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Evaluation of tetraploid big trefoil (*Lotus uliginosus* Schkuhr.) for rust resistance

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Introduction

Several species of *Lotus* are cultivated in Uruguay; however, the prevalence of diseases has only been thoroughly studied on birdsfoot trefoil (*Lotus corniculatus* L.; Altier, 1997). The widespread cultivation of susceptible cultivars almost always results in an increase of disease severity. The area of big trefoil (*Lotus uliginosus* Schkuhr.) has increased in recent years, mainly due to the good adaptation of the tetraploid cultivar Grasslands Maku. This synthetic cultivar developed in New Zealand is based on 13 elite plants selected after three generations of recurrent selection of an induced tetraploid developed by colchicine treatment (Charlton, 1983). Rust outbreaks caused by *Uromyces* spp. have been observed on this cultivar since 2000 and its severity ranged from trace to severe, where all the lower leaves were killed by the rust and upper leaves had open pustules (Ciliuti *et al*, 2003).

Rust on birdsfoot trefoil develops mostly in summer, from February to April (Altier, 1997), whereas the disease has been observed on big trefoil from November onwards (Ciliuti *et al*, 2003). Mild winters allow overwintering of the pathogen in Uruguay; therefore the disease may appear at any time during warm and wet weather conditions. Host specificity experiments have identified two races of rust on big trefoil in Uruguay (Ciliuti *et al*., 2003).

The recent appearance of rust on the main big trefoil cultivar utilized in Uruguay and the lack of information of the pathogen reaction within the tetraploid cultivar raised the need to study its genetic variability and to compare the effectiveness of field selection for rust tolerance with selection under artificial inoculation in greenhouse conditions.

Materials and Methods

Big trefoil germplasm utilized in this study came from seed stocks of tetraploid cultivar Grasslands Maku harvested either in New Zealand (NZ 1 to NZ 3) or Uruguay (UR 1, UR 2), and a sample of the diploid accession G4701 (D) from New Zealand that gave origin to the tetraploid cultivar (Charlton, 1983). An experiment was set on the Forage Nursery Experimental Field at La Estanzuela, Colonia, Uruguay. Plots of 25 plants, spaced 1 m apart, were distributed in a complete randomized block design with 10 replicates. Seedlings were established in the greenhouse in June 2000 and transplanted to the field on the second week of August 2000.

Individual plants were characterized in the field during the second year. Date of flowering was recorded twice a week (coded as initial date 1= November 27 2001 to 14= January 28 2002). Plants were individually harvested on September 19 2001 to evaluate early spring forage yield. Mature pods were harvested twice a week from the first week of January to the end of February and the seed accumulated for total yield. Rust was scored in mature plants during the second week of December, when epidemic was evenly established and the most susceptible plants showed symptoms of the disease in all leaves. Plants were rated on a 1 to 5 visual scale (1 – no foliar symptoms, 2 – few pustules on lower leaves, 3 – many pustules on lower leaves, 4 – pustules in mid leaves, 5 – pustules in all leaves). Seed stocks were compared on a plot-mean basis and the association between rust and other characteristics was estimated.

Selected plants were vegetatively multiplied in March 2002. Chromosomes dyed with acetocarmine were counted from the root tips of these plants to verify the ploidy level after conservation in a 3:1 solution of alcohol and acetic acid in the fridge (Sass, 1958). Tetraploid plants were separated into three groups, according to rust resistance: MR - moderately resistant (classes 1 and 2), MS – moderately susceptible (class 3) and S - susceptible (classes 4 and 5). Diploid plants with rust scores between 1 and 2 were grouped in a forth category (D). Each group was polycrossed by manual pollination and seed harvested from individual plants. Rust resistance was evaluated for each half-sib family, artificially inoculating at least 40 seedlings distributed in 10 pots. Urediniospores from cv Grassland Maku were collected at La Estanzuela, Colonia. Rust collection and inoculation procedures were described by Ciliuti *et al* (2003). Inoculation was carried out on January 2 2003 at the seedling stage (5 to 8 leaflets). First symptoms of the disease appeared 10 days after inoculation. Rust was evaluated 14 and 17 days after inoculation (13 January and 16 January) in the most severely infected leaf per individual seedlings. The scale described by Skinner and Stuteville (1995) was used: 1 - resistant (no symptoms), 2 - moderately resistant (flecks and closed pustules), 3 - moderately susceptible (closed pustules and small open pustules), 4 - susceptible (small open pustules), 5 - highly susceptible (medium to large open pustules). Plants recorded as class 1 and 2 were inoculated again on February 11 2003. Polycross groups were compared on a half sib family mean basis.

Results and Discussion

Field observations of the pathogen have been reported on several *Lotus* species in the region (Altier, 1997; Ciliuti *et a*l, 2003; Juan *et al*, 2000). The disease severely damaged spaced plants of Grassland Maku in 2002 (Figure 1). Uredinia were mostly found on leaflets and stems, which turned yellow and drop or dried off (Figure 2). Maximum damage was observed during dry spells, when vegetative growth was stunted and pustules developed up to the top leaves. Seed stocks were ranked based on the mean rust leaf ratings averaged over 10 replications (Table 1). Rust ratings were lower ($P<0.01$) in one of the seed stocks of G.Maku harvested in Uruguay (UR 2) and the diploid accession (D). The latter was easily recognized by its small leaves and late flowering, in addition to the lower rust ratings compared with Grassland Maku. Based on the rust ratings, 28% of plants were resistant (class 1) for the diploid, whereas the resistant plants ranged from 6 to 20% within G.Maku seed stocks (Figure

3). With the exception of UR 2, all seed stocks of G.Maku had 50% or higher proportion of susceptible and highly susceptible plants (classes 4 and 5). Despite the differences in rust rating under field conditions, there were no differences in other evaluated traits for the tetraploid seed stocks (Table 1).

Figure 1. Spaced plants in early December.

Figure 2. Foliar damage caused by rust.

Table 1. Field rust ratings, date of flowering, spring forage yield (g DM per plant) and seed yield (g per plant). Average data for seed stocks.

Seed stocks	Rust ratings	Date of flowering	Forage yield	Seed yield
NZ 1	3.62 a	7.23 bc	19.3	3.6
NZ 2	3.61 a	7.54 b	17.7	3.0
NZ 3	3.46 a	7.06 bc	18.1	2.9
UR 1	3.55 a	6.88 c	15	4.2
UR 2	2.86 b	7.57 b	17.5	3.0
D	2.78 b	9.33 a	9.2	2.0
F-test	0.003	<.001	n.s.	n.s.
s.e.d.	0.280	0.315	3.97	0.94

For each column, any value with the same letter were not significantly different (P=0.05) with Fisher LSD test.

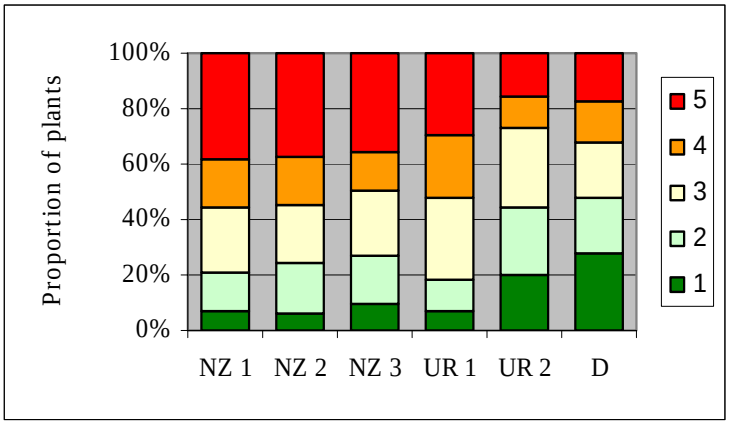

Figure 3. Proportion of plants with rust scores within the different seed stocks (Scale 1 – resistant to 5 – highly susceptible).

Severe damage caused by foliar diseases usually affects plant performance. Barbetti and Nichols (1995) studied forage yield and seed losses for several cultivars of *Trifolium subterraneum* associated with rust scores. However, in this study rust severity was not correlated ($P<0.05$) with growth or seed production, despite the fact that the disease progress throughout all the flowering period. This result probably reflects the low potential for seed production that the tetraploid cultivar has shown in Uruguay (Formoso, 2001). Seed yield correlated with forage yield ($r= 0.65$, $P<0.05$) and date of flowering ($r= -0.43$), $P<0,05$), indicating higher seed production in larger plants with early flowering.

The severity of foliar diseases under field conditions could be affected by factors such as the physiological stage and the microenvironment of the plant (density of foliage), climatic conditions, etc. Emery and English (1994) demonstrated that the incidence and severity of alfalfa foliar diseases, including rust, depended on moisture conditions and so, varied significantly along the growing season. Disease on the lower half of the plant was correlated with leaf wetness duration and atmospheric moisture conditions while disease on the upper half of the plants was correlated with cumulative rainfall. Furthermore, climatic factors such as temperature altered the genetic behavior of alleles involved in alfalfa resistance to rust (Skinner and Stuteville, 1989). In this field study, rust rating was negatively correlated with flowering date ($r= -0.39$, $P<0.05$), indicating that the disease severity was higher in early materials. Since rust was evaluated only once during the growth season in this study, it is not possible to attribute this relationship to either environment or physiological stage.

Variation for field rust rating suggested that opportunities may exist for improvement of rust resistance in the tetraploid cultivar (Figure 3). However, since rust severity could have been affected by the interaction of plant physiological stage and environmental conditions previous to the field evaluation, progenies of selected plants were further tested with artificial inoculation. The selected groups exhibited different patterns of rust scoring under artificial inoculation. The dipliod had lower values of rust score ($P<0.001$) than all three tetraploid

groups 14 days after inoculation (Table 2), indicating a similar ranking as in the field evaluation. However, three days later rust score was over 4 for all groups giving a different group ranking. Although there were significant differences (P<0.001) between groups at this stage, all of them were ranked susceptible (class 4) to highly susceptible (class 5) for most of the plants. Few plants classified as moderately resistant (class 2) in the first inoculation, were reclassified as susceptible after the second inoculation. These plants may have not been properly inoculated previously because their foliage was covered by other plants.

Table 2. Average data and range of rust rating at 14 and 17 days after artificial inoculation (Scale 1 to 5) for half sib families within crossing groups.

	Day 14		Day 17	
	Mean	Range	Mean	Range
S	3.53 a	3.41-3.62	4.36 b	4.20-4.67
MS	3.54 a	2.46-4.52	4.68 a	4.40-4.96
MR	3.50 a	2.65-4.42	4.20 b	3.37-4.84
D	2.80 b	2.38-3.03	4.84 a	4.48-5.0
F- test	<.001		<.001	
sed	0.14		0.09	
cv	26.4		13.5	

For each column, any value with the same letter were not significantly different (P=0.05) with Fisher LSD test.

Genetic resistance is the only practical method to deal with diseases in perennial forage species in the region. The availability of genetic diversity and a proper method of evaluation and genetic diversity are the basic tools to select for acceptable levels of resistance. The results of the present study indicate that phenotypic evaluation of rust severity in the field is not an appropriate method to identify resistant genotypes. This is possibly due to the existence of an important environmental effect that generates microenvironment variability in the field that hides the genotypic performance. The artificial inoculation made under uniform environmental conditions and performed at a uniform physiological stage (seedlings is more appropriate for the characterization of genetic resistance to *Uromyces* than field evaluation.

Genetic uniformity can result in *Lotus* cultivars being vulnerable to the increase and spread of *Uromyces* pathotypes with virulence to deployed resistance genes. The present study determined that G.Maku was susceptible to the rust strain collected in commercial fields, since all half-sib families tested had high values of rust ratings. This uniformity could be explained by the narrow genetic base of this cultivar (Charlton, 1983). The disease has not been reported in New Zealand, where the tetraploid cultivar was bred. Therefore, selection did not take place during the breeding process in the absence of the pathogen.

No reference about genetic resistance to rust in *Lotus uliginosus* has been found in the literature. However, *Medicago* and *Trifolium* rusts are good examples of case studies where resistance has been identified and the genetic basis has been determined (Diachun and Henson, 1974; Engelke et al, 1977; Elgin *et al*, 1988; Barbetti and Nichols, 1991). Future

studies should involve accessions from different geographical origins in order to expand the possibilities to identify genotypes with rust resistance, since this research only involved two accessions. Breeding in Uruguay and neighbouring countries is likely to be based on parents adapted to the regional specific environment. However, this study further emphasises the need to have variability in the basis of effective genetic resistance, which reduce the selection for virulence in the pathogen and minimize the possibility of appearance of a pathotype that affect all cultivars at the commercial level.

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