Development of new tetraploid *Chloris gayana* cultivars with improved salt tolerance from 'Callide' and 'Samford'

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Abstract

Chloris gayana (Rhodes grass) is an outbreeding species with both diploid and tetraploid genotypes. Repeated mass selection for improved salt tolerance during germination and during growth and for forage quality was applied to 3 breeding populations derived from the tetraploid cultivars 'Callide' and 'Samford'. Three new synthetic tetraploid cultivars, each based on elite single-plant clones selected from the final generation, were produced. 'Sabre' (10 clones, 5 generations) and 'Toro' (13 clones, 4 generations) were derived from 'Callide', while 'Mariner' (12 clones, 4 generations) was derived from 'Samford'.

Introduction

Rhodes grass (*Chloris gayana*) is among the more salt-tolerant subtropical/tropical pasture grasses, and is a major forage species, both in Australia and overseas (Loch *et al.*, 2004). It is well adapted to a wide range of medium-textured soils in the 600-1500 mm rainfall belt in the subtropics and tropics, and has been widely sown for the past 100 years in grazed pastures or short-term pasture leys, for hay or silage production, and for soil conservation and remediation. Rhodes grass is an outbreeding species with a great deal of between-plant variation in salt tolerance and other agronomic attributes, which could be exploited by breeding to tap market opportunities for salt-tolerant grass cultivars with good agronomic characteristics. The commercial success of 'Finecut' (bred in the early 1990s) demonstrates the gains that can come through targeted selection for improved agronomic attributes.

Over the past 30 years, the tetraploid cultivar 'Callide' has become the premier grazing type among the current Australian Rhodes grass cultivars, and seed is also sold into the Middle East where it is grown under irrigation for fresh forage and hay. Despite this, there has been no formal breeding work on 'Callide', which is quite variable in terms of flowering time, growth habit, leafiness, coarseness of stem, and many other agronomic attributes. Similarly, 'Samford' (which also produces high quality forage, but has not achieved the same market penetration as 'Callide') is derived from East African germplasm represented internationally by 'Boma' and 'Elmba'. This paper reports on a breeding program to improve salt tolerance while at the same time selecting for improved agronomic attributes in 'Callide' and 'Samford'.

Materials and Methods

Breeding Strategy

Mass selection was applied to seedling populations derived from 'Callide' and 'Samford' Rhodes grass (2001-06) with the aim of improving both the level of salt tolerance and the agronomic attributes. Based on first generation performance, seedlings selected from 'Callide' were divided into two breeding populations: one comprising leafy, early flowering (less daylength-sensitive) plants with a preference for fine-stemmed types; and the other based on leafy, late flowering plants, rejecting only the very coarse-stemmed types. Selection within the single 'Samford' population focused on leafy, late-flowering, finer-stemmed genotypes.

In the first 'Callide' generation, selection for salt tolerance related only to plant growth and survival under high salinity, and was followed by selection for improved agronomic characters. Selection for germination under saline conditions was then added, giving a three-stage selection process for generations 2-4(-5) in 'Callide' and for generations 1-4 in 'Samford': (i) germination under saline conditions; (ii) growth and survival under saline conditions; and (iii) agronomic performance under non-saline field conditions.

Breeding Procedures

2000 'Callide' seedlings were grown individually in a peat-vermiculite mix in 50 mm square tubes. At early tillering, the lower one-third of the tube was immersed in a NaCl solution, and the concentration progressively raised (0.2 M every 2-3 days) to 0.7 M NaCl, which was maintained for c. 2 months during which approximately 85-90% of the seedlings died. The surviving plants were then established on a spaced-plant grid for field agronomic evaluation.

Because salt tolerance for germination and salt tolerance for growth tend to be unrelated, screening for germination under saline conditions was applied to generations 2-4(5) in 'Callide' and generations 1-4 in 'Samford'. Preliminary trials showed that germination was progressively delayed (by osmotic effects) and then reduced as the level of salt increased, reaching virtually nil germination by the 0.3 M NaCl level. At the start of each new generation, seed of the 3 breeding populations from the preceding generation was sown and lightly covered by sand in a "flood-and-drain" hydroponic system with 0.2 M NaCl plus complete plant nutrients. Despite heavy seeding rates, low numbers of germinated seedlings were obtained due to the heavy selection pressure exerted for salt tolerance. Following germination, the trays of young seedlings (up to c. 1-2 cm tall) growing in sand were placed in salt solutions to c. 3 cm depth. Salt levels were progressively increased to 0.7 M NaCl, and the most visibly tolerant seedlings later transferred to the field as spaced plants.

Results and Discussion

Following 4 (or 5) cycles of selection, 3 new synthetic Rhodes grass cultivars (each based on 10-13 elite plants selected from the final generation) were constituted as shown in Table 1. In each case, the selected single-plant clones were divided vegetatively and established as balanced polycross breeder's blocks (each 500-1000 m^2, with the component clones each contributing equally to the overall cultivar) for seed multiplication in isolation from all other Rhodes grass plants in north Queensland. The aim was to reduce the risk of genetic drift by taking only 2 of generations of seed increase to produce commercial seed.

Table 1. New synthetic Rhodes grass cultivars constituted following repeated mass selection.

Cultivar	No. of clones	Origin	No. of generations	Agronomic selection criteria
Sabre	10	Callide	5	Early flowering, dense, leafy growth habit; fine stems
Toro	13	Callide	4	Late flowering, dense, leafy growth habit
Mariner	12	Samford	4	Late flowering with a dense, leafy growth habit; fine stems

A growing trial to describe the new cultivars for Plant Breeder's Rights registration (Loch and Zorin, 2009) showed that 'Toro' and 'Mariner' are later-flowering and 'Sabre' earlier-flowering than 'Callide' and 'Samford'. Probably because of selection against extremely prostrate genotypes, 'Sabre' and 'Toro' spread laterally a little slower than 'Callide' and on average are more erect in habit. They produce stolons with shorter, thinner internodes and more branches at the nodes, and thinner culms than 'Callide'. 'Sabre' produces slightly shorter culms with fewer nodes and longer leaves than 'Callide' and 'Toro', and does not exsert its inflorescences as far above the leaf canopy as the latter two cultivars. Stems of 'Mariner' are thicker than for 'Samford' (reflecting the removal of the finer-stemmed early-flowering genotypes through selection) and comparable to 'Sabre' and 'Toro'. The last 2 cultivars produce smaller inflorescences with fewer raceme branches and ('Toro' only) shorter racemes than 'Callide'. 'Mariner' produces larger inflorescences with longer racemes than 'Samford, comparable in overall size to 'Sabre', but with more (though shorter) racemes.

Additional work is on-going to document the levels of salt tolerance achieved through selection in the 3 new cultivars, both under controlled glasshouse and laboratory conditions and in the field. In this respect, the gains in salt tolerance reported by Malkin and Waisel (1986) from 5 generations of mass selection from 'Pioneer' Rhodes grass are encouraging.
Plants vary in salinity tolerance at different developmental stages. However, available evidence from other species (e.g. Mano and Takeda, 1997) suggests that plants tolerant of salt during germination may not necessarily show a comparable level of tolerance during growth (and vice versa), hence the two-stage selection process for salt tolerance applied from the second 'Callide'

generation onwards. Other breeders have adopted similar approaches when selecting for salt tolerance (e.g. Rose-Fricker and Wipff, 2001; Rose-Fricker *et al.*, 2003).

In conclusion, it is interesting to speculate as to the possible mechanism(s) contributing to higher salt tolerance of Rhodes grass. While a number of possible factors have been identified (Loch *et al.*, 2004), the density of salt glands may be important as suggested by de Luca *et al.* (2001). This is supported by counts of salt gland density made on the selected plants retained from each of the 5 breeding generations, showing that the density of salt glands in each case had been increased 2- to 3-fold by selection for salt tolerance (Figure 1). Nevertheless, more detailed studies of this and other salt tolerance mechanisms in Rhodes grass will obviously remain a fruitful avenue for other researchers in the future.

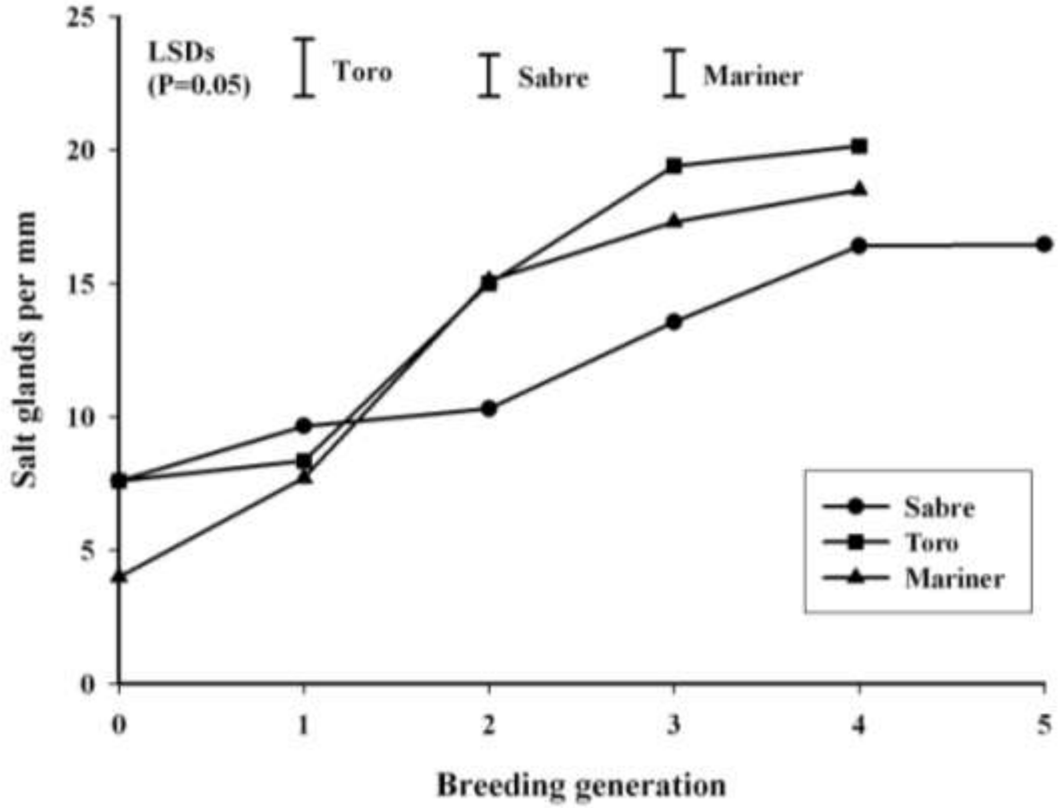

Figure 1. Changes in salt gland density in 3 Rhodes grass breeding populations over 4 or 5 generations of selection for salt tolerance.

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