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MS INTA 416: A new Argentinean wheat cultivar carrying Fhb1 and Lr47 resistance genes

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Abstract: *MS INTA 416 is a hard red winter wheat selected for high yield potential and good bread-making quality, combined with moderate resistance to Fusarium-head-blight and high resistance to leaf-rust, due mainly to presence of resistance genes Fhb1 and Lr47. MS INTA 416 is adapted to main production areas of Central-Argentina.*

Key words*: Backcross, marker-assisted selection, yield, bread-making quality, disease control.*

INTRODUCTION

The diseases Fusarium Head Blight (FHB), caused by *Fusarium graminearum* and leaf rust, caused by *Puccinia triticina,* are widespread and devastating for bread wheat production in the Southern cone of South America (Germán et al. 2007, Bainotti et al. 2013). Damage caused by FHB includes reductions in yield and seed quality, and toxin contamination by deoxynivalenol, threatening human health. Every year, according to the climatic conditions and the area in which susceptible cultivars are planted, leaf rust causes widespread epidemics if no chemical control is applied. Host resistance is considered an efficient and eco-friendly way to manage both diseases; however, progress in breeding for FHB resistance has been limited by the complex inheritance of the partial resistance currently available in wheat. Resistance to the spread of the disease within a spike (Type II resistance) is considered a stable form of FHB resistance, and one of the worldwide best known and most reliable sources of Type II FHB resistance is *Fhb1* from Sumai 3, a major QTL mapped on chromosome 3BS (Anderson et al. 2001, Buerstmayr et al. 2009), still not widely used in Argentina. In the case of leaf rust, several races are generally present in the *P. triticina* populations in the Southern cone of Latin America, probably due to a high acreage where wheat cultivars susceptible or moderately susceptible to leaf rust were sown (Germán and Kolmer 2014). Host resistance is governed by seedling and adult-plant resistance genes, among which the seedling resistance gene *Lr47* is particularly effective (Vanzetti et al. 2011).

MS INTA 416 is a hard red winter wheat (*Triticum aestivum* L.), developed and released by the INTA EEA Marcos Juárez, in 2016. MS INTA 416, previously designated JN12009, was selected for its high yield potential and good bread-

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making quality, aside from moderate resistance to Fusarium head blight and high resistance to leaf rust, conferred mainly by the resistance genes *Fhb1* and *Lr47* introgressed by artificial crosses and selected by marker-assisted selection (MAS). MS INTA 416 is adapted to rainfed and irrigated production areas in the sub-humid and humid plains of the provinces Córdoba, Santa Fé, Buenos Aires, and La Pampa, Argentina.

BREEDING METHODS

MS INTA 416 (JN12009) was selected from a population derived from two backcrosses, using the breeding line R4004 as recurrent parent and Sumai 3 as *Fhb1* donor. Breeding line R4004 was obtained from a population derived from six backcrosses using ProINTA Oasis (pedigree OASIS/TORIM-73) as recurrent parent and PI 603918 (pedigree Pavon 76 *8//T7AS-7S#1S-7S#1S/*ph1b*) as donor of the leaf rust resistance gene *Lr47*. ProINTA Oasis is a hard red spring wheat developed by INTA EEA Saenz Peña, released in 1989. Since the agronomic performance of this cultivar was very good and yields were high, it was readily adopted in the main wheat-producing areas of Argentina until 1997, when it became highly susceptible to leaf rust by the breakdown of resistance gene *Lr26* (Antonelli 2003). The development of line PI 603918 including a *Triticum speltoides* interstitial translocation carrying *Lr47* was previously described (Bainotti et al. 2009). *Lr47* is effective against field leaf rust infection in Argentina, according to information obtained from the commercial cultivar BIOINTA2004 released in Argentina in 2009, which carries this gene (Bainotti et al. 2009, Vanzetti et al. 2011, Campos 2013, Campos and Lopez 2015). For the development of R4004 in 1996, in greenhouse facilities of the Instituto de Recursos Biológicos (IRB) INTA, Hurlingham, PI 603918 was crossed with PROINTA Oasis and then backcrossed with the same cultivar for six generations. RFLP marker *Xabc465* (Dubcovsky et al. 1998) was used to select *Lr47* heterozygous plants from BC_1 to BC_3, and PCR markers (Helguera et al. 2000) from BC_4 to BC_6 (Figure 1). Then, at least three *Lr47*-heterozygous plants were self-pollinated producing BC_6 F_2 seeds. In 2000, BC_6 F_2 seeds were planted at INTA EEA Marcos Juárez for the selection of *Lr47* homozygous plants, using PCR markers as before. About 30 selected BC_6 F_3 head rows were planted in June 2001 in single 1-m rows, and evaluated in a non-replicated leaf rust screening nursery.

In June 2002, 18 BC_4 F_5 lines were advanced to a non-replicated observation plot trial in Marcos Juárez (plots of six 3-m rows) and in 2003, the same lines were advanced to a Multilocation Trial (MLT) at Pergamino, Corral de Bustos and Marcos Juárez. At this point, R4004 was selected for further backcrossing with Sumai 3 on the basis of grain yield, leaf rust resistance, uniformity, and general agronomic appearance. For the development of MS INTA 416, in 2004, by IRB INTA, R4004 was crossed with cultivar Sumai 3 (Funo/Taiwan Xiaomai), the *Fhb1* donor, kindly provided by Dr Jim Anderson (Dpt. of Agronomy and Plant Genetics, University of Minnesota), and backcrossed with R4004 for three generations. In heterozygous plants, locus *Fhb1* was traced with SSRs *Xgwm533* and *Xgwm493* (Figure 2). Then, at least three *Fhb1*-heterozygous plants were self-pollinated, producing BC_3 F_2 seeds. During 2007, the BC_3 F_3 plants were planted in Marcos Juárez for selection of homozygous *Fhb1* plants using SSRs *Xgwm533* and *Xgwm493,* as before. About 30 selected BC3 F4 head rows were planted in Marcos Juárez in June 2008 and 2009, in single 1-m rows under 15x2x1.5-m greenhouses, covered by nylon mesh 35, and the agronomic appearance of the plants was evaluated.

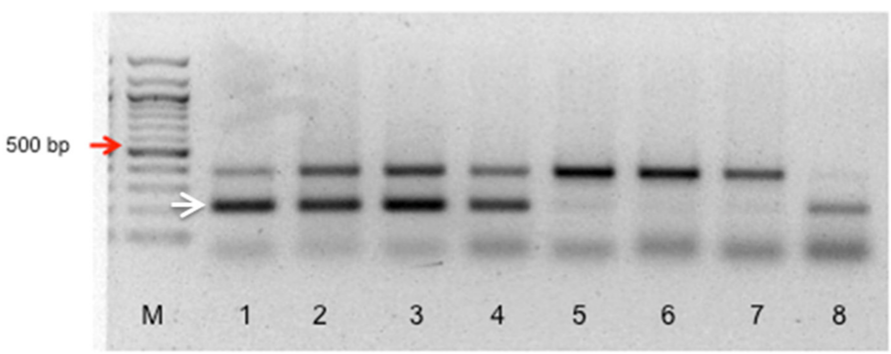

Figure 1. 1% agarose gel electrophoresis showing PCR amplification with *Lr47*-specific primers using genomic DNA from BC_6 plants. The *Lr47* DNA fragment is indicated by a white arrow. The 500bp fragment of the DNA marker (lane M) is indicated with a red arrow. Lanes 1-4: *Lr47*-heterozygous plants, 5-6: homozygous negative plants, 7: ProINTA Oasis, 8: Pavon S3 (*Lr47* donor).

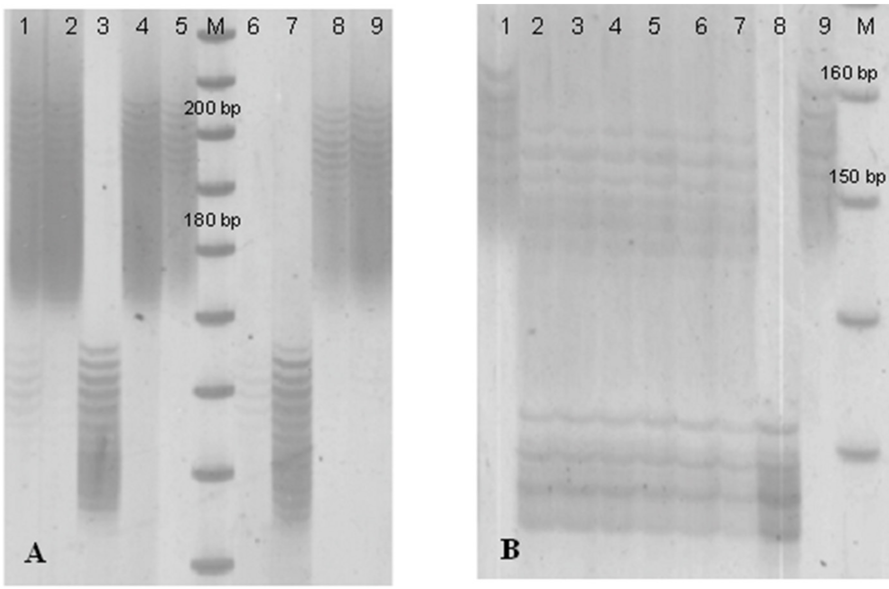

Figure 2. PCR profiles of *wms493* (A) and *wms533* (B) microsatellite markers flanking *Fhb1,* obtained from BC_3F_2 plants. For (A), in lane M, (size standard DNA), 200bp and 180bp fragments are indicated. Lane 1: *Fhb1* heterozygous plants; lanes 2, 4, 8, 9: *Fhb1* homozygous plants; lane 3: R4004; lane 5: Sumai 3 (*Fhb1* donor); lanes 6-7: negative homozygous plants. For (B), in lane M, 160bp and 150bp fragments are indicated. Lane1: *Fhb1* homozygous plant; lanes 2-7: *Fhb1* heterozygous plants; lane 8: R4004; lane 9 Sumai 3 (*Fhb1* donor).

In 2010, 28 BC_3 F_6 lines were planted at the same location in non-replicated observation plot trials (six 5-m rows) and, in 2011, 27 lines were advanced to the Preliminary Yield Trials (PYT) in Marcos Juárez. The PYT were arranged in a 10×9 alpha lattice design with two replications and the above plot size. In 2012, based on its yield potential, JN12009 was advanced to the Regional Yield Trials (RYT) in the provinces Buenos Aires (five locations), Córdoba (two locations), Entre Ríos (one location) and Chaco (one location), for three years (2012, 2013 and 2014), under rainfed conditions. A 6x8 alpha lattice design was used for the RYT at all locations, with three replications per trial (plots with seven 5-m rows). Seeding rates were standardized based on the seed size (300 seeds per m^2). The RYT trials at Marcos Juárez were also used to measure: (1) days to heading (days from emergence until 50% of the spikes emerged from the boot), (2) plant height (measured at maturity as the mean stem length from the soil to the tip of the spike, excluding the awns), (3) leaf rust (*Puccinia triticina*) and stem rust (*Puccinia graminis tritici*) severities based on the modified Cobb Scale (Peterson et al. 1948), (4) head blight (*Fusarium graminearum spp*), tan spot (*Drechslera tritici* spp) and bacterial stripe (*Xanthomonas translucens* p.v. *undulosa*) incidence and severity, both on 0–9 scales (Stubbs et al. 1986).

MS INTA 416 in the seedling stage was also evaluated for resistance to stem rust and leaf rust at the Cereal Disease Laboratory in INTA Bordenave, in 2013 and 2014. Local leaf rust races MDP 10-20, MFP 10, MKT 10-20 were inoculated on seedlings as described by Long and Kolmer (1989). Leaf rust severity was evaluated on a 0 to 4 scale, as proposed by Stakman et al. (1962). The infection types identified by the symbols 0, 1, 2, or combinations were considered low infection types, indicating resistance, while 3 and 4 were considered high infection types, indicating susceptibility. Seedlings were also inoculated with the local stem rust races QHFTC, QRFTF, QRFTC, and evaluated according to Stakman et al. (1962). The molecular basis of vernalization and photoperiod response were determined using *Vrn-A1, Vrn-B1, Vrn-D1, Ppd-B1,* and *Ppd-D1* allele-specific PCR markers, as previously described (Yan et al. 2004, Fu et al. 2005, Beales et al. 2007, Díaz et al. 2012). To assess the bread-making quality, grain harvested in RYT trials in 2012, 2013 and 2014, in Marcos Juárez (unreplicated samples) was used. Samples were analyzed by standard AACC methods at the Quality Laboratory of INTA Marcos Juárez, for milling, volume weight, protein content, Chopin Alveograph, and bread baking as previously described (Bainotti et al. 2009). High-molecular-weight subunits of glutenin (HMWGs) in the composition of MS INTA 416 were determined by SDS-PAGE as before (Lawrence and Shepherd 1980).

AGRONOMIC AND BOTANICAL DESCRIPTION

Juvenile plants of MS INTA 416 (JN12009) (growth stages 22 to 29, according to Zadoks et al. (1974)) are semi-erect, with a recurved flag leaf at the beginning of inflorescence emergence (GS 52). At maturity (GS 90), the spikes are semi-short (81-100 mm), white yellow and dense, with inclined position. The glumes are white, long (9mm), have medium width (3.5 mm) and a straight shoulder shape. The kernel is red, vitreous, and ovate; the germ is medium-sized; the brush is medium-sized and has no collar. According to data from the RYT trials between 2012 and 2014 in Marcos Juárez, MS INTA 416 had a mean plant height of 91.6 cm and developed within 114.6 days from emergence to heading (HD). Comparisons of these values with other frequently grown varieties in Argentina are presented in Table 1. As expected, the HD values observed in our study were significantly longer than those in cooler regions of Brazil (Marchioro et al. 2007, Franco et al. 2014, Franco et al. 2015, Marchioro et al. 2016). MS INTA 416 is uniform for plant type, without obvious phenotypic variants, and remained stable over five generations of evaluation (2011-2015). Molecular data obtained from *Vrn-1* and *Ppd-1* adaptation genes defined MS INTA 416 as a winter (carrying the triple combination of "winter" -recessive- alleles within *Vrn-1* homoeologs) - insensitive (carrying at least one "insensitive" allele within *Ppd-1* homoeologs, with low photoperiod response - in this case *Ppd-D1*) wheat. Local cultivars with the same combination of *Vrn-1* /*Ppd-1* adaptation genes (winter insensitive) are Baguette 21, BIOINTA 2004, Buck Ranquel, PROINTA Puntal, SRM Nogal, and Themix, among others (Vanzetti et al. 2013, Gomez et al. 2014). Phenological data (heading time) obtained from a subset of local winter-insensitive wheats sown on six dates between April 29 (mid-autumn) and August 11 (mid-winter), at approximately fortnightly intervals between sowings, indicated two clear groups: BIOINTA 3003, BIOINTA 3005 and Baguette 31, with high vernalization requirements (did not flower when planted after June 26), and, SRM Nogal and MS INTA 416 with milder vernalization requirements, as they did not flower when planted on August 11, the last tested sowing date (Table 2). Variation in the duration of cold requirements to complete vernalization has been described previously for *Vrn-1* copy number variation (Díaz et al. 2012). Related with the photoperiod response, the relatively low frequency of winter-sensitive (1/11) compared to winter-insensitive (10/11) commercial wheats released in Argentina (Vanzetti et al. 2013) suggests a better adaptation of the second than the first group. Typical winter-sensitive wheats are normally grown in environments with a longer growing season than is being explored by most of the wheat cultivars sown in the wheat belt of Argentina (Gomez et al. 2014).

Table 1. Performance[1] of MS INTA 416 and other hard wheat cultivars in Marcos Juárez, Argentina, 2012-2014

Cultivars	**HD**	**PH**	**LR**	**FHB**	**LS**
MS INTA 416	114.6	91.6	0	0,5.1	7.2
Baguette Premium 11	119	80	60S	1.1	7.2
Baguette 17	116.3	85	70S	4.2	8.2
Klein Yarará	120	96.6	10MR	7.3	7.3
	TW	**GP**	**LV**	**W**	**GW**
MS INTA 416	74.73	137	712	264	27.9
Baguette Premium 11	72.27	128	658	264	25.9
Baguette 17	68.83	125	612	257	27.2
Klein Yarará	74.73	134	708	336	28.2

[1] HD = days from emergence to heading; PH = plant height without awns in cm; LR = leaf rust incidence and severity based on modified Cobb scale (Peterson et al. 1948); FHB = Fusarium head blight incidence and severity based on the scale of Stubbs et al. (1986); LS = leaf spot diseases incidence and severity based on the scale of Stubbs et al. (1986); TW = test weight in kg hl^{-1}; GP = grain protein content in g kg^{-1}; LV = loaf volume in cm^3; W = general gluten strength by the Chopin Alveograph and GW = 1000-grain weight in grams.

Table 2. Heading date of Argentinian winter wheat cultivars considering eight successive sowing dates (in bold). Data obtained at the experimental station of Marcos Juárez, 2014

	Sowing date							
Cultivars	**Apr 27**	**May 9**	**May 26**	**Jun 10**	**26 Jun**	**Jul 16**	**Jul 28**	**Aug 11**
BIOINTA 3003	Sep 28	Sep 29	Oct 6	Oct 12	Oct 26	dnf	dnf	dnf
BIOINTA 3005	Sep 23	Sep 25	Oct 4	Oct 14	Oct 25	dnf	dnf	dnf
BAGUETTE 31	Oct 4	Nov 7	Oct 10	Oct 18	Nov 3	dnf	dnf	dnf
SRM NOGAL	Sep 13	Sep 15	Sep 24	Oct 3	Oct 13	Oct 24	Nov 5	dnf
MS INTA 416	Sep 9	Sep 10	Sep 20	Oct 1	Oct 13	Oct 26	Oct 31	dnf

dnf: did not flower

Table 3. Grain yield (kg ha^{-1}) of MS INTA 416 and controls at 18 trial locations (RYT), in 2012, 2013 and 2014. In bold, grain yields of MS INTA 416 ranked highest or second highest

Cultivars	Marcos Juárez	Paraná	Pergamino	Balcarce	Tres Arroyos	Bordenave
MS INTA 416 (2012)	**3272**	2742	**3222**	4576	4396	5517
Baguette Premium 11	2774	3079	3663	4911	4901	5719
Baguette 17	2954	2978	2814	5573	5124	5615
Klein Yarará	2636	2967	2693	4895	4593	5258
Mean	2826	2467	2561	4703	4811	5688
$LSD_{0.05}$ (kg ha^{-1})	401	367	357	639	586	541
CV (%)	9.38	10.9	9.09	8.38	9.81	7.83
MS INTA 416 (2013)	**4295**	2981	4916	**8961**	4638	**1985**
Baguette Premium 11	4261	3557	5576	9718	4655	1110
Baguette 17	4294	3461	4446	8528	4680	1622
Klein Yarará	4126	4320	5386	8355	4575	1452
Mean	4432	3254	5024	8397	4736	1972
$LSD_{0.05}$ (kg ha^{-1})	570	330	691	802	688	530
CV (%)	9.37	7.52	8.49	5.9	8.96	14.3
MS INTA 416 (2014)	**5008**	**3800**	**4905**	**4580**	**5695**	**5659**
Baguette Premium 11	3223	2589	4798	4331	4543	4307
Baguette 17	3308	3166	4863	4832	4466	4370
Klein Yarará	3669	3486	5315	4520	6321	6048
Mean	4091	3219	4757	4721	5610	5199
$LSD_{0.05}$ (kg ha^{-1})	368	369	595	580	522	709
CV (%)	5.50	7.07	8.41	7.00	6.90	9.14

Yield performance

MS INTA 416 was tested at 18 trial locations of the provinces Buenos Aires, Córdoba, Santa Fé and Entre Ríos (RYT trials, in 2012, 2013 and 2014). In this analysis, the yield of MS INTA 416 was ranked highest or second highest in relation to the test control varieties Baguette Premium 11, Baguette 17 and Klein Yarará in 11 trials, including in Marcos Juárez in 2012, 2013, 2014; Pergamino in 2012, 2014; Balcarce in 2013, 2014, Bordenave in 2013, 2014, Paraná in 2014 and Tres Arroyos in 2014 (Table 3). In view of this good performance, cultivar MS INTA 416 was indicated for cultivation in rainfed and irrigated production areas in the sub-humid and humid plains of the provinces Córdoba, Santa Fé, Buenos Aires, and La Pampa, Argentina.

Disease resistance

Evaluations of dominant leaf rust races on seedlings in Argentina showed that MS INTA 416 is highly resistant, with an infection type (IT) of 0 (Table 4). The IT indicating high resistance observed in MS INTA 416 can be explained by the presence of *Lr47,* from R4004, confirmed by a gene-specific PCR marker (Helguera et al. 2000). In line with this hypothesis, in advanced breeding lines carrying *Lr47* J12012, J13013 and JN12015, IT was 0. Our data agree with previous studies describing no virulence of the major *P. triticina* populations in relation to *Lr47* in Argentina, México and the Southern cone (Huerta-Espino et al. 2011, Vanzetti et al. 2011, Campos 2013, Campos and Lopez 2015). Evaluations of seedlings infected with dominant stem rust races in Argentina also showed that MS INTA 416 is highly resistant, with ITs between 0 and 1+ (Table 4), based on an unknown source of genetic resistance. As expected, field observations for disease resistance showed that MS INTA 416 was highly resistant to the prevalent leaf rust races in Marcos Juárez in 2012, 2013 and 2014. A similar situation was observed for stem rust in Marcos Juárez in 2014. Leaf spot diseases (tan spot and Septoria leaf blotch) were detected in Marcos Juárez, in 2012, and MS INTA 416 showed moderate resistance (7/2), similar to Baguette Premium 11 (7/2), Baguette 17 (8 /2) and Klein Yarará (7/3). Bacterial stripe severity indices were intermediate to high (9/6), classifying the cultivar as moderately susceptible. In 2012, FHB was observed in Marcos Juárez and MS INTA 416 showed moderate resistance (0.5/1). Fusarium head blight was also evaluated in Marcos Juárez, in 2014, in a nursery with natural infection, where MS INTA proved moderately resistant (2/2), similar to Baguette Premium 11 (2/2) and Klein Yarará (4/2) (data not shown). The good performance of MS INTA 416 in response to FHB can be explained by the

Table 4. Seedling stage infection types[1] of wheat cultivars and MS INTA 416 after inoculation with dominant leaf rust and stem rust races in Argentina in 2014

	Leaf rust strains			Stem rust strains		
Cultivars	**MDP 10-20**	**MFP 10**	**MKT 10-20**	**QHFTC**	**QRFTF**	**QRFTC**
MS INTA 416	0	0	0	0	0;	1+
SY 041	3-	3	0	4	3	4
SY 015	2=	1	3	4	4	4
Buck Bellaco	;1	0;	1	0	;1	1
Klein Serpiente	1+	22+	3	2=	0	2=
ACA 307	0;	3+	4	4	4	4
Cambium	3	1++	4	1+	0	11+

[1] '0'= no visible uredia; ';'= hypersensitive flecks; '1'= small uredia with necrosis; '2'= small to moderate-sized uredia with green islands, surrounded by necrosis or chlorosis; '3'= moderate uredia size, with or without chlorosis; '4'= large uredia without chlorosis; 'N'= necrosis. Symbols '-' and '+' indicate smaller or larger uredinia. The most common infection is listed first. For example, 11+; indicates infection types 1 and 1+.

presence of *Fhb1* confirmed by the flanking markers *Xgwm533* and *Xgwm493*. Marker-assisted selection of *Fhb1* has been used successfully used in the development of germplasm adapted to specific environments (Bainotti et al. 2013, Bernardo et al. 2014, Anderson et al. 2015). MS INTA 416 is the first wheat cultivar released in Argentina carrying the genetic resistance sources *Fhb1* and *Lr47* against FHB and leaf rust, respectively.

Milling and baking quality

Since 1998, bread wheat cultivars from Argentina are being classified based on their commercial and industrial quality performance as Quality Group (QG)1, cultivars with extra strong gluten suitable for blending; QG 2, cultivars adapted to traditional baking (fermentation time longer than 8 hours); and QG 3, cultivars suitable for direct baking methods (fermentation time less than 8 hours) (Cuniberti and Ottamendi 2004). MS INTA 416 belongs to QG 2, with W values of 264, similarly to Baguette Premium 11 (QG 2 check), lower than Klein Yarará (W=336, QG 1 check) but higher than Baguette 17 (W= 257, QG 3 check). The mean test weight of MS INTA 416 and Klein Yarará was 74.73 kg hL^{-1}, higher than that of Baguette Premium 11 and Baguette 17 (72.27 and 68.83 kg hL^{-1}, respectively). The mean grain protein content (137 g kg^{-1}) was higher than of the check cultivars Klein Yarará, Baguette Premium 11 and Baguette 17 (134, 128 and 125 g kg^{-1}, respectively). Additional quality parameters for MS INTA 416 and check cultivars are listed in Table 1.

MS INTA 416 contains the subunits 1, 7+9 and 5+10 in *Glu-A1*, *Glu-B1* and *Glu-D1* loci respectively, where the subunits 1 and 5+10 were associated with good and 7+9 with intermediate bread-making quality (Payne 1987). The possibility of 1BL/1RS rye translocation affecting wheat quality (Dhaliwal et al. 1987) was not tested and can therefore not be excluded.

BASIC SEED PRODUCTION

The Argentinian National Institute of Agricultural Technology - INTA (Rivadavia 1439, Ciudad Autónoma de Buenos Aires, Argentina) has licensed the seed company LDC Semillas SA (Olga Cossettini 240, Ciudad Autónoma de Buenos Aires, Argentina) to multiply and sell protected wheat cultivars developed by the INTA Wheat Breeding Program for 10 years, as of June 14, 2014. Cultivar MS INTA 416 will be released on the market in 2018.

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