



Inheritance of glyphosate resistance in *Lolium perenne* and hybrids with *Lolium multiflorum*



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ABSTRACT

Glyphosate-resistant *Lolium* species have been selected in weed communities where glyphosate is the herbicide used almost exclusively for weed control. The rate of evolution of herbicide resistance is highly influenced by the mating system and the inheritance type. Given the relevance of *Lolium* spp. as major weeds of winter cereal crops, it is important to know the basis of how they inherit glyphosate resistance. During three years of testing, we studied *Lolium perenne* plants from a glyphosate-resistant population in Argentina. Plants with different glyphosate sensitivity were forced to self-fertilize and breed. In addition, inter-specific hybridizations were obtained using glyphosate-susceptible *Lolium multiflorum* and glyphosate-resistant *L. perenne*. Moderately resistant *L. perenne* plants, when selfed, produced offspring in three phenotype classes: susceptible, moderately resistant and highly resistant plants in a 1:2:1 ratio, respectively. When moderately glyphosate-resistant plants and susceptible ones were crossed, the offspring showed the same parental phenotypes in a 1:1 ratio. In crosses between highly resistant plants with susceptible individuals, all offspring showed moderate resistance, while crosses of susceptible plants produced 100% glyphosate-susceptible individuals. Glyphosate resistance therefore appears to be controlled by a single locus with incomplete dominance and maternal effects are unlikely to play a major role.

Moreover, glyphosate resistance was inherited in hybrids between susceptible *L. multiflorum* and resistant *L. perenne* with a similar type of inheritance pattern as that indicated above. Considering these cross-pollinated species, glyphosate resistance may be transmitted not only among plants of the same species but also to related species such as *L. multiflorum*.

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1. Introduction

Glyphosate [*N*-(phosphonomethyl) glycine], which is the most extensively used herbicide worldwide, has a key role in current extensive agriculture (Baylis, 2000; Gianessi, 2008). In this context, cases of glyphosate-resistant weed populations may emerge, thus threatening the long-term efficacy of this important herbicide (Duke and Powles, 2008).

The first glyphosate-resistant biotype was reported in a population of *Lolium rigidum* in 1996 (Powles et al., 1998). Since then, several other weeds have shown cases of glyphosate resistance (Heap, 2015). Among them, glyphosate-resistant *Lolium* species

have been selected in situations where glyphosate is used almost exclusively for weed control (Preston et al., 2009). In Argentina, glyphosate resistance was reported in a *Lolium perenne* population after 12 years of successful use of this active principle (Yannicari et al., 2012a).

Herbicide resistance is an evolutionary process and its dynamics and impact depend on the biology of the weed species, the properties of the herbicide, operational factors, and genetic aspects such as frequency, number, dominance and fitness costs of resistance genes (Preston et al., 2009; Powles and Yu, 2010; Manalil, 2014). In addition, the rate of evolution of herbicide resistance is highly influenced by the mating system and the inheritance type (Jasieniuk et al., 1996).

L. perenne and *Lolium multiflorum* are frequent weeds in cereal crops from the Pampas (Argentina) and have a constancy (proportion of fields in which the species occurs in the region) of 40%

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(Istilar and Yannicari, 2012; Scursoni et al., 2014). In both species, some biotypes are difficult to control at normally lethal doses of glyphosate (Diez de Ulzurrun and Leaden, 2012; Yannicari et al., 2012a,b,c). Both *L. perenne* and *L. multiflorum* are allogamous, which manifest more than 90% natural out-crossing and can produce inter-specific hybrids spontaneously (Arcioni and Mariotti, 1983; Ryan et al., 2006).

The inheritance of glyphosate resistance has been addressed in a few weeds and several models have been found. Several authors found that resistance to glyphosate is controlled either by single genes with different levels of dominance (Ng et al., 2004a; Zelaya et al., 2004; Wakelin and Preston, 2006) or by multiple genes (Simarmata et al., 2005; Chandi et al., 2012). This information is particularly relevant in understanding and modelling the evolution of resistance (Gressel and Segel, 1978; Neve et al., 2011).

While the gene flow from transgenic herbicide-resistant crops to wild and naturalized compatible weed species has been well reported (Ellstrand, 2003; Légère, 2005; Zapiola et al., 2008; Zapiola and Mallory-Smith, 2012), the transfer of glyphosate resistance between related weed species has received less attention. The potential for glyphosate resistance to be transferred via pollen in weed-to-weed interactions has nevertheless been reported in several cases, such as from *Conyza canadensis* to *Conyza ramosissima* (Zelaya et al., 2007) and from *Amaranthus palmeri* to five other *Amaranthus* weed species (Gaines et al., 2012).

Given the relevance of *Lolium* species as major weeds of winter cereal crops and the importance of genetic factors in herbicide-resistance evolution (Powles and Yu, 2010), it is important to know the basis of the inheritance of glyphosate resistance. This information can then be used to interpret the dynamics of the problem and support the development of management strategies.

2. Materials and methods

2.1. *Lolium perenne* materials and resistance screening

Seeds from a glyphosate-resistant *Lolium perenne* ($2x = 2n = 14$) population (problem population) from southern Buenos Aires province, Argentina, were employed in the current work. In this population, a 10.8-fold greater dose of glyphosate had been necessary to match the control efficiency on a susceptible standard (Yannicari et al., 2012a). The plant materials obtained and used in crosses had been previously phenotyped according to their glyphosate sensitivity as indicated below.

The seeds were sown in 250 cm³ pots with sterile soil in a greenhouse. Eight weeks later, when the plants showed at least four tillers, they were propagated by tiller separation to obtain four plants of each genotype. When the clones had three to four tillers, each one was treated with 0.0, 0.75, 1.0, or 3.0 kg ae ha⁻¹ formulated glyphosate (isopropylamine salt of glyphosate, Roundup, 360 g ae L⁻¹, Monsanto Argentina), following Baerson et al. (2002). The applications were performed using a laboratory belt sprayer equipped with flat-fan nozzles (Teejet® 11002) and calibrated to deliver 200 L ha⁻¹ at 300 kPa. On the basis of these preliminary screenings, at 20 days post-application, the plants were characterized as 'susceptible' (did not survive at 0.75 kg ae ha⁻¹ glyphosate or higher doses), 'moderately resistant' (plants surviving at 1.0 kg ae ha⁻¹ glyphosate and lower doses, but were controlled at the highest dose) or 'highly resistant' (plants surviving at 3.0 kg ae ha⁻¹ glyphosate and lower doses).

The control plants (0.0 kg ae ha⁻¹) of each phenotype selected for making crosses were further subdivided into new propagules and grown until flowering in 5-L pots with sterile soil. The plants were grown in a greenhouse and pots were irrigated daily to field capacity. Fertilizer (12:10:20, Nitrofoska®, Compo Argentina) (1 g L⁻¹) was added to the irrigation water every 15 days.

The materials used in the crosses and their characteristics are shown in Table 1. The *L. perenne* plants were obtained from seeds of the problem population or their offspring: Progeny obtained from controlled crosses (*Ri10-5* × *Ri10-5*; *Ri10-1* × *Ri10-1* and *Re12* × *Sc12*) or out-breeding of a moderately glyphosate-resistant plant (*Ri11-1*), which was grown together with individuals with the same glyphosate-sensitivity in a greenhouse (Table 1).

2.2. Self-fertilization

Plants with different glyphosate sensitivities (susceptible, moderately resistant or highly resistant plants) were forced to self-fertilize. For this purpose, one spike per plant was selected before anthesis. Next, 10 to 12 spikelets from the middle of every spike were selected and the remaining spikelets were removed together with the apical florets due to their delayed development. Thereafter, the spikes were bagged into wax paper bags (10 × 5 cm) and supported by stakes. During the following 15 days, every bag was gently stirred twice a day to promote the release of pollen inside the bag. Twenty days after the bagging, the bags were opened and the growth of grains corroborated. The plants were grown until harvest maturity when the spikes were cut and individualized.

Table 1

Plants used for self-fertilization and crosses, their glyphosate sensitivity, origin of each plant material and year in which these were obtained.

Plant	Glyphosate sensitivity	Origin	Year
Ri10-1	Survivor at 1.0 kg ae ha ⁻¹	Yannicari et al., 2012a	2009
Ri10-3	Survivor at 1.0 kg ae ha ⁻¹	Yannicari et al., 2012a	2009
Ri10-5	Survivor at 1.0 kg ae ha ⁻¹	Yannicari et al., 2012a	2009
Ri10-7	Survivor at 1.0 kg ae ha ⁻¹	Yannicari et al., 2012a	2009
Ri11-1	Survivor at 1.0 kg ae ha ⁻¹	Yannicari et al., 2012a	2010
Ri11-2	Survivor at 1.0 kg ae ha ⁻¹	Yannicari et al., 2012a	2010
Sb11	Did not survive at 0.75 kg ae ha ⁻¹	Yannicari et al., 2012a	2010
Ra11	Survivor at 3.0 kg ae ha ⁻¹	Ri10-5 × Ri10-5	2010
Sa11	Did not survive at 0.75 kg ae ha ⁻¹	Ri10-1 × Ri10-1	2010
Re12	Survivor at 1.0 kg ae ha ⁻¹	Ri11-1 (out-breeding)	2011
Sc12	Did not survive at 0.75 kg ae ha ⁻¹	Ri11-1 (out-breeding)	2011
Ri13-1	Survivor at 1.0 kg ae ha ⁻¹	Re12 × Sc12	2012
Ri13-2	Survivor at 1.0 kg ae ha ⁻¹	Re12 × Sc12	2012
Ri13-3	Survivor at 1.0 kg ae ha ⁻¹	Re12 × Sc12	2012
Sc13-1	Did not survive at 0.75 kg ae ha ⁻¹	Re12 × Sc12	2012
Sc13-2	Did not survive at 0.75 kg ae ha ⁻¹	Re12 × Sc12	2012
Re13	Survivor at 3.0 kg ae ha ⁻¹	Re12 × Re12	2012
Lm13	Did not survive at 0.5 kg ae ha ⁻¹	Susceptible population from La Plata (Argentina)	2013

Spikes were preserved in paper bags at room temperature for six months.

Plants resistant to 1.0 kg ae ha⁻¹ glyphosate (*Ri10-1*, *Ri10-3*, *Ri10-5*, *Ri10-7*, *R11-1*, *R11-2*, *Ri13-1*, *Ri13-2* and *Ri13-3*; [Table 1](#)) were forced to self-fertilize during 2009, 2010 and 2012. In addition, other plants with different glyphosate sensitivities were also self-pollinated in 2012 (*Sc13-1*, *Sc13-2* and *Re13*; [Table 1](#)).

2.3. Cross breeding

At the heading stage (7 days before anthesis), plants to be crossed were manually emasculated. First, 10 to 12 central spikelets were chosen from each spike, while apical and basal spikelets were discarded using fine scissors. In turn, three to five florets from each spikelet were selected.

The selected florets were emasculated (using a fine-tipped forceps) and all three stamens of every floret were carefully removed. Immediately, each spike from the mother plant was bagged together with a non-emasculated spike from another plant, used as the father plant. Thereafter, the spikes were treated following the previous description until their harvest and storage.

During 2010, a highly glyphosate-resistant plant (*Ra11*, a survivor at 3.0 kg ae ha⁻¹) and a susceptible one (*Sa11*, did not survive at 0.75 kg ae ha⁻¹) obtained from the offspring of self-fertilized plants ([Table 1](#)) were crossed. In addition, plants with the same glyphosate sensitivity, i.e. moderately resistant plants (*Ri11-1* and *Ri11-2*, [Table 1](#)) and susceptible plants (*Sa11* and *Sb11*, [Table 1](#)), were crossed between them.

In 2011, two plants from the offspring obtained by out-breeding of a moderately resistant plant (*Ri11-1*) were selected: one moderately resistant plant (*Re12*, [Table 1](#)) and a susceptible one (*Sc12*, [Table 1](#)). These materials were propagated by tiller separation and numerous crosses were conducted to obtain a large number of descendants. Plants of the *Sc12* clone were crossed with *Re12* and the reciprocal cross was also performed. Finally, one moderately resistant plant (*Re13-1*, [Table 1](#)) and a susceptible one (*Sc13-1*, [Table 1](#)), selected from the progeny of *Re12* × *Sc12*, were crossed in 2012.

2.4. Hybrids between a glyphosate-susceptible *Lolium multiflorum* and a glyphosate-resistant *Lolium perenne*

Five plants of *L. multiflorum* ($2x = 2n = 14$) at the tillering stage were collected from a volunteer vegetation in La Plata, Buenos Aires province (34° S, 58° W). These plants were propagated by tiller separation and glyphosate susceptibility was subsequently assessed by using a dose of 0.5 kg ae ha⁻¹ glyphosate. No clones survived at this dose.

At the heading stage, control clones (without application of glyphosate) were emasculated as indicated above. Immediately, inter-specific hybridizations were forced using a glyphosate-resistant *L. perenne* plant (*Re13*, [Table 1](#)) as father. As indicated in the preceding section, spikes of the mother and father plants were bagged together. Afterwards, the spikes were handled as described previously until their harvest and storage.

In the following season, the hybrid progeny were grown and forced to self-fertilize using the methodology indicated above. Later, the F₂ obtained was also cultivated in the same way. Individuals of both filial generations were propagated and tested for sensitivity to glyphosate as described below.

2.5. Glyphosate sensitivity test

Seeds obtained by self-fertilization, cross breeding and inter-specific hybridization were germinated in Petri dishes containing

filter paper with distilled water when the dormancy period ended (6 months after harvest maturity). Germination was performed in a growth chamber with 75 μmol m⁻² s⁻¹ of photosynthetically active radiation, in a regime of 12/12 h of light/darkness and temperatures of 25°C/15 °C day/night, respectively. After 7 days, every seedling was transferred to a 500-cm³ pot with soil as substrate in a greenhouse.

When the plants had at least four tillers, they were propagated by tiller separation to obtain four propagules per plant. These were grown in 250-cm³ pots with soil. As indicated above, a propagule of every genotype was treated with 0.0, 0.75, 1.0, or 3.0 kg ae ha⁻¹ glyphosate at the stage of three to four tillers. Twenty days post-application, plants with severe visual injury (wilting, chlorosis of newly emerged leaves and general brownish colour) were recorded as 'controlled plants' (i.e., did not survive). In contrast, plants with green young leaves and without wilt symptoms were considered as 'survivors'. On this basis, plants with 'high glyphosate resistance' were those that survived at 3.0 kg ae ha⁻¹ glyphosate, plants with moderate glyphosate resistance were those that survived at 1.0 kg ae ha⁻¹ glyphosate and susceptible genotypes were those controlled at 0.75 kg ae ha⁻¹ glyphosate.

2.6. Inheritance models tested

Based on the segregation results, three levels of glyphosate-sensitivity were detected and the following inheritance models, which show three expected phenotypic classes, were postulated and tested:

1. One locus of incomplete dominance (*RR*: 'glyphosate susceptibility', *RR'*: 'moderate glyphosate resistance' and *R'R'*: 'high glyphosate resistance' in a 1:2:1 ratio, respectively).
2. Two interacting loci with susceptible, moderately glyphosate resistant and highly glyphosate resistant segregants: recessive epistasis where resistant genes are recessive (9:3:4, susceptible, moderate glyphosate resistance and high glyphosate resistance, respectively) or dominant (4:3:9, respectively); dominant epistasis where the resistance trait is recessive (12:3:1, respectively) or dominant (1:3:12, respectively); duplicate loci with cumulative effects where the resistance is recessive (9:6:1, respectively) or dominant (1:6:9, respectively) and two loci with additive effects where individuals with three or four resistant genes constitute one phenotypic class (high resistance), genotypes with two resistant genes form a class with moderate resistance and plants with one or no resistant alleles comprise a susceptible class (5:6:5, respectively).

2.7. Statistical analysis

Chi-squared (χ^2) tests were used to determine the goodness-of-fit of each model. Then, χ^2 tests for heterogeneity were also performed to examine whether different progeny from the same type of cross or self-fertilization displayed similar genetic behaviour ([Liu, 1997](#)).

3. Results

3.1. Inheritance of glyphosate resistance in *Lolium perenne*

Most plants obtained through self-fertilization of moderately resistant individuals showed the same glyphosate sensitivity as their mother plant ([Table 2](#)). Thus, the most frequent plants among the progeny were those that survived at 0.75 and 1.0 kg ae ha⁻¹ (moderate glyphosate resistance). Plants resistant to a dose of

Table 2

Segregation analysis of the glyphosate sensitivity of the offspring obtained from self-fertilized plants. Chi-square testing following an inheritance model controlled by one locus (*R*) of incomplete dominance with the 1:2:1 expected ratio. Chi-square analysis for heterogeneity testing different segregation ratios among the progeny ($P < 0.05$).

Self-fertilization		Susceptible plants	Moderately glyphosate-resistant plants	Highly glyphosate-resistant plants	Total	P
<i>RR' x RR'</i>	Ri10-1	10	16	4	30	
	Ri10-3	6	9	4	19	
	Ri10-5	6	23	10	39	
	Ri10-7	1	7	4	12	
	Ri11-1	7	10	2	19	
	Ri11-2	3	8	4	15	
	Ri13-1	4	7	2	13	
	Ri13-2	3	4	4	11	
	Ri13-3	3	5	4	12	
	Total	Observed	43	89	38	170
	Expected (1:2:1)	42.5	85	42.5		
χ^2					0.67	0.71
Heterogeneity					11.32	0.18
<i>RR x RR</i>	Sc13-1	16	0	0	16	
	Sc13-2	18	0	0	18	
	Total	Observed	34	0	0	34
	Expected (1)	34	0	0		
<i>R'R' x R'R'</i>	Re13	0	0	19	19	
	Total	Observed	0	0	19	
		Expected (1)	0	0	19	

Susceptible (*RR*): did not survive at a dose of glyphosate of 0.75 kg ae ha⁻¹.

Moderately glyphosate-resistant (*RR'*): survivor at a dose of glyphosate of 1.0 kg ae ha⁻¹.

Highly glyphosate-resistant (*R'R'*): survivor at a dose of glyphosate of 3.0 kg ae ha⁻¹.

0.75 kg ae ha⁻¹ glyphosate were also resistant to 1.0 kg ae ha⁻¹. Moreover, glyphosate-susceptible and highly glyphosate-resistant plants were found in the offspring (Table 2).

A model for the inheritance of glyphosate sensitivity based on a single locus with incomplete dominance was tested according to the segregation obtained (Table 2). The segregation of highly resistant plants (at a frequency of 1/4), moderately resistant plants (with a frequency of 2/4) and susceptible ones (with a frequency of 1/4) in the offspring fitted in the segregation expected for one Mendelian gene with incomplete dominance. In this way, there was no statistical evidence to discard the null hypothesis based on χ^2 tests ($P = 0.71$) and the heterogeneity analysis indicated that all nine progeny had a homogeneous segregation pattern ($P = 0.18$; Table 2). In contrast, the effect of two loci on the control of glyphosate resistance was discarded. The proportions of susceptible, moderately resistant and highly resistant plants did not fit in the two-locus models tested, considering resistance as a dominant or recessive trait (Table 3).

The segregation detected in the progeny obtained from the crosses between resistant plants at a dose of 1.0 kg ae ha⁻¹ of glyphosate (*R'R' x R'R'*) was similar to that obtained for self-fertilized plants with a moderately resistant phenotype (Table 4). In both cases, the segregation detected fitted with that expected for F_2 offspring.

In the crosses between moderately glyphosate-resistant plants (*R'R'*; *Re12* and *Ri13-1*) and susceptible ones (*RR*; *Sc12* and *Sc13-1*), the offspring showed the same parental phenotypes in a 1:1 ratio (Table 4). When a highly resistant plant (*R'R'*; *Ra11*) was crossed with a susceptible individual (*RR*; *Sa11*), the offspring was homogeneous with respect to glyphosate sensitivity (Table 4). All plants survived at a dose of 1.0 kg ae ha⁻¹ (moderate glyphosate resistance) and the genotype would be *RR'* (Table 4). In addition, the crosses of susceptible plants (*Sb11 x Sa11*) produced 100% glyphosate-susceptible individuals with no variations in glyphosate sensitivity (Table 4).

The progeny obtained from self-pollinated susceptible plants were 100% glyphosate-susceptible individuals (Table 2). Finally, the offspring obtained from a self-fertilized highly resistant plant showed no segregation and all plants survived at a dose of

Table 3

Segregation analysis of the offspring obtained by self-fertilization of moderately glyphosate-resistant plants (survivors at a dose of glyphosate of 1.0 kg ae ha⁻¹): number of expected and observed individuals (susceptible, moderately resistant and highly resistant plants, respectively) and *P*-values of chi-square testing following different inheritance models controlled by the interaction of two loci where resistance is a recessive or a dominant trait.

Two-locus models	Expected ^a	<i>P</i>
<i>Recessive epistasis:</i>		
Recessive resistance (9:3:4) ^a	95.6:31.9:42.5	<0.001
Dominant resistance (4:3:9) ^a	42.5:31.9:95.6	<0.001
<i>Dominant epistasis:</i>		
Recessive resistance (12:3:1) ^a	127.5:31.9:10.6	<0.001
Dominant resistance (1:3:12) ^a	10.6:31.9:127.5	<0.001
<i>Duplicate loci with cumulative effects:</i>		
Recessive resistance (9:6:1) ^a	95.6:63.8:10.6	<0.001
Dominant resistance (1:6:9) ^a	10.6:63.8:95.6	<0.001
Two loci with additive effects (5:6:5) ^a	53.1:63.8:53.1	<0.001
Observed ^a : 43:89:38		
Total: 170		

^a Ratio of susceptible, moderately glyphosate-resistant and highly glyphosate-resistant plants.

3.0 kg ae ha⁻¹ glyphosate (Table 2).

3.2. Interspecific hybrids between glyphosate-susceptible *Lolium multiflorum* and glyphosate-resistant *Lolium perenne*

After determining a model of inheritance of glyphosate resistance in *L. perenne*, we studied the possible transmission of the character via pollen from *L. perenne* to a hybrid offspring in a cross with *L. multiflorum*. In these crosses, only 20% of the plants produced viable seeds and ten hybrid plants were obtained.

The glyphosate sensitivity tested in the hybrid plants at the three tiller stage showed that every plant survived at a dose of 0.75 and 1.0 kg ae ha⁻¹ of glyphosate. However, 100% of the hybrids were controlled at 3.0 kg ae ha⁻¹. At the heading stage, other traits such as the number of spikelets per spike, number of florets per spikelet, and length of glumes and awns showed intermediate values in the hybrids (Table 5).

Finally, the second filial generation obtained from self-

Table 4
Segregation analysis of the offspring obtained from crosses: glyphosate sensitivity of the progeny produced and chi-square testing following an inheritance model controlled by one locus (*R*) with incomplete dominance. Chi-square analysis of heterogeneity testing different segregation ratios among the progeny ($P < 0.05$).

Cross-breeding		Susceptible plants	Moderately glyphosate-resistant plants	Highly glyphosate-resistant plants	Total	P
<i>RR' x RR'</i>	Ri11-1 x Ri11-2	13	24	11	48	
	Ri11-2 x Ri11-1	4	13	2	19	
Total	Observed	17	37	13	67	
	Expected (1:2:1)	16.75	33.5	16.75		
χ^2					1.20	0.54
Heterogeneity					1.94	0.16
<i>RR' x RR</i>	Re12 x Sc12	39	40	0	79	
	Ri13-1 x Sc13-1	7	5	0	12	
Total	Observed	46	45	0	91	
	Expected (1:1)	45.5	45.5	0		
χ^2					0.01	0.99
Heterogeneity					0.32	0.57
<i>RR x RR'</i>	Sc12 x Re12	21	27	0	48	
	Sc13-1 x Ri13-1	13	12	0	25	
Total	Observed	34	39	0	73	
	Expected (1:1)	36.5	36.5	0		
χ^2					0.34	0.84
Heterogeneity					0.45	0.50
<i>R'R' x RR</i>	Ra11 x Sa11	0	26	0	26	
	Observed	0	26	0	26	
Total	Expected (1)	0	26	0	26	
<i>RR x RR</i>	Sb11 x Sa11	17	0	0	17	
	Observed	17	0	0	17	
Total	Expected (1)	17	0	0	17	

Susceptible (*RR*): did not survive at a dose of glyphosate of 0.75 kg ae ha⁻¹.

Moderately glyphosate-resistant (*RR'*): survivor at a dose of glyphosate of 1.0 kg ae ha⁻¹.

Highly glyphosate-resistant (*R'R'*): survivor at a dose of glyphosate of 3.0 kg ae ha⁻¹.

pollinated hybrid plants showed susceptible, moderately resistant and highly resistant individuals in a 1:2:1 ratio, respectively (Table 6).

4. Discussion

Pioneering studies addressed the genetic control of a system of obligate allogamy in *L. perenne* that is controlled by a pair of genes called *S* and *Z* (Spoor, 1976; Cornish et al., 1979; Fearon et al., 1983; Lawrence et al., 1983). Despite this apparently strict system that prevents autogamy, self-compatibility is not unusual in this species (Thorogood et al., 2002). Currently, it is known that a gene called *T* restores self-fertility (Thorogood et al., 2005; Manzanares, 2013). This background supports the ability of some genotypes to produce viable seeds. In the current work, self-pollination was used to evaluate different models of inheritance of glyphosate resistance.

Considering the results obtained during three years of tests, self-fertilized moderately resistant plants produced three classes of phenotypes in 1:2:1 proportions: susceptible, moderately resistant and highly resistant plants, respectively (Table 2). These results suggest that glyphosate resistance could be controlled by a single locus with incomplete dominance.

Both self-fertilized susceptible and highly resistant plants produced homogeneous offspring, which consisted of susceptible and highly resistant individuals, respectively (Table 2). Thus, these phenotypes would be determined by a locus in the homozygous state.

Therefore, we tested the model of incomplete dominance in the offspring of several crosses among plants with different glyphosate sensitivity and found no significant differences between the expected and observed frequencies (Table 4). Moreover, maternal effects should not play an important role in glyphosate resistance, considering that not all of the progeny have the same phenotype as the maternal parent (Tables 2 and 4).

These results are consistent with evidences found by Lorraine-Colwill et al. (2001) in an Australian population of glyphosate-resistant *Lolium rigidum*. The authors suggested that glyphosate resistance is conferred by a single nuclear gene that is inherited in a semi-dominant manner, but that other genes may also contribute to plant survival at low doses of glyphosate (Lorraine-Colwill et al., 2001). However, in other biotypes of this species from California, the inheritance of glyphosate resistance appears to be multigenic and the most resistant plants survived at a dose 8-times higher than the field dose (1.12 kg ae ha⁻¹) (Simarmata et al., 2005).

On the other hand, Vargas et al. (2007) found that a *L. multiflorum* F₁ showed intermediate glyphosate sensitivity compared with its susceptible and glyphosate-resistant parents from Brazil. Moreover, when the F₂ population was obtained and treated with a dose of 0.72 kg ae ha⁻¹ glyphosate, a 3:1 ratio was found for resistant and susceptible plants, respectively. In this case, the authors indicated that a single gene controlled glyphosate sensitivity (Vargas et al., 2007).

Crosses between moderately resistant plants and susceptible ones produced progeny composed of moderately resistant and susceptible plants in a 1:1 ratio (Table 4). In addition, when a highly resistant individual was crossed with a susceptible one, the offspring was composed of a single phenotype with moderate glyphosate resistance. In this sense, neither glyphosate

Table 5

Morphological traits recorded on glyphosate-resistant *Lolium perenne*, glyphosate-susceptible *L. multiflorum* and hybrid offspring.

Trait	<i>L. perenne</i>	Hybrids	<i>L. multiflorum</i>
Spikelets per spike	17.5 (±1.3)	20.7 (±1.3)	27.5 (±1.5)
Florets per spikelet	5 (±0.3)	9.3 (±0.6)	13.5 (±0.8)
Length of glumes (mm)	11 (±0.9)	9 (±0.9)	7 (±0.5)
Awns	Absent	Short awns, only in apical florets	Present

Table 6

Segregation analysis of the F₂ offspring obtained from self-pollinated hybrids from glyphosate-susceptible *L. multiflorum* (Lm13) and glyphosate-resistant *Lolium perenne* (Re13): glyphosate sensitivity and chi-square testing following an inheritance model controlled by one locus (R) with incomplete dominance with the expected 1:2:1 frequency.

Self-fertilization		Susceptible plants	Moderately glyphosate-resistant plants	Highly glyphosate-resistant plants	Total	P
Hybrids (Lm13 × Re13)	Observed	16	38	12	66	
RR' × RR'	Expected (1:2:1)	16.5	33	16.5		
	χ^2				1.98	0.37

Susceptible (RR): did not survive at a dose of glyphosate of 0.75 kg ae ha⁻¹.

Moderately glyphosate-resistant (RR'): survivor at a dose of glyphosate of 1.0 kg ae ha⁻¹.

Highly glyphosate-resistant (R'R): survivor at a dose of glyphosate of 3.0 kg ae ha⁻¹.

susceptibility nor glyphosate resistance was dominant over the other. In connection with this, several populations of glyphosate-resistant *L. rigidum* have shown that the inheritance of resistance observed in dose–response experiments ranged from partial to total dominance (Wakelin and Preston, 2006).

Lorraine-Colwill et al. (2001) also found that the glyphosate-resistant population of *L. rigidum* exhibits strong resistance to normal field application rates of glyphosate when the resistance allele is present in either the homozygous or heterozygous condition. In this sense, the current results are consistent with those previously found. The glyphosate doses normally used to control ryegrass in Argentina are around 1 kg ae ha⁻¹, and plants with moderate or high glyphosate resistance could survive field applications.

In Argentina, the massive adoption of no-till practices and the increased utilization of glyphosate-resistant soybean crops are factors that have encouraged glyphosate use. The evolution of glyphosate resistance has clearly taken place in those agroecosystems where the herbicide exerts a strong and continuous selection pressure on weeds (Vila-Aiub et al., 2008).

Considering the current results on glyphosate resistance inheritance, the high level of allogamy of *L. perenne* and the possible gene flow via pollen (Giddings et al., 1997), the frequency of resistant plants will increase in a few generations under continuous herbicide selection pressure. This could explain the evolution of glyphosate resistance after 12 years of continuous use of glyphosate in the population studied and the spread of this problem in the region (Yannicari et al., 2012a). In this population, preliminary results have shown that the target site of the glyphosate (EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase) from glyphosate-resistant plants was as sensitive as the enzyme from glyphosate-susceptible plants and the resistance was associated with a higher EPSPS activity (Yannicari, unpublished results).

Glyphosate resistance is explainable by the nuclear genome and this phenotype is dominant over glyphosate susceptibility in the glyphosate-resistant weeds *Eleusine indica* and *C. canadensis* (Ng et al., 2004a; Zelaya et al., 2004). In these cases, the target-site mechanism of resistance detected in *Eleusine indica* and the selective sequestration of glyphosate into vacuoles (non-target site mechanism) observed in *C. canadensis* would be similarly inherited (Ng et al., 2004b; Ge et al., 2010). On the other hand, the variable number of EPSPS copies explains the different glyphosate sensitivities in resistant *A. palmeri* and could influence the type of glyphosate resistance inheritance, determining whether it is monogenic or polygenic (Chandi et al., 2012; Mohseni-Moghadam et al., 2013).

As indicated above, *L. multiflorum* and *L. perenne* are cross-pollinated species and their ability to hybridize spontaneously has been previously reported (Diekmann et al., 2012). The current results demonstrate that inheritance of glyphosate resistance can occur in hybrids between glyphosate-susceptible *L. multiflorum* and glyphosate-resistant *L. perenne*. These findings are important considering that both species are principally weeds of cereal crops

on most continents (Charmet et al., 1996) and that about forty cases of glyphosate-resistant *Lolium* spp. have been reported (Heap, 2015).

Improved knowledge of weed biology and ecology is essential for developing effective weed management tactics (Norsworthy et al., 2012; Chauhan and Gill, 2014). The present work warns that glyphosate resistance may be transmitted not only among *L. perenne* plants but also towards related species such as *L. multiflorum*.

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