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Francisco Ayala, Griselda V. Fedrigo, Moises Burachik & Patricia V. Miranda

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Compositional equivalence of event IND-ØØ412-7 to non-transgenic wheat

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Abstract Wheat is the most widely grown cereal grain, occupying a significant portion of the total cultivated land. As drought is the major environmental stressor affecting crop production, yield maintenance under water deficit conditions appears as a highly desirable phenotype for crop improvement. The *HaHB4* (*Helianthus annuus* homeobox 4) gene from sunflower encodes for a transcription factor involved in tolerance to environmental stress. The introduction of *HaHB4* in wheat led to the development of event IND-ØØ412-7 (HB4® wheat), which displayed higher yield in production environments of low productivity potential. Compositional analysis of IND-ØØ412-7 wheat, including 41 nutrients and 2 anti-nutrients for grain and 10 nutrients in forage, was performed. Results of these studies indicated that IND-ØØ412-7 is compositionally equivalent to non-transgenic wheat.

Keywords Wheat · IND-ØØ412-7 · Food safety evaluation · Compositional analysis · Transgenic wheat

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F. Ayala · G. V. Fedrigo · M. Burachik · P. V. Miranda (✉)
Instituto de Agrobiotecnologia Rosario (INDEAR), Ocampo Bis 210, Rosario, Santa Fe, Argentina
e-mail: patricia.miranda@indear.com

P. V. Miranda
Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET), Buenos Aires, Argentina

Introduction

Wheat (*Triticum aestivum*) is the largest food crop in terms of area allocation (nearly 25% of global arable land) and is the second most produced cereal crop after maize (Velu and Singh 2013). With an estimated production of 760 million tons for the 2016–2017 season (FAO 2016), wheat accounts for 56% of the global coarse grain production. Wheat is the staple food for 35 percent of the world's population, and provides more calories and protein in the human's diet than any other crop (Curtis 2002; IDRC 2010).Based on 2013 production figures (711 million tons) it has been estimated that wheat production will need to increase by 60% to 110% if it is to meet the demand of a growing human population of more than 9.7 billion by 2050 (Ray et al. 2013; UN 2015). Although wheat productivity is improving at an annual rate of 1% (Velu and Singh 2013), a higher rate will be needed to supply meat and dairy to an increasing human population, as well as to meet the demand of the bio-fuel industry (Godfray et al. 2010; Ray et al. 2013). As the potential of increasing arable land is limited, future increases in wheat production must be achieved by

enhancing the productivity per unit area. Attention should therefore be given to drought, the most important environmental stress that limits crop productivity around the world. Low water availability at critic stages of crop development leads to great yield losses (Duque et al. 2013). Estimates indicate that 25% of the world's agricultural land (Jajarmi 2009) and up to 32% in developing countries (IDRC 2010) are now affected by high levels of water stress. In addition, it has been anticipated that water deficit, already a serious worldwide problem, is likely to increase as a consequence of climate change, further reducing arable land in rainfall-depended regions (Ahuja et al. 2010; Vergara et al. 2014; Elliott et al. 2014). Tolerance to drought stress is therefore a highly desired goal of wheat genetic improvement.

Plants have developed several response mechanisms to survive drought stress. A variety of genes are induced in the plant under conditions of environmental stress (Reynolds and Langridge 2016). The products of these genes are involved not only in stress tolerance (cells protection) but are also channeled into stress response pathways. Some of these genes have been tested in crop-breeding programs (Khan and Iqbal 2011; Mwadzingeni et al. 2016; Reynolds and Langridge 2016). Great efforts are being made to develop wheat varieties with drought tolerance through conventional breeding (Pfeiffer et al. 2005; Vinocur and Altman 2005; Witcombe et al. 2008; Gupta et al. 2012; Velu and Singh 2013; IWYP 2016). However, none of these approaches have reached the market yet, because drought tolerance is a complex trait that is controlled by many genes (Naeem et al. 2015). Against this complex background, it was found that the superior survival under severe drought is often associated with constitutive activation of water-saving mechanisms, such as stomatal closure, that can lead to growth penalty (Skirycz et al. 2011) or to the appearance of undesired phenotypic features (Yang et al. 2017). Adding to these difficulties, it must be realized that wheat has a structurally intricate and large genome.

Consequently, breeding for drought tolerance requires the integration of various knowledge systems and methodologies from multiple disciplines in plant sciences (Deikman et al. 2012; Budak et al. 2015; Mwadzingeni et al. 2016). New approaches have been sought (Shinozaki et al. 2003; Wang et al. 2003, 2016; Verma 2016), including high-throughput phenotyping (Saint Pierre et al. 2012), next generation sequencing and genetic engineering (Bhalla 2006).

Environmental stress tolerance involves signal transduction networks from perception of stress signals to stress-responsive gene expression, in which various transcription factors (TFs) and *cis*-acting elements in stress-responsive promoters function for plant adaptation (Yamaguchi-Shinozaki and Shinozaki 2006; Host et al. 2016). Therefore, the relevant TFs constitute likely targets for engineering crops for stress tolerance (Kasuga et al. 1999; Morran et al. 2011; Host et al. 2016; Wang et al. 2016). Members of the HD-Zip family of TFs, unique to plants, have been shown to be involved in regulating developmental processes associated with the response of plants to environmental stress (Schena and Davis 1992). In particular, expression of genes of the HD-Zip subfamily I is regulated by external factors such as drought, extreme temperatures, osmotic stresses and light conditions (Ariel et al. 2007; Chan 2009). When introduced in *Arabidopsis,* the *HaHB4* (*Helianthus annuus* homeobox 4) gene, a member of the HD-Zip sub-family I encoding for the sunflower TF HAHB4, provides increased tolerance to drought (Dezar et al. 2005). Similarly, the introduction of *HaHB4* gene in wheat led to the drought stress tolerance phenotype. Phenotypic and field performance selection of several *HaHB4*-containing lines allowed us to develop a transgenic wheat (termed IND-ØØ412-7 according with OECD Unique Identifier nomenclature), which was shown to provide an increased yield opportunity under conditions of environmental stress.

Field tests of event IND-ØØ412-7 across wheat-producing areas in Argentina have shown that yield gains were significant in production environments of low yield potential whereas HAHB4 expression caused no penalty in high yield potential areas, suggesting a tight environment-dependent regulation of the tolerance pathway regulated by HAHB4. The wheat event IND-ØØ412-7 also contains the *bar* gene from *Streptomyces hygroscopicus,* expressing the glufosinate-inactivating enzyme phosphinothricin N-acetyl transferase (PAT), which confers glufosinate herbicide tolerance.

From the food safety perspective, the use of HAHB4 in wheat event IND-ØØ412-7 results in several relevant favorable characteristics. First, the source of this protein (sunflower) has been in the food chain from a long time. Therefore, it has a history of

safe food use. Also, HAHB4 acts as a transcriptional regulator of normal endogenous pathways, that is, it relays on the natural physiology of the plant. Therefore, no metabolites other than the natural plant's ones are expressed in the transgenic event. Finally, being a TF, HAHB4 is expressed at extremely low levels which, added to the safety of the source, makes its presence in foods of no safety concern. The PAT protein, also expressed in HB4 wheat can be also considered as having a history of safe food use as it has been introduced in many crops [233 edible crops, as of May 30, 2018 (ISAAA 2018)] and consumed since the very beginning of crop genetic engineering technology.

The assessment of the compositional equivalence between genetically modified crops and their non-modified counterparts is considered the keystone of the food/feed safety evaluation of genetically modified crops (Kuiper et al. 2001; Privalle et al. 2013). Here, the results of the compositional assessment of wheat event IND-ØØ412-7 with and without glufosinate treatment are presented.

Materials and methods

Field trials and samples

Field trials were conducted in Argentina during three different growing seasons (2012, 2013 and 2015), at nine locations representing the environmental diversity over the range of the wheat producing regions. Field sites were distributed among three Provinces: Buenos Aires (Villa Saboya, Carmen de Areco, Daireaux, Balcarce and Pergamino), Cordoba (Monte Buey and Corral de Bustos) and Santa Fe (Landeta and Roldan). A randomized complete block design with four replicates was used in the trials. Samples were collected from the transgenic event IND-ØØ412-7, the parent non-transgenic variety Cadenza (SASA 1993) as the near-isogenic comparator, and from five commercial reference varieties with desirable characteristics, currently in use in each region. The reference varieties were grown together to provide the range of natural variability of the crop, thereby giving the appropriate context for the interpretation of the experimental results in terms of the biological significance. They include Biointa 1005, 3005 and 3006 (Bioceres Semillas), SY100 (Buck Semillas), Baguette 30 and 601 (Nidera Semillas) and Nogal (Sursem). Grain samples were collected at maturity (2012 and 2015 trials), and forage samples were taken at tillering (2013 and 2015 trials).

Statistical analysis

Results for each analyte were expressed as the value of the mean plus/minus the standard error of the mean. The occurrence of statistically significant differences between event IND-ØØ412-7 and the non-transgenic control line were analyzed using a two-way ANOVA and the Least Significant Difference post-test using the InfoStat software (http://www.infostat.com.ar). Values from IND-ØØ412-7 and Cadenza were compared in search for statistical differences at a 5% level of significance ($\alpha = 0.05$) across all sites (combined-site analysis).

Analytical methods

Compositional analysis were performed according with OECD Consensus Document recommendations (OECD 2003). Nutrients and micronutrients measured in grain (total 41 analytes) included proximates (moisture, protein, fat, ash, and carbohydrates), starch, dietary fiber, minerals (calcium, iron, phosphorous, selenium and zinc), fatty acids and amino acids profiles and vitamins (thiamine, riboflavin, niacin, pyridoxine, folic acid and α-tocopherol). Two anti-nutrients were measured in grain: phytic acid and gliadin. Nutrients measured in forage (total 10 analytes) included proximates, acid detergent fiber (ADF), neutral detergent fiber (NDF), dietary fiber (DF) and minerals (phosphorous and calcium).

Proximate analysis

Moisture was determined in grain or forage as the weight loss of samples heated for 72 h at 130 °C (AACC Method 44-15.02). For the determination of ash content, samples were incinerated in an oven at 585 °C until a constant weight (AOAC Method 923.03). Total protein nitrogen was determined through Kjeldahl analysis by digesting the sample in sulfuric acid-copper catalyst mixture. The percent nitrogen was determined and converted to equivalent protein using a factor of 6.25 (AACC Method 46-11A). Total fat was determined by Soxhlet extraction

with diethyl ether (AACC Method 30-20.01). Carbohydrates were calculated from the proximate analysis, as the difference in percent weight using the following equation:

$$\%\ \text{carbohydrates} = 100\% - (\%\ \text{protein} + \%\text{fat} + \%\text{moisture} + \%\text{ash}).$$

Acid detergent fiber (ADF)

An acidified quaternary detergent solution was used to dissolve cell solubles, hemicellulose and soluble minerals leaving a residue of cellulose, lignin, damaged protein and a portion of cell wall protein and minerals (ash). ADF was determined gravimetrically as the residue remaining after extraction with acetone (AOAC Method 973.18).

Neutral detergent fiber (NDF)

Samples were defatted by Soxhlet extraction and boiled in a neutral sodium lauryl sulphate buffered solution. The residue obtained after boiling was washed and dried. NDF was calculated as the weight loss after incineration of the washed and dried residue (Van Soest et al. 1991; FAO 2011).

Total dietary fiber (DF)

Duplicated samples were suspended in buffer and sequentially digested with heat stable α-amylase, protease and amyloglucosidase to remove starch and protein. The digested sample was treated with ethanol to precipitate soluble dietary fiber. The resulting suspension was filtered, and the residue was washed sequentially with ethanol and acetone, dried and weighed (Residue Weight = R). One of the duplicates was used to determine protein (P) and the second duplicate was used to determine ash (A) as previously described. Total dietary fiber (DF) was calculated by subtracting protein and ash contents to the Residue Weight: DF = R − P − A (Method AOAC 991.43).

Fatty acids profile

Fat and fatty acids were extracted and saponified by alkaline hydrolysis. Pyrogallic acid was added to minimize oxidative degradation of fatty acids. Triglyceride, tri-undecanoin (C11:0), was added as internal standard. Fat was extracted into ether and methylated to fatty acid methyl esters (FAMEs) using boron trifluoride in methanol. FAMEs were quantitatively measured by capillary gas chromatography (GC) against the C11:0 internal standard (AOAC Method 996.06).

Amino acids profile

The amino acids profile was determined after protein hydrolysis with 6 N hydrochloric acid at 100 °C for 24 h. Amino acids in the hydrolysate were derivatized in borate buffer with fluorenyl-methyl-oxycarbonylchloride (FMOC-Cl) for proline and orthophthalaldehyde/mercaptoethanol (OPA/ME) for the other amino acids. The amino acids were isolated and quantified using HPLC with a fluorescence detector and acetonitrile/water as organic and aqueous phase, respectively (Zhou et al. 2011).

Starch

Ground grain sample was extracted with 80% ethanol and the pellet recovered after centrifugation was hydrolyzed to maltodextrins with thermostable α-amylase at 95–100 °C. Maltodextrins were then hydrolyzed to glucose with amyloglucosidase. Glucose was determined with glucose oxidase–peroxidase reagent and spectrophotometric measurement at 540 nm (AOAC Method 996.11).

Minerals

Following conversion of the material into ash in a 500 °C oven, the residues were dissolved in nitric acid and analyzed by Inductively Coupled Plasma (ICP) emission spectroscopy. The concentration of each mineral was determined by reading at 3179 Å (calcium), 2149 Å (phosphorous), 2383 Å (iron), 2138 Å (zinc) and 1960 Å (selenium) (AOAC Method 985.01).

Group B vitamins

Vitamins were released from the sample matrix by acid or enzymatic (amylase and papain) hydrolysis (for thiamin, riboflavin, pyridoxine and folic acid) or both (for niacin). After purification, the hydrolysate

Table 1 Proximates, starch, fiber, minerals and vitamins of grain from drought tolerant IND-ØØ412-7 wheat

Component[a]	IND-ØØ412-7 mean (SE) (range)	Cadenza mean (SE) (range)	Commercial references range[b]	Literature range[c]
Ash	2.37 (0.09) (1.37–2.90)	2.32 (0.07) (1.69–2.79)	1.91–2.09	1.2–3.0
Carbohydrates	65.4 (0.0) (62.5–70.2)	65.8 (0.48) (63.0–70.3)	65.4–67.5	65.4–78.0
Moisture	13.09 (0.12) (12.14–14.75)	12.99 (0.16) (11.83–14.63)	13.99–14.30	8.0–18.0
Protein	16.2 (0.4) (12.3–18.4)	15.9 (0.3) (13.1–18.7)	14.2–15.2	10.0–16.0
Total fat	2.3 (0.0) (1.8–2.6)	2.2 (0.1) (1.6–2.7)	2.1–2.3	1.5–2.0
Starch	63.7 (0.5) (60.8–68.6)	63.7 (0.4) (61.1–69.3)	63.6–66.0	59–72
Dietary fiber	13.8 (0.2) (12.0–15.5)	13.9 (0.2) (11.6–16.0)	14.0–15.3	11.0–14.6
Calcium	461 (12) (373–573)	458 (12) (374–548)	441–501	250–538[d]
Iron	49 (2) (31–65)	50 (2) (30–76)	38–43	33–79[d]
Phosphorus	4912 (167) (3194–6146)	4961 (160) (3466–6061)	3970–4534	3320–5160[d]
Selenium	0.55 (0.03) (0.35–0.78)	0.55 (0.03) (0.37–0.82)	0.53–0.58	0.04–0.71[d]
Zinc	42 (2)* (22–63)	46 (2) (28–56)	32–35	24–47[d]
Thiamine	4.0 (0.1) (3.1–4.7)	4.1 (0.1) (3.2–5.0)	4.0–4.3	1.3–9.9
Riboflavin	0.43 (0.03) (0.25–0.81)	0.40 (0.02) (0.25–0.62)	0.48–0.66	0.6–3.1
Niacin	60.4 (2.2) (45.7–83.8)	58.8 (1.8) (46.7–80.8)	57.9–68.0	22.0–111.0
Pyridoxine	4.0 (0.1) (3.3–4.9)	4.1 (0.1) (3.3–4.8)	3.9–4.2	0.9–7.9
Folic acid	0.29 (0.01)* (0.17–0.38)	0.31 (0.01) (0.16–0.40)	0.27–0.33	0.2–0.9
α-Tocopherol	10.7 (0.4) (6.5–14.0)	10.6 (0.3) (7.7–13.7)	8.4–9.5	9–18

Numbers represent mean of 24 values measured in samples from field trials developed during 2012 in six different locations (four replicates)

[a]Results are expressed as % dry weight, except for moisture (% fresh weight) and minerals (ppm dry weight)

[b]Values measured in commercial varieties grown in the same trials

[c]OECD (2003) unless otherwise indicated

[d]Obert et al. (2004); SE: standard error of the mean

*Significant difference ($p < 0.05$)

was analyzed by HPLC, after derivatization (for thiamin and folic acid). Detection was carried out by fluorescence (for thiamin, riboflavin, pyridoxine and folic acid) or with a photodiode array (for niacin) (Method NOM-131-SSA1-1995).

Vitamin E

Oil from wheat grains was recovered by Soxhlet extraction using hexane supplemented with 0.1% butylated hydroxytoluene (BHT) to prevent oxidation. Alpha-tocopherol was quantified by HPLC with fluorescence detection (excitation 290 nm, emission 330 nm) (AACC Method 86-06.01).

Phytic acid

Ground grains were extracted with 3% trichloroacetic acid. Phytate was precipitated as the ferric salt by addition of ferric chloride solution. The precipitate was dissolved in nitric acid and iron measured by colorimetric determination at 480 nm after potassium thiocyanate addition. Phytate concentration was calculated assuming a 4 Fe: 6 P molecular ratio (Wheeler and Ferrel 1971).

Gliadin

Ground grain samples were extracted with water at 40 °C. Prolamins (gliadins) were then selectively solubilized with 80% ethanol (1 h at room temperature). After centrifugation, gliadins were measured in the supernatant with a monoclonal antibody–based ELISA (AACC, Method 38.50.01).

Results

Among the nutrients measured in grain including proximates, starch, dietary fiber, five minerals, and six vitamins, no significant differences were found in the levels of all but two components when IND-ØØ412-7 wheat was compared to its non-transgenic parental line Cadenza (Table 1). The two components showing differences were zinc and folic acid and, in both cases, the levels measured in the transgenic wheat were slightly below the control (Table 1). However, values measured in IND-ØØ412-7 wheat were within the range displayed by the local reference varieties (folic acid), and/or within those reported in literature (folic acid and zinc, respectively).

Table 2 Fatty acid profile of grain from drought tolerant IND-ØØ412-7 wheat

Component[a]	IND-ØØ412-7 mean (SE) (range)	Cadenza mean (SE) (range)	Commercial references range[b]	Literature range[c]
Palmitic acid	16.5 (0.2) (15.0–19.0)	16.2 (0.2) (15.0–17.7)	17.4–18.9	11–32
Stearic acid	1.7 (0.2)* (1.0–3.7)	1.4 (0.1) (1.0–2.9)	1.9–2.1	0–4.6
Oleic acid	20.4 (0.5)* (17.8–24.3)	19.4 (0.3) (16.0–21.4)	15.8–18.7	11–29
Linoleic acid	56.8 (0.6)* (51.4–59.8)	58.5 (0.2) (56.4–60.7)	56.6–59.1	44–74
Linolenic acid	3.6 (0.1) (2.9–4.3)	3.6 (0.1) (3.1–4.2)	3.5–4.0	0.7–4.4

Numbers represent mean of 24 values measured in samples from field trials developed during 2012 in six different locations (four replicates)

[a]Results are expressed as % of total fatty acids

[b]Values measured in commercial varieties grown in the same trials

[c]OECD (2003); SE: standard error of the mean

*Significant difference ($p < 0.05$)

Table 3 Amino acid composition of grain from drought tolerant IND-ØØ412-7 wheat

Component[a]	IND-ØØ412-7 mean (SE) (range)	Cadenza mean (SE) (range)	Commercial references range[b]	Literature range[c]
Alanine	3.42 (0.04) (3.04–3.85)	3.48 (0.05) (3.19–4.33)	3.22–3.51	3.4–3.7
Arginine	4.27 (0.04) (3.85–4.90)	4.21 (0.03) (4.03–4.58)	4.03–4.29	4.0–5.7
Aspartic acid	5.08 (0.06) (4.32–5.66)	5.06 (0.03) (4.68–5.29)	5.01–5.13	4.8–5.6
Cysteine	2.60 (0.04) (2.40–3.09)	2.53 (0.02) (2.20–2.91)	2.50–2.59	1.7–2.7
Glycine	3.42 (0.03) (3.08–3.92)	3.41 (0.03) (3.17–3.94)	3.30–3.50	3.8–6.1
Glutamic acid	27.43 (0.28) (24.92–29.58)	27.47 (0.36) (24.89–32.54)	26.25–27.86	29.9–34.8
Histidine	2.57 (0.04) (2.21–2.99)	2.54 (0.02) (2.20–2.84)	2.48–2.58	2.0–2.8
Isoleucine	3.39 (0.04) (2.79–3.81)	3.39 (0.03) (3.10–3.77)	3.27–3.45	3.0–4.3
Leucine	6.61 (0.09) (5.44–7.46)	6.72 (0.08) (5.71–7.71)	6.66–6.85	5.0–7.3
Lysine	2.58 (0.03) (2.34–3.07)	2.56 (0.03) (2.20–2.93)	2.48–2.58	2.2–3.0
Methionine	1.65 (0.02) (1.40–1.78)	1.70 (0.03) (1.42–1.98)	1.63–1.77	1.3–1.7
Phenylalanine	4.25 (0.03) (3.90–4.51)	4.21 (0.03) (3.74–4.56)	4.22–4.29	3.5–5.4
Proline	8.60 (0.06) (8.14–9.43)	8.50 (0.08) (7.34–9.29)	8.36–8.62	9.8–11.6
Serine	3.45 (0.04)* (2.93–4.05)	3.33 (0.03) (2.94–3.55)	3.25–3.43	4.3–5.7
Threonine	2.58 (0.02)* (2.49–2.90)	2.48 (0.02) (2.20–2.61)	2.43–2.58	2.4–3.2
Tryptophan	1.71 (0.02) (1.49–1.93)	1.69 (0.03) (1.39–2.05)	1.63–1.73	1.0–2.1
Tyrosine	2.55 (0.02) (2.29–2.80)	2.52 (0.03) (2.14–2.89)	2.40–2.58	1.8–3.7
Valine	4.25 (0.03) (3.88–4.51)	4.26 (0.04) (3.83–4.71)	4.10–4.29	4.4–4.8

Numbers represent mean of 24 values measured in samples from field trials developed during 2012 in six different locations (four replicates)

[a]Results are expressed as % of total protein

[b]Values measured in commercial varieties grown in the same trials

[c]OECD (2003); SE: standard error of the mean

*Significant difference ($p < 0.05$)

Three of the five fatty acids included in the analysis showed statistically significant differences when levels measured in IND-ØØ412-7 wheat were compared to those found in the parental line (Table 2). For linoleic acid, the major fatty acid in wheat, values were within both the range of values for the reference varieties and those reported in the literature. In the other two cases (stearic and oleic acids), the content in the transgenic event was greater than in Cadenza (Table 2), with both showing values that were outside the range of the local reference varieties, but still within those reported in the literature (Table 2).

The analysis of the amino acids levels revealed that serine and threonine contents were statistically higher in IND-ØØ412-7 wheat when compared to the parental control Cadenza (Table 3). While the threonine content was within the range of the local reference varieties, the serine level was slightly above it, but with a difference too small to be of biological significance.

Levels of the anti-nutrients gliadin and phytic acid did not show significant differences between the transgenic event IND-ØØ412-7 and the parental control line Cadenza (Table 4).

A compositional analysis was also performed with wheat materials taken from field trials involving glufosinate treatment, as IND-ØØ412-7 displays tolerance to this herbicide. Overall, the analysis of grain obtained from herbicide-treated plants also showed a picture of compositional equivalence to the parental control. Values of the contents of proximates, starch, fiber, minerals, and vitamins (Online Resource 1) revealed that only two components (total protein and zinc) exhibited significant differences when IND-ØØ412-7 wheat was compared to Cadenza. For both of these analytes, levels were lower in the event when plants were treated with the herbicide (Online Resource 1). In these two instances, however, the values obtained for the transgenic event, whether or not treated with herbicide, were within the range provided by both the local commercial varieties and the literature (Online Resource 1). No statistically significant differences between the wheat event IND-ØØ412-7 and the parental control Cadenza were found in the fatty acids (Online Resource 2) or amino acids (Online Resource 3) profiles, or in the anti-nutrients content (Online Resource 4), with the only exception of leucine. The level of this amino acid was higher in the transgenic event with no herbicide treatment. However, the value measured in IND-ØØ412-7 wheat was within the range provided by the reference varieties and the one reported in the literature (Online Resource 3).

Concerning forage nutrients, three parameters showed statistically significant differences when the levels measured in IND-ØØ412-7 wheat were compared to the parental control (Table 5). The carbohydrate content was greater in the transgenic but fell within the range provided by the reference varieties. Moisture, on the other hand, was slightly below the reference range, but the difference was too small to be considered of biological significance. The third component showing a significant difference was calcium, whose content in the event was lower than the one measured in Cadenza. However, the level found in the

Table 4 Anti-nutrients composition of grain from drought tolerant IND-ØØ412-7 wheat

Component[a]	IND-ØØ412-7 mean (SE) (range)	Cadenza mean (SE) (range)	Commercial references range[b]	Literature range
Phytic acid	1.5 (0.1) (1.0–1.9)	1.5 (0.1) (1.0–2.0)	1.3–1.5	0.5–0.9[c]
Gliadin	6.9 (0.2) (5.1–8.8)	7.1 (0.2) (5.7–8.5)	5.9–6.8	3.9–9.1[d]

Numbers represent mean of 24 values measured in samples from field trials developed during 2012 in six different locations (four replicates)

[a]Results are expressed as % of dry weight

[b]Values measured in commercial varieties grown in the same trials

[c] Obert et al. (2004)

[d]Huebner and Rothfus (1968); SE: standard error of the mean

Table 5 Proximates, fiber and minerals of forage from drought tolerant IND-ØØ412-7 wheat

Component[a]	IND-ØØ412-7 mean (SE) (range)	Cadenza mean (SE) (range)	Commercial references range[b]	Literature range[c]
Ash	11.24 (0.26) (11.04–11.45)	11.64 (0.20) (11.34–11.95)	11.27–12.79	NA
Carbohydrates	48.4 (1.4)* (47.6–49.2)	46.7 (1.48) (45.8–47.8)	45.6–49.6	NA
Moisture	81.32 (0.24)* (81.13–81.71)	81.89 (0.27) (81.54–82.16)	81.56–82.51	NA
Total Protein	22.2 (1.0) (21.5–22.7)	22.3 (0.9) (21.8–23.2)	21.6–23.7	22.5–30.9
Total Fat	2.7 (0.1) (2.6–2.7)	2.7 (0.1) (2.5–2.9)	2.4–2.9	NA
ADF	23.8 (0.6) (23.5–23.9)	24.1 (0.6) (23.7–24.6)	23.1–24.8	25.1–40.3
NDF	50.6 (0.7) (48.9–52.5)	49.1 (0.9) (46.7–50.5)	41.1–46.9	46.1–63.8
Dietary fiber	16.3 (0.5) (15.4–17.2)	16.8 (0.5) (16.1–17.1)	14.7–15.2	NA
Calcium	0.35 (0.01)* (0.32–0.37)	0.38 (0.01) (0.37–0.39)	0.33–0.37	0.24
Phosphorus	0.29 (0.02) (0.28–0.30)	0.30 (0.02) (0.29–0.31)	0.27–0.30	0.35

Numbers represent mean of 24 values measured in samples from field trials developed during 2013 in six different locations (four replicates)

[a]Results are expressed as % of dry weight except for moisture (% fresh weight) and minerals (ppm dry weight)

[b]Values measured in commercial varieties grown in the same trials

[c]Obert et al. (2004). SE: standard error of the mean, ADF: acid detergent fiber, NDF: neutral detergent fiber, NA: not available

transgenic wheat was within the range of the reference varieties (Table 5).

In the analysis of forage from the glufosinate-treated wheat, only one significant difference was found between the event and its control parental line (Online Resource 5). The ash content was significantly lower in IND-ØØ412-7 wheat under both treatments (with or without glufosinate) when compared to the parental non-transgenic line. However, values measured in the event were within the reference range provided by the commercial varieties for both, the herbicide-treated and untreated plants.

Discussion

Results of the comparative compositional analysis show that most of the nutrient, micronutrient and anti-nutrient levels measured in grain (43 analytes) and the nutrient levels in forage (10 analytes) from the transgenic wheat event IND-ØØ412-7 were similar to those found in the parental non-transgenic control Cadenza. In the few occasions in which significant differences were found between the transgenic event and the control, levels measured in the event were within the range provided by the local commercial reference varieties planted in the same locations, and/or the values reported in the literature.

Compositional equivalence was also confirmed by the analysis of materials obtained from plants treated

with glufosinate herbicide. In this specific analysis the differences found were few and not for the same analytes as in the study without herbicide treatment. This lack of consistency suggests a chance nature of the differences observed, what was also supported by the statistical analysis at each individual site (data not shown).

Occasionally, the values of some of the few parameters showing different levels between the event and the parental line were both outside the range of values of the commercial reference varieties but still within the literature range, suggesting an effect of their common genetic background. This was the case for zinc, stearic and oleic acids. For the fatty acids, variations may be expected as they may arise from adaptive strategies for seed survival and seedling establishment under contrasting local conditions [see, for example, Zhang et al. (2015)]: lower stearic acid and higher oleic acid may lead to a net increase in fatty acid unsaturation which is consistent with the origin's colder climate (Scotland) to which plants with the genetic background common to both IND-ØØ412-7 and the parent variety (Cadenza) would be exposed.

An appropriate context for the interpretation of the comparative compositional assessment should consider the relative magnitude of the differences in the mean values of the components, the range of natural variability defined by the reference varieties grown in the test sites, the reproducibility (consistency) of the statistically significant differences across individual sites, and the range of values published in the literature. When analyzed within this context, the results presented here support the compositional equivalence of the transgenic event IND-ØØ412-7 with conventional wheat.

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Compliance with ethical standards

Conflict of interest All the authors are affiliated to INDEAR, the R&D area of Bioceres, working for Trigall Genetics in the development of the transgenic event involved in this study.

References

Ahuja I, de Vos RCH, Bones AM, Hall RD (2010) Plant molecular stress responses face climate change. Trends Plant Sci 15:664–674. https://doi.org/10.1016/j.tplants.2010.08.002

Ariel FD, Manavella PA, Dezar CA, Chan RL (2007) The true story of the HD-Zip family. Trends Plant Sci 12:419–426. https://doi.org/10.1016/j.tplants.2007.08.003

Bhalla PL (2006) Genetic engineering of wheat–current challenges and opportunities. Trends Biotechnol 24:305–311. https://doi.org/10.1016/j.tibtech.2006.04.008

Budak H, Hussain B, Khan Z et al (2015) From genetics to functional genomics: improvement in drought signaling and tolerance in wheat. Front Plant Sci 6:1012. https://doi.org/10.3389/fpls.2015.01012

Chan RL (2009) The use of sunflower transcription factors as biotechnological tools to improve yield and stress tolerance in crops. Phys Int J Exp Bot 78:5–10

Curtis B (2002) Wheat in the world. In: Curtis B, Rajaram S, Gómez Macpherson H (eds) Bread wheat. Improvement and Production. Food and Agricultural Organization of the United Nations, Rome, p 544

Deikman J, Petracek M, Heard JE (2012) Drought tolerance through biotechnology: improving translation from the laboratory to farmers' fields. Curr Opin Biotechnol 23:243–250. https://doi.org/10.1016/j.copbio.2011.11.003

Dezar CA, Gago GM, Gonzalez DH, Chan RL (2005) Hahb-4, a sunflower homeobox-leucine zipper gene, is a developmental regulator and confers drought tolerance to Arabidopsis thaliana plants. Transgenic Res 14:429–440

Duque AS, de Almeida AM, Bernardes da Silva A et al (2013) Abiotic stress responses in plants: unraveling the complexity of genes and networks to survive. In: Vahdati K, Leslie C (eds) Abiotic stress—plant responses and applications in agriculture. InTech, Rijeka, p 418

Elliott J, Deryng D, Müller C et al (2014) Constraints and potentials of future irrigation water availability on agricultural production under climate change. Proc Natl Acad Sci USA 111:3239–3244. https://doi.org/10.1073/pnas.1222474110

FAO (2011) Quality assurance for animal feed analysis laboratories. FAO Animal Production and Health Manual no. 14. Rome, Italy

FAO (2016) http://www.fao.org/worldfoodsituation/csdb/en/. Accessed 17 May 2017

Godfray HCJ, Beddington JR, Crute IR et al (2010) Food security: the challenge of feeding 9 billion people. Science 327:812–818. https://doi.org/10.1126/science.1185383

Gupta PK, Balyan HS, Gahlaut V, Kulwal PL (2012) Phenotyping, genetic dissection, and breeding for drought and

heat tolerance in common wheat: status and prospects. In: Janick J (ed) Plant breeding reviews. Wiley, Hoboken, pp 85–168

Host R, David A, Ceasar SA et al (2016) Genetic engineering of crop plants for drought tolerance: role of transcription factors. South Indian J Biol Sci 2:272–286

Huebner FR, Rothfus JA (1968) Gliadin proteins from different varieties of wheat. Cereal Chem 45:242–253

IDRC (2010) Facts & figures on food and biodiversity. IDRC - International Development Research Centre. https://www.idrc.ca/en/article/facts-figures-food-and-biodiversity. Accessed 25 May 2018

ISAAA (2018) GM events with glufosinate herbicide tolerance. GM Approval Database-ISAAA.org. In: International Service for the Acquisition of Agri-biotech Applications. http://www.isaaa.org/gmapprovaldatabase/gmtrait/default.asp?TraitID=1&GMTrait=Glufosinateherbicidetolerance. Accessed 30 May 2018

IWYP (2016) International wheat yield partnership. International Wheat Yield Partnership. http://iwyp.org/. Accessed 25 May 2018

Jajarmi V (2009) Effect of water stress on germination indices in seven wheat cultivar. World Acad Sci Eng Technol 49:105–106

Kasuga M, Liu Q, Miura S et al (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat Biotechnol 17:287–291. https://doi.org/10.1038/7036

Khan MA, Iqbal M (2011) Breeding for drought tolerance in wheat (*Triticum aestivum* L.): constraints and future prospects. Front Agric China 5:31–34. https://doi.org/10.1007/s11703-010-1054-2

Kuiper HA, Kleter GA, Noteborn HP, Kok EJ (2001) Assessment of the food safety issues related to genetically modified foods. Plant J 27:503–528

Morran S, Eini O, Pyvovarenko T et al (2011) Improvement of stress tolerance of wheat and barley by modulation of expression of DREB/CBF factors. Plant Biotechnol J 9:230–249. https://doi.org/10.1111/j.1467-7652.2010.00547.x

Mwadzingeni L, Shimelis H, Dube E et al (2016) Breeding wheat for drought tolerance: progress and technologies. J Integr Agric 15:935–943. https://doi.org/10.1016/S2095-3119(15)61102-9

Naeem MK, Ahmad M, Kamran M et al (2015) Physiological responses of wheat (*Triticum aestivum* L.) to drought stress. Int J Plant Soil Sci Greece Rev 6:1–9. https://doi.org/10.9734/IJPSS/2015/9587

Obert JC, Ridley WP, Schneider RP, Riordan SG, Nemeth MA, Trujillo WA et al (2004) The composition of grain and forage from glyphosate tolerant wheat MON 71800 is equivalent to that of conventional wheat (*Triticum aestivum L.*). J Agric Food Chem 52:1375–1384

OECD (2003) Consensus document on compositional considerations for new varieties of bread wheat (*Triticum aestivum*): key food and feed nutrients, anti-nutrients and toxicants. ENV/JM/MONO(2003). Series on harmonization of regulatory oversight in biotechnology. Environment Directorate. Organisation for Economic Co-operation and Development. Paris, France. Available at: https://www.oecd.org/env/ehs/biotrack/46815206.pdf

Pfeiffer W, Trethowan R, van Ginkel M et al (2005) Breeding for abiotic stress tolerance in wheat. In: Ashraf M, Harris PJC (eds) Abiotic stresses: plant resistance through breeding and molecular approaches. Food Products Press, New York, p 725

Privalle LS, Gillikin N, Wandelt C (2013) Bringing a transgenic crop to market: where compositional analysis fits. J Agric Food Chem 61:8260–8266. https://doi.org/10.1021/jf400185q

Ray DK, Mueller ND, West PC, Foley JA (2013) Yield trends are insufficient to double global crop production by 2050. PLoS ONE 8:e66428. https://doi.org/10.1371/journal.pone.0066428

Reynolds M, Langridge P (2016) Physiological breeding. Curr Opin Plant Biol 31:162–171. https://doi.org/10.1016/j.pbi.2016.04.005

Saint Pierre C, Crossa JL, Bonnett D et al (2012) Phenotyping transgenic wheat for drought resistance. J Exp Bot 63:1799–1808. https://doi.org/10.1093/jxb/err385

SASA (1993) The scotish wheat variety database. http://wheat.agricrops.org/varieties/view/Cadenza. Accessed 25 May 2018

Schena M, Davis RW (1992) HD-Zip proteins: members of an Arabidopsis homeodomain protein superfamily. Proc Natl Acad Sci USA 89:3894–3898

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 6:410–417

Skirycz A, Vandenbroucke K, Clauw P et al (2011) Survival and growth of Arabidopsis plants given limited water are not equal. Nat Biotechnol 29:212–214. https://doi.org/10.1038/nbt.1800

UN (2015) World population prospects: the 2015 revision. Key Findings and Advance Tables, New York

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Velu G, Singh RP (2013) Phenotyping in wheat breeding. In: Panguluri SK, Kumar AA (eds) Phenotyping for plant breeding: applications of phenotyping methods for crop improvement. Springer, New York, p 211

Vergara W, Rios AR, Trapido P, Malarín H (2014) Agriculture and future climate in Latin America and the Caribbean: systemic impacts and potential responses. Inter-American Development Bank. Climate Change and Sustainability Division. Environment, Rural Development, Disaster Risk Management Division. Discussion Paper, No. IDB-DP-329. Available at: https://www.researchgate.net/publication/265368765_Agriculture_and_Future_Climate_in_Latin_America_and_the_Caribbean_Systemic_Impacts_and_Potential_Responses

Verma AK (2016) Abiotic stress and crop improvement: current scenario. Adv Plants Agric Res 4:1–2. https://doi.org/10.15406/apar.2016.04.00149

Vinocur B, Altman A (2005) Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr Opin Biotechnol 16:123–132. https://doi.org/10.1016/j.copbio.2005.02.001

Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218:1–14. https://doi.org/10.1007/s00425-003-1105-5

Wang H, Wang H, Shao H, Tang X (2016) Recent advances in utilizing transcription factors to improve plant abiotic stress tolerance by transgenic technology. Front Plant Sci 7:67. https://doi.org/10.3389/fpls.2016.00067

Wheeler EL, Ferrel RE (1971) A method for phytic acid determination in wheat and wheat fractions. Cereal Chem 48:312–320

Witcombe JR, Hollington PA, Howarth CJ et al (2008) Breeding for abiotic stresses for sustainable agriculture. Philos Trans R Soc Lond B Biol Sci 363:703–716. https://doi.org/10.1098/rstb.2007.2179

Yamaguchi-Shinozaki K, Shinozaki K (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu Rev Plant Biol 57:781–803. https://doi.org/10.1146/annurev.arplant.57.032905.105444

Yang Y, Luang S, Harris J et al (2017) Overexpression of the class I homeodomain transcription factor TaHDZipI-5 increases drought and frost tolerance in transgenic wheat. Plant Biotechnol J 16:1227–1240. https://doi.org/10.1111/pbi.12865

Zhang J-L, Zhang S-B, Zhang Y-P, Kitajima K (2015) Effects of phylogeny and climate on seed oil fatty acid composition across 747 plant species in China. Ind Crops Prod 63:1–8. https://doi.org/10.1016/J.INDCROP.2014.10.045

Zhou W, Zhanga X-Y, Duana G-L (2011) Liquid-chromatography quantitative analysis of 20 amino acids after derivatization with FMOC-Cl and its application to different origin radix isatidis. J Chin Chem Soc 58:509–515. https://doi.org/10.1002/jccs.201190014