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Field evaluation and agronomic performance of transgenic wheat

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Abstract Seven transgenic lines of wheat have been evaluated under field conditions during 2 agonomic years. Four lines contained the transgenes for β-glucuronidase (*uid*A), herbicide resistance (*bar*) and for one high-molecular-weight (HMW) subunit, and three lines contained only one transgene for one HMW glutenin subunit and no marker genes. Agronomic traits and yield components were studied in transgenic lines and compared with the non-transgenic parent and null segregant lines. Although phenotypic differences for many traits have been found, only heading date and the number of spikelets per spike showed clear genotypic differences for both field trials. All transgenic lines had a longer heading date than parent lines whereas the number of spikelets per spike in transgenic lines was around that for L88-31 and higher for L88-6 than the corresponding parent lines. No differences were found between lines constitutively expressing the *uid*A and *bar* genes from those which only expressed the HMW genes. We conclude that differences between transgenic lines and their parents are small, and could be eliminated by backcrossing transgenic lines with their parents and selecting for the wanted genotype.

Keywords Transgenic wheat · Field trials · Agronomic performance · Somaclonal variation · Transformation

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Introduction

Genetic transformation has become an important approach for the introduction of novel agronomic traits into crops. For many desirable traits from unrelated plants or other organisms, genetic transformation is clearly the only source of variation for breeding programs. Methods for the transformation of major cereals, including rice, maize, barley and wheat, are now available. In wheat, the application of such technology has resulted in the production of transgenic plants with increased resistance to fungi (Bliffeld et al. 1999; Clausen et al. 2000), insects (Altpeter et al. 1999; Stoger et al. 1999) and virus (Sivamani et al. 2000), and in the modification of important quality traits like breadmaking (Altpeter et al. 1996; Barro et al. 1997) or starch content (Chibbar et al. 1998). However, a major requirement for the application of this technology is that transgenic plants bearing the new genetic combinations only differ from untransformed crops in the new traits added by transformation, leaving undisturbed the basic genetic background in which these new traits are expressed. Thus, the agronomic performance of transgenic plants under field conditions needs to be evaluated in order to establish the impact of transformation on agronomic traits.

Nowadays, the production of transgenic plants implies the gene transfer and the recovery of transgenic plants after an in vitro culture procedure. The latest process causes important genetic changes, termed somaclonal variation, that can negatively affect transgenic plants. These changes are unpredictable and can affect any of the agronomic traits of the plant. In wheat, important changes in the agronomic characteristics have been addressed to somaclonal variation, among them a reduction in the yield (Hanson et al. 1994), a lower 1,000-seed weight and a longer spike size (Symillides et al. 1995), and even chromosomal structural changes and meiotic abnormalities (Whelan 1990).

The objective of the present work was to evaluate the effect of two transgene combinations on the agronomic performance of wheat under field conditions. Transgene

expression, plant development and agronomic performance were evaluated in transgenic lines and were compared with the non-transgenic parent and tissue-culture null segregant lines.

Materials and methods

Field trials of seven transgenic and four non-transgenic lines of wheat have been conducted during 1998/99 and 1999/00 in the south of Spain. Transgenic lines were obtained by particle bombardment as described by Barro et al. (1997). The wheat lines L88-6 and L88-31 form part of a series of near-isogenic lines derived from crossing mutants of the Australian spring cultivars Olympic and Gabo (Lawrence et al. 1988). Wheat lines were transformed with the plasmid pAHC25 (Christensen and Quail 1996), which contains the *bar* and *uid*A genes, in combination with the plasmid p1Ax1 (Halford et al. 1992) or plasmid p1Dx5 (Halford et al. 1989), which contain the genes encoding for the HMW glutenin subunits 1Ax1 and 1Dx5 respectively. The *bar* and *uid*A genes were under the control of the constitutive maize ubiquitin promoter, whereas both HMW subunit genes were driven by their own endosperm-specific promoters. Four T_0 transgenic lines containing the *bar* and *uid*A genes, and one of the genes encoding for the HMW subunits 1Ax1 or 1Dx5, were selected and self-pollinated in order to obtain non-segregant lines. After five generations of self-pollination, no segregation was observed for both marker genes (*bar* and *uid*A) and the HMW subunit genes. In addition, it was possible to separate the marker from the HMW genes in different lines, resulting in lines containing the *bar* and *uid*A genes and one of the HMW genes (lines 1-1, 2-1, 6-1 and 9), and lines which contain only one of the HMW genes (lines 1-2, 2-2 and 6-2), and no marker genes (see Table 1). In each generation the presence of the *bar* and *uid*A genes were determined by PCR (Barro et al. 1998) and the presence of the HMW glutenin subunit genes were analysed in single half-grains by SDS-PAGE (Williams et al. 1988). Four non-transgenic lines of wheat were included in the field trials as controls. Two of them were the corresponding L88-6 and L88-31 parent lines, and the others were one null segregant line of each genotype from in vitro culture (see Table 1).

Field trials were grown at Córdoba, under irrigation, using a randomized complete block design with three replicates. Each plot consisted of four rows, 2-m long, with 50 seeds per row. The space between rows was 30 cm, and the separation between plots was 50 cm. Plant height, heading date, the number of spikelets per spike, the number of flowers per spike, and the number of seeds per spike was determined from ten individual plants collected from the two central rows of each plot. For biomass production, yield, the harvest index, 1,000-seed weight, test weight and the grain protein content mean values were calculated by bulking the plants from the two central rows of each plot.

Protein content was calculated from the nitrogen content by the Kjeldahl method (%N × 5.7). The β-glucuronidase (GUS) expression was assayed as described by Barcelo and Lazzeri (1995). Determination of BASTA resistance was carried out by spraying with a 1% BASTA solution containing 0.1% Tween-20 onto the leaves of plants.

General analysis of variance and Fisher's least significant difference (LSD) comparison of means were calculated using the SPSS statistical software.

Results

Transgene expression

The expression of transgenes was contrasted and monitored during plant development in both field trials. As expected, the constitutive expression of the *uid*A gene was detected at high levels in lines 1-1, 2-1, 6-1 and 9, and in tissues like roots, leaves, flowers and seeds (Table 1). The application of 1% BASTA solution to leaves showed that those lines were also resistant to the herbicide, whereas lines 1-1, 2-1, 6-1, the null segregant and the parent were highly sensitive (Table 1).

Plant development

The observation of the field plots showed no differences for germination among most of the transgenic, parent and null segregant lines, but transgenic line 9 showed about a 20% lower germination capacity in the 98/99 field trial than the rest of the lines. However, this lower germination of line 9 was not observed in 99/00. Further plant development showed that, in both field trials, lines 2-1, 2-2, 6-1 and 6-2 exhibited a spreading growth habit whereas the rest of the lines showed a normal growing habit. No other morphological differences were observed during the rest of the plant growth.

Agronomic performance

Field evaluation has shown a range of variation for most of the agronomic traits studied in this work; first among

Table 1 Transgene composition and transgene expression of wheat lines used in the field trials. NA = not applicable

Genotype	Line	Transgenes			Transgene expression		
		bar	*uid*A	HMW subunit	BASTA	GUS[a]	HMW subunit
L88-31	Parent	NA	NA	NA	–	–	–
	Null segregant	–	–	–	–	–	–
	1-1	–	–	1Ax1	–	–	+
	2-1	–	–	1Ax1	–	–	+
	6-1	–	–	1Dx5	–	–	+
	1-2	+	+	1Ax1	+	+	+
	2-2	+	+	1Ax1	+	+	+
	6-2	+	+	1Dx5	+	+	+
L88-6	Parent	NA	NA	NA	–	–	–
	Null segregant	–	–	–	–	–	–
	9	+	+	1Dx5	+	+	+

[a] Determined in roots, leaves, flowers and seeds

Table 2 Agronomic performance of the L88-31 and L88-6 parent, and null segregant and transgenic lines in the 98/99 and 99/00 field trials. The least significant difference (LSD) value at the 0.05 level is indicated. ND = not determined

Genotype	Line	Plant height (cm)		Heading date (days)		No. of spikelets per spike		No. of flowers per spike (A)		No. of seeds per spike (B)		Fertility (B/A)	
		98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
L88-31	Parent	118.8	131.4	133.1	132.1	23.9	23.1	93.5	84.5	65.5	59.0	0.70	0.70
	Null segregant	ND	128.7	ND	133.6	ND	23.2	ND	81.6	ND	57.4	ND	0.72
	1-1	121.3	134.7	134.5	132.3	22.5	22.2	88.7	81.7	69.0	61.8	0.78	0.75
	2-1	113.5	124.7	137.1	133.4	23.6	22.6	104.1	84.9	71.0	57.3	0.68	0.68
	6-1	116.2	126.2	139.1	135.6	23.6	22.9	102.2	82.3	63.6	56.6	0.62	0.69
	1-2	112.0	134.2	134.5	132.7	23.5	21.9	95.8	80.4	68.6	56.7	0.72	0.71
	2-2	122.2	125.3	136.7	133.0	23.4	23.0	81.0	85.7	72.6	57.8	0.79	0.67
	6-2	120.3	125.9	140.5	134.5	24.6	23.1	104.3	87.7	76.5	58.6	0.73	0.67
	LSD ($P = 0.05$)	12.1	11.6	1.1	0.9	0.9	0.9	11.2	12.1	12.4	8.2	0.15	0.08
L88-6	Parent	110.6	117.2	132.2	129.5	21.2	20.5	78.3	72.3	61.5	51.8	0.78	0.72
	Null segregant	ND	118.7	ND	130.6	ND	21.3	ND	76.9	ND	53.4	ND	0.70
	9	106.6	120.4	136.6	133.3	23.1	21.5	97.3	80.4	69.8	56.8	0.72	0.71
	LSD ($P = 0.05$)	11.8	11.1	0.7	0.7	0.5	0.4	5.5	10.4	12.9	9.9	0.11	0.09

Genotype	Line	Biomass (kg/ha)		Yield (kg/ha)		Harvest index (%)		1,000-seed weight (g)		Test weight (kg/100 l)		Protein content (% DW)	
		98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
L88-31	Parent	12,231	21,756	2,706	3,578	22.1	16.5	27.0	33.6	74.7	75.6	16.7	15.4
	Null segregant	ND	18,111	ND	3,089	ND	17.1	ND	31.5	ND	75.4	ND	15.3
	1-1	12,410	17,489	2,355	2,711	19.0	15.7	25.6	30.3	72.8	73.7	16.2	15.4
	2-1	10,411	18,678	2,376	3,133	23.1	16.8	25.5	30.8	74.4	73.9	16.5	15.9
	6-1	10,089	20,956	1,976	3,311	19.6	15.7	25.1	31.0	76.1	75.4	17.1	16.2
	1-2	12,064	16,856	2,338	2,800	19.5	16.7	25.9	32.0	74.3	74.5	16.5	15.2
	2-2	10,738	20,544	2,586	3,422	24.1	16.7	26.8	30.5	73.6	75.0	16.8	15.9
	6-2	10,400	19,156	2,320	2,922	22.3	15.3	24.0	31.3	75.8	73.5	16.4	16.3
	LSD (P = 0.05)	1,688	5,108	333	798	3.4	2.9	2.7	1.5	1.8	1.7	0.7	0.2
L88-6	parent	13,699	19,233	2,799	2,667	20.2	13.8	29.7	31.2	75.1	72.2	15.4	15.9
	Null segregant	ND	20,933	ND	3,233	ND	15.5	ND	30.5	ND	71.2	ND	14.5
	9	9,627	22,778	1,760	2,867	18.3	12.7	27.6	31.9	75.3	72.9	16.0	16.2
	LSD (P = 0.05)	4,851	9,827	2,243	1,401	11.0	5.1	7.7	3.3	0.8	2.9	2.0	2.1

lines tested, and second between field trials (Table 2). For example, among lines, traits like the heading date, the number of flowers per spike and the number of grains per spike showed a wider range of variation in 98/99, while for the content of biomass and harvest index, the variation range was greater in the 99/00 field trial (Table 2). Despite this variation, the analysis of variance has shown that most differences were either not significant or significant for one field trial but not for the other, and only for heading date and the number of spikelets per spike there were clear genotypic differences for both field trials (Table 3). All L88-31 and L88-6 transgenic and null segregant lines had a longer heading date than parent lines (Table 2). With respect to the number of spikelets per spike, the L88-31 transgenic and null segregant lines produced a similar or a lower number of spikelets per spike than their parent lines, whereas for L88-6, transgenic line 9 and the null segregant derived from in vitro culture showed a higher number of spikelets per spike than the corresponding parent line (Table 2).

The number of flowers per spike, the fertility, the harvest index, the test weight and the grain protein content of transgenic and null segregant lines were around that of the parent lines (Table 2). These traits did not show any tendency, and the genotypic differences detected were not very consistent since they were only observed in one of the two field trials (Table 3). Besides that, genotypic differences for fertility, harvest index and test weight were present only among transgenic lines since there were no significant differences between them and their respective wheat parent lines (Table 2).

The genotypic differences for yield and 1,000-seed weight were only observed in one of the two field trials for the genotype L88-31 (Table 3). However, it is interesting to highlight that for these traits a clear tendency

Table 3 Analysis of variance for wheat lines L88-31 and L88-6 in the 98/99 and 99/00 field trials. NS = non significant

Trait	L88-31		L88-6	
	98/99	99/00	98/99	99/00
Plant height	NS	NS	NS	NS
Heading date	***	***	**	***
No. of spikelets	*	***	**	**
No. of flowers (A)	*	NS	***	NS
No. of seeds (B)	NS	NS	NS	NS
Fertility (B/A)	NS	*	NS	NS
Biomass	NS	NS	NS	NS
Yield	*	NS	NS	NS
Harvest index	*	NS	NS	NS
1,000-seed weight	NS	**	NS	NS
Test weight	*	NS	NS	NS
Protein content	NS	**	NS	NS

*, **, ***Significant at 0.05, 0.01 and 0.001 probability levels, respectively

was observed, and both the yield and the grain size of the parental L88-31 were, in both years, higher than that of its transgenic and null segregant lines (Table 2).

Discussion

Evaluation of transgenic plants under field conditions is necessary to determine the effect that genetic transformation could have on the agronomic traits of crops. However, the nature of some traits, like yield and the content of biomass, was very influenced by the environment, and the fact that the transgenic plants are obtained by an in vitro culture process make these traits difficult for evaluation.

The results obtained from this work, after 2 years of field evaluation with transgenic lines of wheat, have shown differences for important agronomic traits between field trials and genotypes. However, most of these differences were either non-significant or significant for one agronomic year but not for the other. Therefore, most of the variation observed could be addressed to the environment and weather conditions. In this work, the environmental component of the variance for yield was high, partly due to the small size of the plot and to the necessity to carry out the field trials in a confined environment. Although there were no significant differences for biomass and yield, it is difficult to conclude that the yields of transgenic lines and their parents are identical.

Clear and consistent differences were found for heading date and the number of spikelets per spike. In addition, heading date showed a clear tendency, and all the L88-31 and L88-6 transgenic lines showed a longer heading date than the parent lines. It is difficult to address the basis of the genotypic variation observed for these traits. However, the fact that, for both genotypes, null segregant lines also exhibited a longer heading date could indicate that variation for this trait could be more related with somaclonal variation, induced by in vitro culture, rather than with the transformation procedure.

The production of transgenic plants involved a three-step process: the transformation method, tissue culture, and the selection of transgenic plants. Each step is probably providing the conditions for chromosome changes such as mutations and chromosomal breakage or rearrangements. This is well-documented for tissue culture, where somaclonal variation has been widely described. However, the transformation method and the selection of transgenic plants may represent additional stressing steps for favouring somaclonal variation. Phan et al. (1996) gave evidence for the occurrence of stable genomic changes in transgenic rice plants and point to in vitro cell culture as the causative agent. In barley transgenic plants obtained by particle bombardment Bregitzer et al. (1998) have shown that differences for important agronomic traits, including heading date, were due to somaclonal variation and that the transformation procedure appears to induce greater somaclonal variation than tissue culture. In addition, important changes in the ploidy number, associated with transformation, have also been reported (Choi et al. 2000). Furthermore, Svitashev et al. (2000) reported the association of transgene integration with chromosome breakage and rearrangements in oat plants produced by particle bombardment. One could consider that those rearrangements may be related with the transformation procedure used to produce the transgenic plants, and that some transformation methods, like particle bombardment, could be more stressing than others, like *Agrobacterium* or cell electroporation, increasing the frequency of rearrangements. However, Arencibia et al. (1999) have also reported somaclonal variation in transgenic sugarcane produced by cell electroporation, and those changes, although affecting a small number of qualitative traits, were related to genomic changes in transgenic plants. Moreover, Sala et al. (1998) transformed rice, poplar and sugarcane by various techniques, and changes in the genomes were analysed. It is concluded that genetic transformation can cause genomic changes in cell cultures, but the lowest level of changes were observed after particle bombardment and electroporation. In our work, variation in chromosome number has not been observed, and all wheat lines tested had 42 chromosomes although chromosome rearrangements like translocation cannot be discarded. In this way, more information about transgene integration and location on chromosomes is needed in order to clearly elucidate the effect of transformation, and to develop efficient transformation and in vitro culture procedures that minimise chromosome rearrangements.

We have not found significant differences for the agronomic traits studied between lines constitutively expressing the *bar* and *uid*A genes along the entire growing period, from those free of marker genes and expressing only the transgenes corresponding to HMW in the grain endosperm. Therefore, the constitutive expression of transgenes in the whole plant does not affect the intrinsic agronomic properties of wheat lines.

We conclude that there are differences between transgenic lines and their parents. However, these differences

are small and some of the transgenic lines showed agronomic traits and yields comparable to, or even higher than, parent lines. Thus, the selection of transgenic lines has to be made for the desirable trait, and changes due to somaclonal variation or transformation could be eliminated by backcrossing transgenic lines by their parents and selecting for the desired genotype.

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