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Effects of region, genotype, harvest year and their interactions on δ^{13}C, δ^{15}N and δD in wheat kernels

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ABSTRACT

The objective of this study was to investigate the influences of region, genotype, harvest year and their interactions on stable carbon, nitrogen and hydrogen isotopic ratio (δ^{13}C, δ^{15}N and δD) fingerprints in wheat kernels. A total of 270 wheat kernel samples including ten genotypes were collected from three different regions of China during 2011–2013 harvest. Analysis of variance was employed to investigate the effects of region, genotype, harvest year and their interactions on the δ^{13}C, δ^{15}N and δD. The results showed that the δ^{13}C and δ^{15}N values in wheat kernels were significantly influenced by the region, genotype, harvest year and their interactions (region × genotype, genotype × year, region × year and region × genotype × year), δD was significantly affected by region, genotype, harvest year and region × year. Region accounted for the largest proportion of the total variation and explained 47.57%, 58.02% and 27.96% for δ^{13}C, δ^{15}N and δD, respectively.

1. Introduction

Agricultural product quality is closely related to its origin (Zia-Ul-Haq, Ahmad, Qayum, & Ercişli, 2013). Plants reflect characteristics of their environment and physiology through the stable isotope ratios of elements (e.g., $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$ and $^{2}H/^{1}H$) that form compounds in the organisms (Anderson & Smith, 2006) Furthermore, isotope signatures are natural fingerprints of biomaterials that can be stored for several years without the isotope composition changing significantly, and many researches have proved that stable isotopic composition can be employed in food geographical origin authenticity and traceability such as meat (Guo, Wei, Pan, & Li, 2010; Liu, Guo, Wei, Shi, & Sun, 2013; Osorio, Moloney, Schmidt, & Monahan, 2011), dairy product (Ehtesham, Hayman, McComb, Van Hale, & Frew, 2013; Scampicchio et al., 2012), fruit juice (Rummel, Hoelzl, Horn, Rossmann, & Schlicht, 2010), olive oil (Camin et al., 2010), wine (Marchionni et al., 2013) and cereals (Brescia et al., 2002; Kawasaki, Oda, & Hirata, 2002).

However, the application of this technique on geographical traceability is never simple, that's because the geographical information in each region not only includes the geologic feature, soil type, latitude and altitude, but also the meteorological factors, such as temperature, precipitation and air humidity, the latter can be uncertain factor along with interannual change. The carbon isotopic ratio in plants are reported to vary in response to air humidity, soil water capacity, nitrogen availability and salinity (Christoph, Rossmann, Schlicht, & Voerkelius, 2006; Dawson et al., 2002). Climate and ecosystem variations such as annual temperatures and precipitation can affect nitrogen isotopic composition (Garten, 1993). Temperature will also influence the δD in rain. Decreasing temperatures cause a progressive heavy-isotope depletion of the precipitation when the water vapour from oceans in equatorial regions moves to higher latitudes and altitudes (Craig, 1961), all the factors above might bring the complexity and uncertain factor to establish the stable isotope fingerprints.

Other than region and crop year, isotopic ratios in biological products may be influenced by many factors, such as genotype and the interaction of genotype and environment (Ngugi, Galwey, & Austin, 1994; Rajabi, Ober, & Griffiths, 2009) whether the characteristic fingerprints will change with different genotypes and different harvest years in the same region are not yet clear, and which factor will dominate the variation of isotopic composition in agricultural products is still unclear too. However, as far as we know, no study has been published on the stability of the isotopic fingerprints under the influences of region, genotype in consecutive years previously. Wheat with its characteristics of widespread, diversification of varieties and adaption for different regions, provide possibility for our study. Here we focus on δ^{13}C, δ^{15}N and δD

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which derive from carbon (C), water (H_2O) and nitrogen (N), because they are three of the most important resources influencing plant function, growth, distribution, and the biogeochemical cycles in which plants participate (Dawson, Mambelli, Plamboeck, Templer, & Tu, 2002) which may cause more volatility while implementing the geographic traceability.

The objective of this paper was to investigate the influences of region (R), genotype (G), harvest year (Y) on the stable carbon, nitrogen and hydrogen isotopes firstly, then combined with the effects of interactions (R × G, G × Y, R × Y and R × G × Y) to find out the contribution (expressed in percentage) of each factor and their interactions to each isotope.

2. Materials and methods

2.1. Sample cultivation and collection

Ten wheat varieties of Han 6172, Heng 5229, Hengguan 35, Xinong 889, Xinong 979, Xiaoyan 22, Xinmai 18, Zhengmai 366, Zhoumai 16, and Zhoumai 18 were cultivated in the years of 2010–2012 during growing seasons in three experimental fields, which were Zhaoxian (Hebei province), Huixian (Henan province) and Yangling (Shaanxi province) respectively. The varieties were arranged randomly in each plot. The typical size of the plot was 10 m^2, and there are ten plots in each field. Wheat samples were collected in three different sites at harvest time in each plot, and a quadrant of 1 m^2 was selected randomly at each site. The sample was subsequently threshed, and the final kernel sample was obtained for laboratory analysis. 30 wheat samples were collected in each year at each field, and totally 270 wheat samples over 3 years. The number of samples, location and soil types are shown in Table 1.

2.2. Sample pretreatment

Picked out the impurities like stones and weeds, washed the wheat kernels with deionized water thoroughly, then dried in an oven (DHG-9140A, Yiheng, China) at 38 °C till constant weight. All the wheat kernel samples were ground in a Cyclotec 1093 sample mill (Foss Tecator, Denmark) to obtain whole wheat flour.

2.3. Stable carbon and nitrogen isotope analysis

3–4 mg of wheat dry samples were weighted into tin capsules and introduced by means of an auto sampler into the elemental analyser (vario PYRO cube, Elementar, Hanau, Germany). The elements of carbon and nitrogen in samples were combusted into CO_2 and N_2 gas, and then the CO_2 was diluted by a dilutor. After that, carrier gas flowed into an isotope ratio mass spectrometer (IsoPrime100, Isoprime, Manchester, Britain). The C and N stable isotope compositions of each sample were determined by the same analysis. The operating conditions were as follows:

The elemental analyser: combustion furnace temperature was 1020 °C, reduction furnace was 600 °C, 230 ml/min carrier gas flow rate.

Dilutor: helium pressure was 4 bar, the pressure of CO_2 reference gas was 4 bar, and pressure of N_2 reference gas was 4 bar.
Isotope Ratio Mass Spectrometry (IRMS): demarcated the CO_2 reference gas with USGS24 ($\delta^{13}C_{PDB} = -16‰$) and correct the results with USGS24 and IAEA600 ($\delta^{13}C_{PDB} = -27.5‰$). Calibrated the N_2 reference gas against the International Atomic Energy Agency (IAEA) standards with IAEA N_1 ($\delta^{15}N_{air} = 0.4‰$) and corrected the results with IAEA N_1 and USGS43 ($\delta^{14}N_{air} = 8.44‰$).

Isotope data were expressed using the international delta notation ($\delta‰$) and were calculated as follows:

$$\delta\ (‰) = \left(R_{sample}/R_{standard} - 1\right) \times 1000$$

where δ (‰) referred to $\delta^{13}C$ and $\delta^{15}N$, and R is the respective $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio.

2.4. Stable hydrogen isotope analysis

Dry samples of wheat were weighed (0.3–0.4 mg) and transferred into 6 × 4 mm isotope grade silver capsules and then were folded into tiny balls. All weights were recorded and the samples were transferred into an elementary analyser (vario PYRO cube, Elementar, Hanau, Germany) by a 120 automatic sampler after 72 h of balancing, by subsequent pyrolysis and produced into CO and H_2 at 1450 °C, then were transferred to an isotope ratio mass spectrometer (IsoPrime100, Isoprime, Manchester, Britain). The flow rate of carrier gas was 120 ml/min, and the pressure of the helium reference gas was 4 bar. The value of δD was expressed in delta (δ) notation in parts per thousands (‰) and was referred to V-SMOW, corrected with IAEA-CH-7 Polyethylene, and the delta values were calculated as follows:

$$\delta D\ (‰) = \left(R_{sample}/R_{standard} - 1\right) \times 1000$$

where R is the ratio of D/H.

2.5. Quality control and statistical analysis

The analytical error of the carbon, nitrogen isotopic determinations was less than 0.2‰, and hydrogen was less than 2‰. Each sample was analysed in triplicate. Statistical analysis was carried out by SPSS for Windows version 18.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (one-way ANOVA) and multiple comparison analysis were carried out to estimate the statistically significant differences between different regions, genotypes and years, multi-way analysis of variance (multiway ANOVA) were carried out to find out the contributions rate of each factors.

3. Results

3.1. Isotopic ratio analysis amongst three regions

The isotopic ratios are presented as the mean and standard deviation (SD) for each of the categories tested (Table 2). The $\delta^{13}C$, $\delta^{15}N$ and δD values showed highly significant differences

Table 1
The sample numbers, location and soil types of wheat growing regions of 3 years.

Region	Number of samples	N latitude (deg)	E longitude (deg)	Altitude (m)	Previous crop	Growth period
Zhaoxian	90	37.83	114.82	39	Maize	October 5, 2010-June 14, 2011; October 6, 2011-June 14, 2012; October 3, 2012-June 18, 2013
Huixian	90	35.39	113.83	82	Maize	October 14, 2010-June 9, 2011; October 22, 2011-June 5, 2012; October 6, 2012-June 5, 2013
Yangling	90	34.29	108.06	513	Soybean	October 23, 2010-June 6, 2011; October 17, 2011-June 9, 2012; October 3, 2012-June 2, 2013

Table 2
The $\delta^{13}C$, $\delta^{15}N$ and δD values in wheat samples amongst three regions in each year.

Isotopes	2010/2011			2011/2012			2012/2013		
	Huixian	Yangling	Zhaoxian	Huixian	Yangling	Zhaoxian	Huixian	Yangling	Zhaoxian
$\delta^{13}C$ (‰)	−27.69 ± 0.39B	−25.77 ± 0.61A	−27.59 ± 0.60B	−28.03 ± 0.36B	−27.74 ± 0.30A	−28.11 ± 0.30B	−28.03 ± 0.40B	−26.28 ± 0.51A	−28.30 ± 0.44B
$\delta^{15}N$ (‰)	2.57 ± 0.87A	−3.30 ± 0.90C	−0.46 ± 0.87B	−0.57 ± 0.74A	−1.42 ± 1.82B	−0.10 ± 0.73A	0.86 ± 0.39B	−3.57 ± 0.30C	1.57 ± 0.66A
δD (‰)	−65.43 ± 6.37A	−65.90 ± 6.46A	−74.07 ± 6.40B	−61.95 ± 4.67B	−56.30 ± 6.57A	−65.50 ± 5.02B	−71.86 ± 3.59B	−61.33 ± 6.75A	−75.17 ± 3.85B

Note: The different letters in rows represent statistically significantly different at $p < 0.01$.

($p < 0.01$) in total 270 samples from the three geographical regions in each year, the samples from Yangling were characterised by the highest $\delta^{13}C$ and lowest $\delta^{15}N$ amongst three regions in each year, whereas the wheat kernels from Zhaoxian were characterised by the lowest δD in each year.

3.2. Isotopic ratio analysis amongst ten genotypes

Irrespective of the variation of harvest year, the isotopic ratios were presented as the mean and standard deviation (SD) for 270 samples in Table 3. The $\delta^{13}C$ values amongst ten genotypes were statistically different ($p < 0.01$), but no highly significant differences were found in the $\delta^{15}N$ and δD values of the samples amongst ten genotypes in each region. As for $\delta^{13}C$, the mean value of Xinong 979 showed the highest amongst ten genotypes in each region, and Zhoumai18 showed the lowest value in Huixian and Yangling, Heng5229 showed the lowest values in Zhaoxian. Overall, compared to the geographic differences, genotype did not appear to affect the isotopic differences seen in $\delta^{15}N$ and δD values, but would affect the $\delta^{13}C$ in wheat kernels.

3.3. Isotopic ratio analysis amongst 3 years

One-way ANOVA for composition of three stable isotopes measured during the three consecutive years was employed and the results were showed in Table 4. The average values of δD exhibited the largest variability (∼10‰) across years, and followed by the $\delta^{15}N$ values. The average values of $\delta^{13}C$ were relatively constant and showed the smallest variation (<2‰) across years within each region.

3.4. Multi-way ANOVA

A combined analysis of variance across 3 regions and 3 years was performed using the general linear model (GLM) procedure of SPSS (Table 5). Regions, genotypes and harvest years were considered as fixed factors, and the effects were portioned into different sources, such as region (R), genotype (G), year (Y), region × genotype (R × G), region × year (R × Y), genotype × year (G × Y), region × genotype × year (R × G × Y). Totally, the $\delta^{13}C$ and $\delta^{15}N$ values in wheat kernel were significantly influenced by R, G, Y, R × Y, R × G, G × Y and R × G × Y, whereas R, G, Y and R × Y had significantly effects on δD.

The relative contribution (expressed in percentage) of each component (including interactions) to the total variation were computed and showed in Fig. 1. The percentages of total square sum of each factor for $\delta^{13}C$ are R (47.57) > Y (16.75) > R × Y (13.53) > G (13.32) > R × Y × G (2.09) > R × G (1.89) > G × Y (1.03). The percentages of total square sum of each factor for $\delta^{15}N$ are R (58.02) > R × Y (24.05) > R × Y × G (2.76) > G × Y (2.75) > R × G (2.65) > G (1.06) > Y (0.48), and the percentages of total square sum of each factor for δD are R (27.96) > Y (20.73) > G (11.61) > R × Y (5.58) > R × Y × G (2.67) > Y × G (2.27) > R × G (1.92). Overall, region showed the largest effects on the $\delta^{13}C$, $\delta^{15}N$ and δD in wheat kernels, and the relative contribution was 47.57%, 58.02% and 27.96% respectively.

4. Discussion

In our study, significant differences were found amongst different regions and the $\delta^{13}C$ value in wheat kernels of Yangling was highest, while no significant differences were found between the other two regions, this might be attributed to the altitude difference, and there are some reports (Hobson et al., 2003; Korner, Farquhar, & Roksandic, 1988) consistent with our results that the

Table 3
The $\delta^{13}C$, $\delta^{15}N$ and δD values in wheat samples amongst ten genotype in each region.

Genotypes	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)			δD (‰)		
	Huixian	Yangling	Zhaoxian	Huixian	Yangling	Zhaoxian	Huixian	Yangling	Zhaoxian
Heng 5229	−28.28 ± 0.35C	−26.90 ± 1.13AB	−28.40 ± 0.43C	0.89 ± 1.35A	−2.84 ± 1.20A	0.41 ± 0.52A	−70.44 ± 3.97A	−67.58 ± 7.45A	−76.89 ± 5.69A
Han 6172	−27.73 ± 0.23ABC	−26.05 ± 1.17AB	−28.00 ± 0.27ABC	0.39 ± 0.83A	−1.30 ± 3.36A	0.66 ± 1.04A	−68.62 ± 4.35A	−58.89 ± 5.89A	−70.94 ± 5.68A
Hengguan 35	−28.11 ± 0.07BC	−26.79 ± 0.72AB	−28.16 ± 0.30BC	1.01 ± 1.94A	−3.34 ± 0.88A	−0.46 ± 0.97A	−69.48 ± 5.99A	−65.22 ± 6.40A	−72.66 ± 6.62A
Xinmai 18	−27.58 ± 0.45A	−26.44 ± 0.76AB	−27.85 ± 0.55ABC	0.56 ± 2.07A	−3.27 ± 0.56A	0.36 ± 0.73A	−63.72 ± 6.85A	−57.68 ± 5.53A	−69.06 ± 5.42A
Xinong 889	−27.58 ± 0.33A	−26.28 ± 1.02AB	−27.66 ± 0.44AB	0.99 ± 1.05A	−2.92 ± 0.69A	0.33 ± 1.47A	−66.82 ± 7.84A	−62.29 ± 8.65A	−69.71 ± 6.49A
Xinong 979	−27.57 ± 0.09A	−26.01 ± 1.03A	−27.44 ± 0.96A	0.91 ± 1.28A	−3.24 ± 0.99A	0.31 ± 1.70A	−65.65 ± 4.00A	−58.37 ± 4.94A	−72.31 ± 4.88A
Xiaoyan 22	−28.07 ± 0.35BC	−26.73 ± 0.87AB	−28.18 ± 0.33BC	1.19 ± 1.82A	−2.73 ± 1.12A	0.77 ± 1.54A	−65.59 ± 7.86A	−61.84 ± 8.28A	−71.68 ± 8.03A
Zhoumai 16	−28.04 ± 0.51BC	−26.83 ± 0.73AB	−28.10 ± 0.42ABC	1.25 ± 1.64A	−2.43 ± 1.76A	0.65 ± 0.51A	−63.69 ± 7.86A	−58.17 ± 7.60A	−69.41 ± 5.58A
Zhoumai 18	−28.29 ± 0.19C	−27.36 ± 0.74B	−28.25 ± 0.32BC	0.93 ± 1.19A	−2.52 ± 1.28A	0.29 ± 1.08A	−66.68 ± 6.44A	−61.88 ± 8.39A	−73.04 ± 8.40A
Zhengmai 366	−27.73 ± 0.38AB	−26.57 ± 1.02AB	−27.97 ± 0.61ABC	1.41 ± 1.49A	−3.03 ± 0.78A	0.04 ± 0.57A	−63.47 ± 6.15A	−59.88 ± 8.97A	−70.11 ± 5.98A

Note: The different letters in columns represent statistically significantly different at $p < 0.01$.

Table 4
The $\delta^{13}C$, $\delta^{15}N$ and δD values in wheat samples amongst 3 years in each region.

Isotopes	Huixian			Yangling			Zhaoxian		
	2010/2011	2011/2012	2012/2013	2010/2011	2011/2012	2012/2013	2010/2011	2011/2012	2012/2013
$\delta^{13}C$ (‰)	−27.69 ± 0.39A	−28.03 ± 0.36B	−28.03 ± 0.40B	−25.77 ± 0.61A	−27.74 ± 0.30C	−26.28 ± 0.51B	−27.59 ± 0.60A	−28.11 ± 0.30B	−28.30 ± 0.44B
$\delta^{15}N$ (‰)	2.57 ± 0.87A	−0.58 ± 0.74C	0.86 ± 0.39B	−3.30 ± 0.90B	−1.42 ± 1.82A	−3.57 ± 0.30B	−0.46 ± 0.87B	−0.10 ± 0.73B	1.57 ± 0.66A
δD (‰)	−65.43 ± 6.37A	−61.95 ± 4.63A	−71.86 ± 3.59B	−65.90 ± 6.46C	−56.30 ± 6.57A	−61.33 ± 6.75B	−74.07 ± 5.40B	−65.50 ± 5.02A	−75.17 ± 3.85B

Note: The different letters in rows represent statistically significantly different at $p < 0.01$.

Table 5
Analysis of variance for the stable carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and hydrogen (δD) of wheat kernels.

Source of Variation		$\delta^{13}C$		$\delta^{15}N$		δD	
	DF	MS	F	MS	F	MS	F
Region (R)	2	56.425**	1121.018	356.754**	634.146	2433.951**	92.326
Genotype (G)	9	3.512**	69.777	1.455**	2.586	224.504**	8.516
Year (Y)	2	19.875**	394.866	2.927**	5.202	1804.029**	68.432
R × G	18	0.249**	4.955	1.808**	3.214	18.568	3.214
G × Y	18	0.136**	2.695	1.878**	3.338	21.989	0.834
R × Y	4	8.026**	159.454	73.949**	131.448	242.739**	9.208
R × G × Y	36	0.137**	2.732	0.944*	1.677	12.928	0.490
Error	180	0.050		0.563		26.362	

Note: DF indicates degree of freedom, MS indicates mean squares, *indicates significant differences ($p < 0.01$), **indicates highly significant differences ($p < 0.01$).

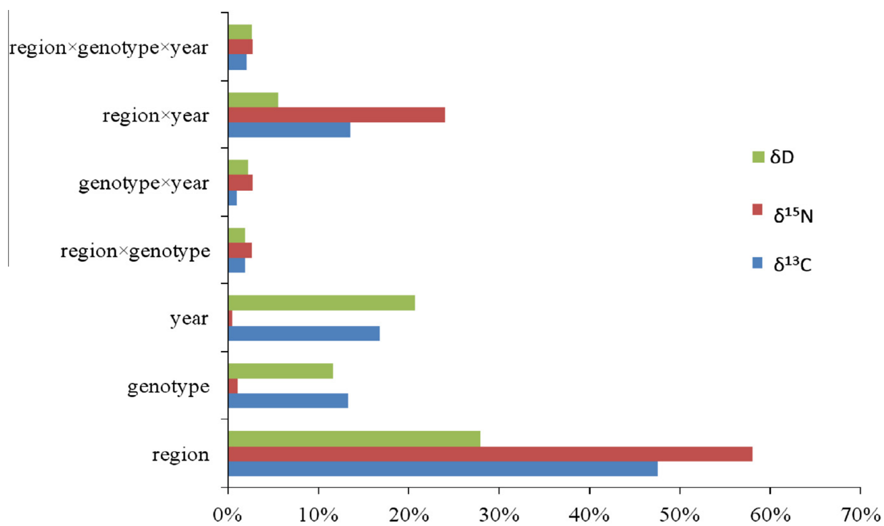

Fig. 1. The percentage of total square variance of each factor for $\delta^{13}C$, $\delta^{15}N$ and δD in wheat kernels.

$\delta^{13}C$ value in plants increased as the altitude increased, this can make sense because wheat as C_3 plant uses the Calvin photosynthetic pathway to assimilate CO_2, and the $\delta^{13}C$ values in plants are reported to be correlated with the ratio of intercellular CO_2 and CO_2 from the environment (P_i/P_a) (Farquhar, O'leary, & Berry, 1982), reflecting the fact that CO_2 source (both concentrations and $\delta^{13}C$) at different region had different values, higher altitude may be associated with lower concentration of atmospheric CO_2, as a result of different intensities of physiological process and different values of $\delta^{13}C$ in wheat. We also found significant differences amongst different genotypes for $\delta^{13}C$. Fewer studies focused the relationship between $\delta^{13}C$ and genotype, but studies on the relationship between $\Delta^{13}C$ and genotype increased in recent years, and most reported that genotypes with high drought tolerance in wheat showed a lower $\Delta^{13}C$ and higher water use efficiency (WUE) (Lin, Peng, & Li, 2001), and the correlation between $\Delta^{13}C$ and $\delta^{13}C$ is: $\Delta^{13}C = (\delta^{13}C_{air} - \delta^{13}C_{plant})/(1 + \delta^{13}C_{plant})$, indicated that water is another important factor for the variation of stable carbon components in wheat. Except CO_2 and water, sunshine is requisite for the process of photosynthesis, what's more, temperature will influence the rate of reaction. The precipitation, sunshine and annual temperature are not stable across different years even in the same region, and these fluctuating factors might affect the carbon isotope fractionation during photosynthesis (Yun & Ro, 2008). So the significant effects by R × G, G × Y, R × Y and R × G × Y interactions were reasonable, meant that the $\delta^{13}C$ value in wheat was affected by the interaction between genotype and environment, which was consistent with the result of Araus, Cabrera-Bosquet, Serret, Bort, and Nieto-Taladriz (2013), who found that $\delta^{13}C$ in wheat was affected by genotype and year interaction. Combined with the contribution rate of each factor, region contributed most for the variation, which meant that the environmental CO_2 was the main and direct factor for the $\delta^{13}C$ value in wheat.

For $\delta^{15}N$, region was found to be the main factor for variation, followed by the interaction between region and year, and the contribution rates of other factors were slight. At the same time, no significant difference was found amongst different genotypes in the same region, which indicated that the environment (including region and year) was much more important than genotype for the variation of $\delta^{15}N$. Most of studies demonstrated that soil was the main source of nitrogen for plant and soil characteristics, available N sources, and N cycling processes played a strong role in the overall relationship between $\delta^{15}N$ values in soil and plant (Vallano & Sparks, 2013). Furthermore, the $\delta^{15}N$ values in plant are related with the fertiliser used in agricultural practice. Synthetic nitrogen fertilisers generally have nitrogen isotope values between −4‰ and 4‰ and organic fertilisers generally have higher $\delta^{15}N$ values show a much wider range of compositions (2–30‰) than synthetic fertilisers (Kendall, 1998), and different synthetic nitrogen fertilisers can also show different values of $\delta^{15}N$ (Bateman & Kelly, 2007), here we also found significant differences of $\delta^{15}N$ from different regions with different types of synthetic fertilisers, this indicated that the $\delta^{15}N$ in wheat is closely correlated to local agricultural practices, which represents the local characteristic in each region. The values of $\delta^{15}N$ in wheat were also different in different years, and this could be explained by the changing weather conditions, because climate and ecosystem variations such as annual temperatures and precipitation can affect nitrogen isotopic composition (Garten, 1993).

In the case of δD, both region and harvest year influenced the δD in wheat significantly, and no significant differences were found between different genotypes within each region. In addition, the δD values were also highly significantly influenced by the interaction of region and year. When compared the differences between different regions, the averaged values of δD for wheat is Yangling > Huixian > Zhaoxian, while the order of latitude for the three regions was opposite, which indicated that the δD values in wheat decreased with the higher latitude, which was the same as the before results (Guo, Wei, Kelly, Pan, & Wei, 2009; Heaton, Kelly, Hoogewerff, & Woolfe, 2008). But the altitude effect of δD values was not very clear, the reason may be that the altitude differences of three samples regions are not enough for the δD values change, or maybe other factors influenced the variation. In addition, significant differences of δD values in wheat were also found over different years, that was because δD values were closely related with precipitation and temperature (Anderson, 2011). However, the precipitation varies with different seasons, and shifty temperature amongst different years will also affect the process of rainfall, as a result of different δD values.

5. Conclusion

The light isotopes have been employed in identifying the geographic origin of foodstuff for many decades but are unsteady owing to their interannual changing and close relation to plant growth. In our study, the dominant role of region was first confirmed by the contribution rate when compared with genotype and harvest year, indicating that the stable isotopic fingerprint is capable of representing the geographical information and creates a unique fingerprint for wheat. This could provide a powerful theoretical basis for geographical traceability of wheat and other foodstuffs of botanical origin, even if mixed with different genotypes and different years.

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References

Anderson, K. A., & Smith, B. W. (2006). Effect of season and variety on the differentiation of geographic growing origin of pistachios by stable isotope profiling. *Journal of Agricultural and Food Chemistry, 54*, 1747–1752.

Anderson, M. A. (2011). *Isotopes. In encyclopedia of water science* (2nd ed.). New York: Taylor and Francis, p. 684–687.

Araus, J. L., Cabrera-Bosquet, L., Serret, M. D., Bort, J., & Nieto-Taladriz, M. T. (2013). Comparative performance of $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ for phenotyping durum wheat adaptation to a dryland environment. *Functional Plant Biology, 40*, 595.

Bateman, A. S., & Kelly, S. D. (2007). Fertilizer nitrogen isotope signatures. *Isotopes in Environmental and Health Studies, 43*(3), 237–247.

Brescia, M. A., Di Martino, G., Guillou, C., Reniero, F., Sacco, A., & Serra, F. (2002). Differentiation of the geographical origin of durum wheat semolina samples on the basis of isotopic composition. *Rapid Communications in Mass Spectrometry, 16*, 2286–2290.

Camin, F., Larcher, R., Nicolini, G., Bontempo, L., Bertoldi, D., Perini, M., et al. (2010). Isotopic and elemental data for tracing the origin of European olive oils. *Journal of Agricultural and Food Chemistry, 58*(1), 570–577.

Christoph, N., Rossmann, A., Schlicht, C., & Voerkelius, S. (2006). Wine authentication using stable isotope ratio analysis: Significance of geographic origin, climate, and viticultural parameters. In S. E. Ebeler, G. Takeoka, & P. R. Winterhalter (Eds.), *Authentication of food and wine* (Vol. 952, pp. 166–179). Washington, DC: American Chemical Society.

Craig, H. (1961). Isotopic variations in meteoric waters. *Science, 133*, 1702–1703.

Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H., & Tu, K. P. (2002). Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics, 33*, 507–559.

Ehtesham, E., Hayman, A. R., McComb, K. A., Van Hale, R., & Frew, R. D. (2013). Correlation of geographical location with stable isotope values of hydrogen and carbon of fatty acids from New Zealand milk and bulk milk power. *Journal of Agricultural and Food Chemistry, 61*, 8914–8923.

Farquhar, G. D., O'leary, M. H., & Berry, J. A. (1982). On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology, 9*, 121–137.

Garten, C. T. (1993). Variation in foliar 15N abundance and the availability of soil nitrogen on walker branch watershed. *Ecology, 74*, 2098–2113.

Guo, B. L., Wei, Y. M., Pan, J. R., & Li, Y. (2010). Stable C and N isotope ratio analysis for regional geographical traceability of cattle in China. *Food Chemistry, 118*, 915–920.

Guo, B. L., Wei, Y. M., Kelly, S. D., Pan, J. R., & Wei, S. (2009). Application of stable hydrogen isotope analysis in beef geographical origin traceability. *Chinese Journal of Analytical Chemistry, 37*, 1333–1336 (In Chinese).

Heaton, K., Kelly, S. D., Hoogewerff, J., & Woolfe, M. (2008). Verifying the geographical origin of beef: The application of multi-element isotope and trace element analysis. *Food Chemistry, 107*, 506–515.

Hobson, K. A., Wassenaar, L. I., Mila, B., Lovette, I., Dingle, C., & Smith, T. B. (2003). Stable isotopes as indicators of altitudinal distributions and movements in an Ecuadorean hummingbird community. *Oecologia, 136*, 302–308.

Kawasaki, A., Oda, H., & Hirata, T. (2002). Determination of strontium isotope ratio of brown rice for estimating its provenance. *Soil Science and Plant Nutrition, 48*, 635–640.

Kendall, C. (1998). Tracing nitrogen sources and cycling in catchments. In C. Kendall & J. J. McDonnell (Eds.), *Isotope tracers in catchment hydrology* (pp. 519–576). Elsevier: Amsterdam.

Korner, C., Farquhar, G. D., & Roksandic, Z. (1988). A global survey of carbon isotope discrimination in plants from high altitude. *Oecologia, 74*, 623–632.

Lin, Z. F., Peng, C. L., & Li, G. Z. (2001). Carbon isotope discrimination and water use efficiency in different soybean and wheat genotypes. *Acta Agronomica Sinica, 27*, 409–414 (In Chinese).

Liu, X., Guo, B., Wei, Y., Shi, J., & Sun, S. (2013). Stable isotope analysis of cattle tail hair: A potential tool for verifying the geographical origin of beef. *Food Chemistry, 140*, 135–140.

Marchionni, S., Braschi, E., Tommasini, S., Bollati, A., Cifelli, F., Mulinacci, N., et al. (2013). High-precision 87Sr/86Sr analyses in wines and their use as a geological fingerprint for tracing geographic provenance. *Journal of Agricultural and Food Chemistry, 61*, 6822–6831.

Ngugi, E. C. K., Galwey, N. W., & Austin, R. B. (1994). Genotype × environment interaction in carbon isotope discrimination and seed yield in cowpea (*Vigna unguiculata* L. Walp). *Euphytica, 73*, 213–224.

Osorio, M. T., Moloney, A. P., Schmidt, O., & Monahan, F. J. (2011). Multielement isotope analysis of bovine muscle for determination of international geographical origin of meat. *Journal of Agricultural and Food Chemistry, 59*(7), 3285–3294.

Rajabi, A., Ober, E. S., & Griffiths, H. (2009). Genotypic variation for water use efficiency, carbon isotope discrimination, and potential surrogate measures in sugar beet. *Field Crops Research, 112*(2–3), 172–181.

Rummel, S., Hoelzl, S., Horn, P., Rossmann, A., & Schlicht, C. (2010). The combination of stable isotope abundance ratios of H, C, N and S with 87Sr/86Sr for geographical origin assignment of orange juices. *Food Chemistry, 118*, 890–900.

Scampicchio, M., Mimmo, T., Capici, C., Huck, C., Innocente, N., Drusch, S., et al. (2012). Identification of milk origin and process-induced changes in milk by stable isotope ratio mass spectrometry. *Journal of Agricultural and Food Chemistry, 60*, 11268–11273.

Vallano, D. M., & Sparks, J. P. (2013). Foliar delta^{15}N is affected by foliar nitrogen uptake, soil nitrogen, and mycorrhizae along a nitrogen deposition gradient. *Oecologia, 172*, 47–58.

Yun, S.-I., & Ro, H.-M. (2008). Stable C and N Isotopes: A tool to interpret interacting environmental stresses on soil and plant. *Journal of Applied Biological Chemistry, 51*, 262–271.

Zia-Ul-Haq, M., Ahmad, S., Qayum, M., & Ercişli, S. (2013). Compositional studies and antioxidant potential of *Albizia lebbeck* (L.) Benth. *Turkish Journal of Biology, 37*(1), 25–32.