frequency was found by assaying seeds collected from wild mustard (Pu *et al.* 2005).

Detecting transgene escape continues to be an important biosafety issue (Wei *et al.* 1999, Wilkinson *et al.* 2003). Unfortunately, there is an absence of easy, effective methods to detect transgene escape in commercial fields, and solving this problem could result in more effective monitoring of risky crop–relative systems. Analysis of gene flow using conventional molecular techniques is not practical in most agricultural situations (Rieger *et al.* 2002). Transgenic plants and other organisms could conceivably be monitored using an *in vivo* genetic marker, such as the green fluorescent protein (GFP), whose emission can be detected under excitation with UV light (Stewart 2001, Di *et al.* 2009), and small experimental trials have identified several examples of transgene flow in several species using this method; however, this is an expensive and time-consuming tool and is difficult to use for monitoring large areas.

By contrast, remote sensing offers a rapid and economical way to monitor large areas in general. Spectral reflectance is closely related to photosynthesis, nutrient status and drought-resistance characteristics of plants (Taize and Zeiger 1998,

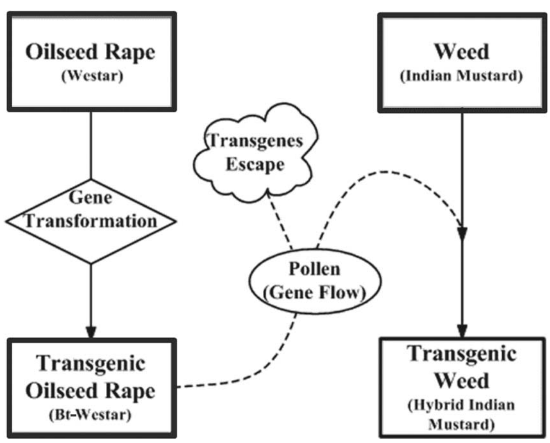

Figure 1. Illustration of gene escape from transgenic oilseed rape.

Daughtry *et al.* 2000, Singh *et al.* 2002, Jiang *et al.* 2004). Changes in genomic composition can influence a plant's phenotype (Campbell *et al.* 2000, Stewart *et al.* 2003, Wilkinson *et al.* 2003). Thus, remote sensing offers the possibility of monitoring transgene escape, provided that significant differences exist between the spectral reflectances of hybridized and un-hybridized plants (Jensen 2000, Huang *et al.* 2004). As a first step towards attempting to use hyperspectral remote sensing to detect transgenic escape, we made hyperspectral field measurements on some of the relevant plants. Thus, in the present study, our goal was to study the reflectance profiles of transgenic and non-transgenic oilseed rape, wild-type Indian mustard and their hybrids using multi-spectral and hyperspectral analysis to determine whether potentially useful differences exist between their spectral signatures for commercial monitoring of transgene escape.

2. Material and methods

In this study we used four plant types:

- non-transgenic maternal plant – a cultivar of oilseed rape (*B. napus*) – Westar;
- transgenic oilseed rape (OSR) containing Bt trangene without GFP gene;
- wild Indian mustard (*Brassica juncea*);
- hybrid wild Indian mustard – hybrids formed between transgenic OSR containing Bt and GFP transgenes and wild Indian mustard.

Seeds of the mustard were kindly provided by Prof. S. Qiang of the Nanjing Agricultural University and originated from a local field collection. Hybrids were obtained by means of hand-crossing (Shen *et al.* 2006) formed between wild Indian mustard and GFP/Bt-oilseed rape. Plants were sown outside in a field between 20 and 25 April 2006, and were transferred into pots at the four- to six-leaf stage to facilitate spectral measurements in the laboratory. All plants were grown together in a common environment and were treated identically. All plants were provided with the standard levels of fertilizer and watering in the study area for such crops to ensure that neither nutrient nor water stress would affect our results.

Hyperspectral data were obtained using a FieldSpec FR spectroradiometer (Analytical Spectral Devices Inc., Boulder, CO, USA). The spectral resolution of this instrument is 3 nm for the region between 350 and 1000 nm and 10 nm for the region between 1000 and 2500 nm. The spectrum of each sample was determined as the average of ten spectral measurements. Thirty samples were collected for both hybrid and wild Indian mustard; 29 samples of Bt-Westar and 30 samples of Westar were collected.

We have used the hyperspectral data obtained to simulate a satellite-flown hyperspectral scanner with eight spectral bands, bands 1–3 of the Landsat Thematic Mapper (TM), bands 1 and 2 of Satellite Pour l'Observation de la Terre (SPOT), bands 1 and 2 of IKONOS and bands 10–14 of Moderate Resolution Imaging Spectroradiometer (MODIS) (Table 1). The eight spectral bands to simulate a satellite flown with a hyperspectral scanner were chosen to provide good discrimination of small areas of crops.

In simulating the reflectances in these bands a simple rectangular response function was assumed, i.e. it takes the value zero outside the wavelength ranges of one of the selected bands and a value of one within that range. The mean reflectance and standard deviation of the reflectance in each band were calculated.

The comparison of hyperspectral and multi-spectral remote sensing features between hybrid Indian mustard and wild Indian mustard and between transgenic oilseed rape (Bt-Westar) were analysed using the analysis of variance (ANOVA) to test the differences among the four plant types, along with Dunnett's two-tailed *t*-test. We also calculated the 95% confidence intervals (CI) for the differences between plants. A 95% CI that differs significantly from 0 indicates that two means differ significantly at $p < 0.05$. Our analyses used the SAS software (version 9.1; SAS Institute, Cary, NC, USA) and Splus (version 7.0; Insightful Corp, Seattle, WA, USA).

3. Results

The spectral characteristics differed significantly among the transgenic and non-transgenic oilseed rape, wild Indian mustard and their transgenic hybrid (table 1, figure 2). The reflectance of wild Indian mustard was highest across the spectrum, followed by Westar, Bt-Westar and hybrid Indian mustard (figure 2). This spectral difference was consistent across the spectrum from 350 to 2500 nm. The overall pattern of spectral compensation was similar for all four plant types, but was most similar between wild-type Indian mustard and hybrid Indian mustard, as well as between Bt-oilseed rape and hybrid Indian mustard. At the base of figure 2(*b*) we show the ranges of the spectral bands of some common satellite remote sensors for reference.

From the analysis of the eight-band simulated satellite hyperspectral scanner bands we can see from table 1 that the differences between hybrid Indian mustard and wild Indian mustard were significant for all eight spectral bands, whereas the differences between hybrid Indian mustard and Bt-Westar were significant for five of the eight spectral bands (i.e. not for the yellow, red and near-infrared (NIR) bands). The spectral characteristics of the plants appeared to be closely related to the differences in their genetic structure.

The differences between the spectral reflectance values in each of the simulated sets of multi-spectral bands for the transgenic plants are given in table 1. From table 1 we can see that for the simulated hyperspectral data the highest degree of spectral difference between wild-type Indian mustard and hybrid mustard occurred in the NIR band, followed by the RE band; the differences between Bt-oilseed rape and hybrid Indian mustard followed the same pattern (table 1). The spectral differences for wild Indian mustard vs. hybrid mustard were larger than those for Bt-Westar vs. hybrid Indian mustard in all bands. From table 1, for the simulated multi-spectral data we can see that there are significant differences between hybrid Indian mustard and wild Indian mustard in all the bands of these four sensors; however, there were partially significant differences between hybrid Indian mustard and Bt-Westar except for band 2 of Landsat TM and band 2 of IKONOS. The results suggest that multi-spectral satellite remote sensing data have the potential for detecting the difference between hybrid Indian mustard and wild Indian mustard. For the difference between hybrid Indian mustard and Bt-Westar, bands 1 and 3 of Landsat TM, bands 1 and 3 of IKONOS, bands 1 and 2 of High Resolution Visible (HRV)/High Resolution Visible and Infrared (HRVIR), as well as bands 10, 13 and 14 of MODIS, are the most sensitive.

Table 1. Comparison of hyperspectral remote sensing features between hybrid Indian mustard and Bt-Westar and wild Indian mustard.

				Hybrid Indian mustard vs. wild Indian mustard		Hybrid Indian mustard vs. Bt-Westar	
Feature waveband (nm)	Hybrid Indian mustard	Wild Indian mustard	Bt-Westar	p	Difference	p	Difference
BR (350–400)	0.047±0.010	0.062±0.014	0.054±0.009	0.000**	–0.015	0.005**	–0.007
BE (490–530)	0.087±0.015	0.116±0.023	0.099±0.015	0.000**	–0.029	0.003**	–0.012
GR (510–560)	0.128±0.023	0.169±0.033	0.141±0.025	0.000**	–0.041	0.044*	–0.013
YE (550–582)	0.137±0.025	0.179±0.036	0.149±0.027	0.000**	–0.042	0.077	–0.012
RV (640–680)	0.072±0.013	0.097±0.019	0.079±0.011	0.000**	–0.025	0.034*	–0.007
RE (670–737)	0.221±0.035	0.290±0.054	0.237±0.039	0.000**	–0.069	0.091	–0.016
VR (350–700)	0.083±0.014	0.110±0.022	0.093±0.014	0.000**	–0.027	0.009**	–0.01
NIR (700–900)	0.470±0.068	0.617±0.105	0.505±0.075	0.000**	–0.147	0.067	–0.035

*Difference significant at $p < 0.05$.
**Difference significant at $p < 0.01$.

Table 2. Comparison of multi-spectral remote sensing features between hybrid Indian mustard and Bt-Westar and wild Indian mustard.

Sensor	Band (nm)	Hybrid Indian mustard	Wild Indian mustard	Bt-Westar	Hybrid Indian mustard vs. wild Indian mustard		Hybrid Indian mustard vs. Bt-Westar	
					p	Difference	*p*	Difference
Landsat TM	1 (450–520)	0.072±0.013	0.096±0.019	0.084±0.012	0.000**	–0.024	0.000**	–0.012
	2 (520–600)	0.128±0.023	0.169±0.033	0.140±0.025	0.000**	–0.041	0.061	–0.012
	3 (630–690)	0.074±0.013	0.100±0.020	0.081±0.012	0.000**	–0.026	0.036*	–0.007
SPOT HRV/HRVIR	1 (500–590)	0.121±0.021	0.159±0.031	0.133±0.023	0.000**	–0.038	0.039*	–0.012
	2 (610–680)	0.079±0.014	0.105±0.021	0.086±0.013	0.000**	–0.026	0.036*	–0.007
IKONOS	1 (450–520)	0.072±0.013	0.096±0.019	0.084±0.012	0.000**	–0.024	0.000**	–0.012
	2 (520–600)	0.128±0.023	0.169±0.033	0.140±0.025	0.000**	–0.041	0.061	–0.012
	3 (630–690)	0.074±0.013	0.100±0.020	0.081±0.012	0.000**	–0.026	0.036*	–0.007
MODIS	10 (483–493)	0.075±0.013	0.100±0.020	0.090±0.011	0.000**	–0.025	0.000**	–0.015
	11 (526–536)	0.144±0.026	0.190±0.037	0.155±0.020	0.000**	–0.046	0.058	–0.011
	12 (546–556)	0.166±0.030	0.217±0.043	0.177±0.023	0.000**	–0.051	0.149	–0.011
	13 (662–672)	0.075±0.013	0.101±0.020	0.084±0.010	0.000**	–0.026	0.006**	–0.009
	14 (673–683)	0.075±0.013	0.101±0.021	0.084±0.010	0.000**	–0.026	0.006**	–0.009

*Difference significant at $p < 0.05$.
**Difference significant at $p < 0.01$.

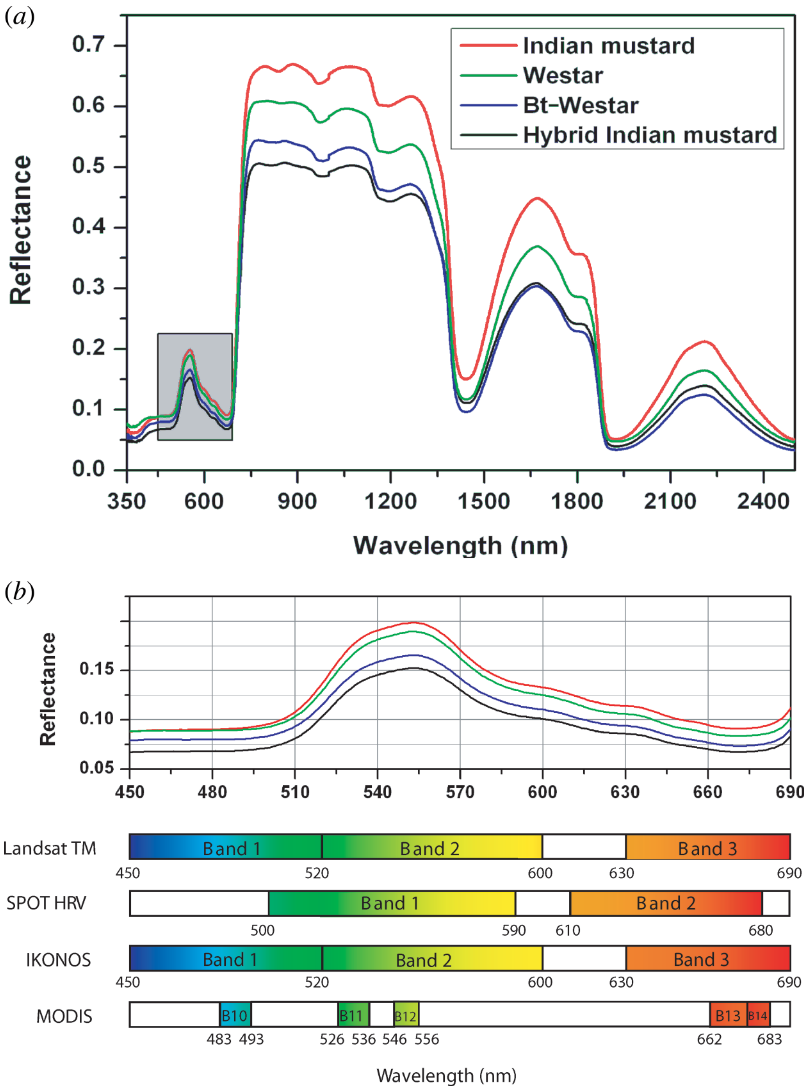

Figure 2. (*a*) The mean hyperspectral and multi-spectral reflectance values for transgenic oilseed rape, wild-type Indian mustard and hybrid Indian mustard. (*b*) An expanded view of the results in the visible spectrum (boxed area in (*a*)).

4. Discussion

While the reflectance values for wild Indian mustard, hybrid Indian mustard, Westar and Bt-Westar were found to be similar in both the simulated hyperspectral and the multi-spectral data, they differed significantly in the blue, red and infrared wavelength bands. Our work thus appears to represent the first report of genetic transformation changing the spectral reflectance of the transgene recipient plant. This leads to the possibility that these differences could be used to detect transgene escape and genomic effects among related taxa from the MODIS hyperspectral scanner and from the

Landsat TM, SPOT HRV and IKONOS multi-spectral scanners. The consistency between the hyperspectral and multi-spectral remote sensing data (figure 2, tables 1 and 2) provides additional evidence that the approach used in our study will become a potentially useful tool in the field, as it provides a strong ability to detect small differences among plants at relatively low cost compared with laboratory techniques.

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