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ABSTRACT

'BIOINTA 2004' (Reg. No. CV-1030, PI 655312) is a hard red winter wheat (*Triticum aestivum* L.) developed and released by the Marcos Juárez Experimental Station from the National Wheat Breeding Program of the National Institute of Agricultural Technology, Argentina. BIOINTA 2004, previously designated R4001, was selected for its excellent grain yield potential, resistance to leaf rust (caused by *Puccinia triticina* Eriks.) conferred mainly by the *Lr47* gene selected by marker assisted selection (MAS), and its good bread-making quality.

'BIOINTA 2004' (Reg. No. CV-1030, PI 655312) is a hard red winter wheat (*Triticum aestivum* L.) developed and released by the Marcos Juárez Experimental Station from the National Wheat Breeding Program of the National Institute of Agricultural Technology, Argentina. BIOINTA 2004, previously designated R4001, was selected for its excellent grain yield potential, resistance to leaf rust (caused by *Puccinia triticina* Eriks.) conferred mainly by the *Lr47* gene present on a 7AS interstitial *Triticum speltoides* (Tausch) Gren. Ex K. Richt. translocation selected by marker-assisted selection (MAS), and its good bread-making quality. BIOINTA 2004 is adapted to nonirrigated production areas in the humid plains from southern Córdoba and Santa Fé, Buenos Aires, and La Pampa Provinces from Argentina. *BIO* refers to Bioceres SA, a private company that has exclusivity in the commercialization of the cultivar, *INTA* refers to the National Institute of Agricultural Technology, which developed the cultivar, and *2004* is the serial number of midmaturity cultivars released under this agreement.

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Abbreviations: A-PAGE, acid polyacrylamide gel electrophoresis; BC, backcross; MAS, marker-assisted selection; MLT, multilocation testing; RYT, regional yield trials; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

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Methods

BIOINTA 2004 was selected from a population derived from four backcrosses (BC) completed in 1998 using 'ProINTA Puntal' [pedigree 'TAM W-101' (CItr 15324)/'CENTURK' (CItr 15075) *3//'AMIGO' (PI 578213)] as the recurrent parent and PI 603918 (pedigree 'Pavon 76' *8//T7AS-7S#1S-7S#1S/*ph1b*) as the donor parent of the leaf rust resistance gene *Lr47*. ProINTA Puntal is a hard red winter wheat developed by W.D. Worrall, Texas Agricultural Experiment Station, Agricultural Research and Extension Center, Vernon, TX, introduced to Argentina in 1989. This cultivar had very good agronomic and yield characteristics, and it was widely planted in the humid plains of Argentina until the late 1990s, when it became highly susceptible to leaf rust.

The interstitial translocation line T7AS-7S#1-7S.7AL carrying *Lr47* from *T. speltoides* (Tausch) Green. was originally transferred to bread wheat by irradiating hybrid seed (CI15092/*T. speltoides*//'Fletcher'/3/5*Centurk) with fast neutrons (Wells et al., 1982). Interstitial segments of chromosome 7S#1 were transferred to chromosome 7A of hexaploid wheat using the *ph1b* mutation (Lukaszewski, 1995). The interstitial translocations were backcrossed into the hard white spring variety Pavon 76 (PI 519847), and plants homozygous for the interstitial translocation were released as germplasm PI 603918 (Lukaszewski et al., 2000). The *T. speltoides* segment is generally transferred as a single linkage block (Helguera et al., 2000). In previous studies, resistance gene *Lr47* was shown to be effective against leaf rust races in field trials at University of

California, Davis, CA, during 2002. Yield comparison in the absence of leaf rust between isolines with and without the *T. speltoides* segment carrying *Lr47* showed variable responses depending on genotype and environment, but on average, the presence of the alien segment was associated with a 3.8% decrease in yield (Chicaiza et al., 2006; Brevis et al., 2008). The presence of this alien segment was also associated with significant increases in grain protein concentration and reduced milling yield (Brevis et al., 2008).

In the backcrossing program, molecular markers developed by Dubcovsky et al. (1998) and Helguera et al. (2000) were used to select at least four plants with one dose of the *T. speltoides* segment carrying *Lr47* in BC_1, BC_2, BC_3, and BC_4 populations (15–20 plants, each population) at Castelar, Buenos Aires. After that, the BC_4F_2 (50 plants) and BC_4F_3 (300 plants) were advanced as bulk populations at Marcos Juárez in 1999 and 2000. In 2000, individual plants were inspected and selected on the basis of leaf rust resistance. About 30 BC_4F_4 head rows were planted at Marcos Juárez in June 2001 as single rows 1 m long, and evaluated in a nonreplicated leaf rust screening nursery. Presence of *Lr47* in BC_4F_4 head rows scored as resistant was confirmed by molecular markers (Helguera et al., 2000).

In 2002, 24 BC_4F_5 lines resistant to leaf rust selected from the screening nursery all showed the *T. speltoides* chromosome segment based on molecular markers. These lines were advanced to a nonreplicated observation plot trial at Marcos Juárez (plots were 3.0 m long by 6 rows wide) in June 2002. Grain yields of these nonreplicated experimental lines were calculated as percentage of the check cultivar ProINTA Puntal.

In 2003 and 2004, eight lines were advanced to a multilocation testing (MLT) at Pergamino, Corral de Bustos and Marcos Juárez on the basis of grain yield, resistance to leaf rust, uniformity, and general agronomic appearance. The experimental design of MLT trials at the three locations used a 6 × 7 alpha lattice design with three replications (plots sizes were similar to those described above).

On the basis of grain yield, the experimental line R4001 was advanced to regional yield trials (RYT) grown in the provinces of Buenos Aires (four locations), Córdoba (two locations), and Entre Ríos and Chaco (one location, each) in 2004, 2005, and 2006 under rainfed conditions. The RYT trials at all locations used 6 × 8 (2004 and 2005) and 6 × 7 (2006) alpha lattice designs with three replications each (plot size was 5.0 m long by 7 rows wide). Seeding rates were standardized based on seed size to 300 seeds m^{-2}.

Days to heading was measured on RYT trials at Marcos Juárez as the number of days from emergence until 50% of the spikes emerged from the boot. Plant height (in centimeters) was measured at maturity as the average length of the stems from the soil to the tip of the spike, excluding the awns.

Resistance to leaf rust of BIOINTA 2004 and check cultivars was evaluated using 19- 20 races by the Cereal Disease Laboratory in INTA Bordenave during 2005, 2006, and 26 races during 2007. For each race, seedlings were inoculated according to Long and Kolmer (1989). Leaf rust severity was evaluated using a scale of 0 to 4 according to Stakman et al. (1962).

Seed harvested in 2004, 2005, and 2006 in RYT trials at Marcos Juárez (unreplicated samples) was analyzed for milling and bread-baking properties using standard American Association of Cereal Chemists (2000) methods at the Wheat Quality Laboratory in INTA Marcos Juárez (AACC 26-21A for milling, AACC 55-10 for volume weight, AACC 39-25 for protein concentration, AACC 54-30 A for Chopin Alveograph, and AACC 10-10B for bread baking). High molecular weight glutenin subunit composition of BIOINTA 2004 was determined by SDS-PAGE as described by Lawrence and Shepherd (1980). Gliadins and secalins were separated by acid polyacrylamide gel electrophoresis (A-PAGE, aluminum lactate buffer, pH 3.1), according to Khan et al. (1985).

Characteristics

Agronomic and Botanical Description

BIOINTA 2004 (R4001) has a semiprostrate growth habit with erect flag leaf. The spikes are long (more than 95 mm) and yellow, with erect position at maturity and lax density. The glumes are yellowish, large (9 mm), and wide, with a straight shoulder shape. The kernel is ovoid, amber, with a medium brush.

Data from the 2004 to 2006 RYT trials at Marcos Juárez, Córdoba (32°42′ S, 62°07′ W), showed BIOINTA 2004 to have an average plant height of 73 cm and to take 115 d from emergence to heading. Comparisons of these values to other frequently grown varieties in Argentina are presented in Table 1. BIOINTA 2004 showed a high vernalization requirement, confirming its winter nature. BIOINTA 2004 is uniform for plant type without obvious phenotypic variants and has remained stable during five generations of evaluation, 2003 to 2007.

Yield Performance

In the 2004 to 2006 RYT trials from Marcos Juárez BIOINTA 2004 average grain yield (4782 kg ha^{-1}) was higher than all the other frequently grown varieties listed in Table 1. Testing in the humid plains of Buenos Aires, Córdoba, Santa Fé, and Entre Ríos Provinces (RYT trials, 21 environments from 2004 to 2006), showed grain yield of BIOINTA 2004 (4783 kg ha^{-1}) to be ranked first out of eight lines tested from 2004 to 2006 in seven locations (Table 2). BIOINTA 2004 yield was not significantly different from that of ProINTA Puntal in the 12 location-years that the two were grown together ($P = 0.73$, ANOVA using locations-years as blocks) .

Disease Resistance

Seedling tests showed that BIOINTA 2004 was highly resistant to the 19, 20, and 26 leaf rust races evaluated during 2005, 2006, and 2007, respectively, which included the most prevalent races in Argentina (Table 3). In the same tests, its recurrent parent ProINTA Puntal was highly susceptible to races MDR 10-20, MCP 10, MCT 10 AG, MFP 20, CHT, MDR 10-20, MDP, and MCT 10.

Table 1. Agronomic performance of five wheat check cultivars and 'BIOINTA 2004' from the 2004–2006 regional yield trials in Marcos Juárez.

Cultivars	Grain yield	Days to heading†	Plant height†
	kg ha^{-1}	d	cm
2004			
BIOINTA 2004	4940	107	75
ProINTA Puntal	4920	112	80
BIOINTA 2002	4569	104	80
Baguette 10	4407	117	75
ACA 303	4294	117	75
BIOINTA B. 2001	4199	113	80
Klein Gavilán	4033	117	85
CV (%)	6,54		
$LSD_{0,05}$ (kg ha^{-1})	492		
2005			
BIOINTA 2004	5675	118	80
BIOINTA 2002	5490	113	85
ProINTA Puntal	5446	118	75
ACA 303	5235	117	75
BIOINTA B. 2001	4762	114	70
Baguette 10	4649	117	90
Klein Gavilán	4509	118	85
CV (%)	7,39		
$LSD_{0,05}$ (kg ha^{-1})	294		
2006			
Klein Gavilán	4310	112	80
BIOINTA 2004	3730	119	65
ACA 303	3727	115	65
ProINTA Puntal	3641	121	60
BIOINTA 2002	3577	110	75
BIOINTA B. 2001	3568	111	65
Baguette 10	3422	115	65
CV (%)	8,83		
$LSD_{0,05}$ (kg ha^{-1})	606		
2004, 2005, 2006 average			
BIOINTA 2004	4782	115	73
ProINTA Puntal	4669	117	72
BIOINTA 2002	4545	109	80
ACA 303	4419	116	72
Klein Gavilán	4284	115	83
BIOINTA B. 2001	4176	113	72
Baguette 10	4159	116	77

†For each cultivar, days to heading and plant height data was collected from a single parcel.

Field observations for disease resistance showed that BIOINTA 2004 is resistant to prevalent races of leaf rust present in the 21 environments tested in the RYT. Average severity under natural infection of *P. triticina* Eriks. in Marcos Juárez trials in 2004 to 2006 was 0 for BIOINTA 2004 and 13.6% for the recurrent parent ProINTA Puntal. In 2004, *Drechslera tritici-repentis* was detected in Marcos Juárez, and BIOINTA 2004 showed moderate susceptibility to this pathogen (similar to ProINTA Puntal and to other frequently grown varieties such as 'Baguette 10' and 'BIOINTA 2001'). In addition to *Lr47*, BIOINTA 2004 is predicted to have the adult plant resistance gene *Lr34* based on the presence of the diagnostic polymerase chain reaction marker developed by Lagudah et al. (2006).

Table 2. Performance of 'BIOINTA 2004' wheat relative to four check cultivars in regional yield trials (RYT), 3-yr average (2004, 2005, 2006) and seven locations (21 environments).†

Cultivars	Grain yield	Additional data
	kg ha^{-1}	
BIOINTA 2004	4783	
Baguette 10	4774	Widely grown, highly productive
BIOINTA 2002	4652	Highly productive
ACA 303	4555	Widely grown, highly productive
BIOINTA B. 2001	4080	Excellent quality
Mean	4574	
CV (%)	8.47	
$LSD_{0,05}$ (kg ha^{-1})	201	

†It included trials at Balcarce, Bordenave, Tres Arroyos, Pergamino, Corral de Bustos, Marcos Juárez and Paraná. Saenz Peña (Chaco Province) was not included because BIOINTA 2004 failed to flower in this environment.

Milling and Baking Quality

SDS-PAGE results show that BIOINTA 2004 has the *Glu-A1b, Glu-B1b, Glu-D1d* alleles combination (2*/7+8/5+10 subunits). The presence of 1RS diagnostic A-PAGE bands indicates that BIOINTA 2004 carries the same 1AL/1RS wheat–rye translocation present in the recurrent parent ProINTA Puntal (coming from AMIGO).

Both milling and baking quality scores of BIOINTA 2004 were very good according to wheat quality standards in Argentina (test weight >79 kg hL^{-1}; protein concentration in grain >11%; alveogram W 340–600; loaf volume >800 cm^3) (Cuniberti, 2004).

The average test weight of BIOINTA 2004 (76.1 kg hL^{-1}) was slightly lower than ProINTA Puntal (77.1 kg hL^{-1}), whereas its average grain protein concentration (127 g kg^{-1}) was higher than ProINTA Puntal (124 g kg^{-1}). BIOINTA 2004 flour yield (670 g kg^{-1}) was slightly lower than ProINTA Puntal (683 g kg^{-1}). The differences in these three parameters between BIOINTA 2004 and ProINTA Puntal are consistent with those reported by other pairs of isogenic lines that differ in the *Lr47* segment (Brevis et al., 2008), but they were not significantly different ($P > 0.05$, ANOVA using year-locations as blocks) likely due to the limited number of replications. Other quality parameters for BIOINTA 2004 and their comparison with the recurrent parent ProINTA Puntal and other check varieties are shown in Table 4.

Availability

Seeds of BIOINTA 2004 are under a Plant Variety Protection agreement for 20 years from the date of its registration in INASE. Seed requests for research and crosses should be

Table 3. Infection types† of wheat check cultivars and 'BIOINTA 2004' after inoculation with dominant (from top to bottom) *Puccinia triticina* races in Argentina during 2005, 2006, and 2007.

Rust race	BIOINTA 2004	ACA 303	ProINTA Puntal	BIOINTA B. 2001	Baguette 10	BIOINTA 2002
			Year 2005			
MDR 10-20	0;1+	1	3	2–	33+	4
MCP 10	;	1	4	22+	4	22+
MCT 10 AG	;	1+	4	2+3	4	3+
MCP 10-19	0;	11+	1+	22–	4	3–
MCD 10	;	1	2+	2 =	4	2
MCT 10	;	1+	4	2+3	4	3+
MCT 10-20	0;	2–	NT	3+4	4	3+4
MFP 20	0;	;1	4	;1	4	;1
			Year 2006			
MCT 10 AG	;	2N	NT	4	4	4
MCP 10	;	1	4	2–	4	22+
MFP 20	0;	;1	4	;1	4	;1
MDR 10-20	0;1	1	3	2–	33+	4
TDD 10-20	;	1N	NT	2–	1+2	2
MDP	0;	;	4	;	4	;1
MCP 10-19	0;	11+	1+	22–	4	3–
CHT	;	;	4	;	4	0;
			Year 2007			
MDT 10-20	;	1+	NT	4	4	4
MFP 20	0;	;1	NT	;1	4	;1
MFR 10-20	0;	1+	NT	2	4	;1
MDP 10-20	0;	;1	NT	1	4	4
MDR 10-20	0;1	1	NT	2–	33+	2
MFT 10-20	;	1+	NT	2+	4	3+
MDP 20	0;	;1	NT	;	4	2
MBD 10-20	;	1+	NT	11+	4	0;

†0 = no visible uredia; ; = hypersensitive flecks; 1 = small uredia with necrosis; 2 = small to moderate size uredia with green islands and surrounded by necrosis or chlorosis; 3 = moderate size uredia with or without chlorosis; 4 = large uredia without chlorosis; N = necrosis. Symbols − and + denote smaller or larger uredinia. The most common infection is listed first. For example, 11+ indicates infection types of 1 and 1+. NT = not tested.

sent to the corresponding author during the period of protection by the a Plant Variety Protection agreement. Seed of this release is deposited in the National Plant Germplasm System, where it will be available after the expiration of the Plant Variety Protection.

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Table 4. Data of milling and baking evaluations† of three wheat check cultivars and 'BIOINTA 2004' from the 2004–2006 regional yield trials in Marcos Juárez.

Characteristic	BIOINTA 2004	ProINTA Puntal	Baguette 10	BIOINTA B. 2001
	2004			
Test weight, kg hL^{-1}	78.4	78.8	74.4	81.1
Weight per 1000 kernels, g	36	37	38	34
Protein concentration, g kg^{-1}	10.9	11.2	11.0	11.9
Flour yield, g kg^{-1}	69.0	69.3	68.9	67.3
Alveogram length, mm	67.0	87.0	49.0	61.0
Alveogram P (height × 1.1), mm	101	97	85	107
Alveogram W, × 10^4 joules	267	320	174	282
Baking absorption, %	62.0	62.5	60	62.5
Baking remix time, min	3.5	3.5	2.5	4
Loaf volume, cm^3	740	715	580	625
	2005			
Test weight, kg hL^{-1}	81.7	82.9	79.8	84
Weight per 1000 kernels, g	30	34	36	32
Protein concentration, g kg^{-1}	13.2	12.8	11.9	13.1
Flour yield, g kg^{-1}	64.5	67.4	71.3	67.0
Alveogram length, mm	83	84	77	89
Alveogram P (height × 1.1), mm	138	126	71	120
Alveogram W, × 10^4 joules	422	399	183	445
Baking absorption, %	63.5	63	60	63.5
Baking remix time, min	3.5	2.5	2	4
Loaf volume, cm^3	795	800	565	723
	2006			
Test weight, kg hL^{-1}	76.1	77.1	73.7	79.9
Weight per 1000 kernels, g	28	28	34	32
Protein concentration, g kg^{-1}	14	13.3	12.9	14.8
Flour yield, g kg^{-1}	67.5	68.1	72.1	69.8
Alveogram length, mm	115	119	144	79
Alveogram P (height × 1.1), mm	94	87	56	92
Alveogram W, × 10^4 joules	358	350	223	322
Baking absorption, %	63	62.5	61	62.5
Baking remix time, min	3	3.5	2	4
Loaf volume, cm^3	875	830	555	805
	2004, 2005, 2006 average			
Test weight, k hL^{-1}	78.7	79.6	76.0	81.7
Weight per 1000 kernels, g	31.3	33.0	36.0	32.7
Protein concentration, g kg^{-1}	127	124	119	133
Flour yield, g kg^{-1}	670	683	708	680
Alveogram length, mm	88.3	96.7	90.0	76.3
Alveogram P (height × 1.1), mm	111.0	103.3	70.7	106.3
Alveogram W, × 10^4 joules	349.0	356.3	193.3	349.7
Bake absorption, g kg^{-1}	628	627	603	628
Baking time, min	3.3	3.2	2.2	4.0
Loaf volume, cm^3	803	782	567	718

†not replicated.

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