

## Developing well adapted *trifolium subterraneum* ssp. *brachycalycinum* cultivars

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**Summary.** At present there are only three commercial cultivars of *Trifolium subterraneum* ssp. *brachycalycinum* (Clare, Rosedale and Nuba). The overall aim of the ssp. *brachycalycinum* programme is to develop and release well adapted, early to mid-season cultivars of ssp. *brachycalycinum* which are persistent in farming systems and resistant to pests and diseases. Seventeen crosses have been made resulting in approximately 800 crossbred lines. The parents used for the crosses were selected for their earliness, general good herbage and seed production and strong winter vigour. Preliminary characterisation of breeding lines in 1991 and 1992 has shown that early flowering, strong winter vigour and good spring production have been recovered from many crossbred lines. Comparisons of F2-bulk, F2-progeny and pedigree selection methods will also be examined.

### Introduction

Despite the success of subterranean clover (*Trifolium subterraneum* L.) in southern Australia, pasture scientists have acknowledged the deficiencies associated with existing cultivars (4, 6 and 7). The potential for improvement in the subspecies *brachycalycinum* group has been identified (1, 4, 6 and 7). At present there are only three commercial cultivars from the ssp. *brachycalycinum* group (cvv. Clare, Rosedale and Nuba). Specific deficiencies identified were the need for greater hardseededness and persistence for low rainfall zones and the ability to regenerate following cropping (4, 6, 7 and 9).

Well adapted early flowering cultivars of ssp. *brachycalycinum* could provide a major pasture legume in cereal-livestock farming systems of central South Australia and in the long-term pastures of northern New South Wales (1 and 2). The potential area of use is 5 million hectares of neutral to alkaline red-brown soils. In NSW the main target soil type is the vertisols and in SA it is the red-brown earths.

A national programme has been established specially for the breeding and selection of ssp. *brachycalycinum*. The selection and evaluation activities of the South Australian Research and Development Institute (SARDI), NSW Agriculture and the Australasian Subterranean Clover and Alternative Legume Improvement Programme (ASCALIP) in Perth have been integrated.

Two breeding methods (F2-bulk and F2-progeny) are being compared (commenced 1992) with the traditional pedigree method which is currently being used. The discussion paper by Nichols (8) provides good background information and an explanation of the three breeding methodologies. Basically the F2-progeny and F2-bulk methods use natural selection in farming systems (including grazing and cropping in early generation selection) as a tool for the selection of suitable lines. This will lead to the selection of more persistent cultivars for the environment and farming systems for which they are intended.

The selection criteria for ssp. *brachycalycinum* lines in South Australia are earlier flowering, more hard-seeded, persistent, disease and insect resistant and vigorous lines than Rosedale and Clare. In northern NSW, seedling vigour, dry matter production and seed production are considered the most important criteria (3), while early flowering is of less importance.

The improvement programme of ssp. *brachycalycinum* has two main components: (i) seed increase and early generation selection of lines; and (ii) the comparison of three selection procedures (pedigree, F2-bulk and F2-progeny).

### Methods

### *Parentage and seed increase*

Seed from approximately 800 F3 ssp. *brachycalycinum* lines of F2 selections was sent from Perth, WA to Turretfield Research Centre (TRC), SA. These lines are the result of 8 crosses made in 1988 and 9 crosses made in 1989, as listed below:

<u>1988</u>		<u>1989</u>	
<u>Cross</u>	<u>Parentage</u>	<u>Cross</u>	<u>Parentage</u>
88B37	19451/24417	89B60	70056B/24417
88B40	70056B/19451	89B61	70100/19451
88B42	70056B/70100	89B63	70100/62325
88B43	Rosedale/19451	89B64	70100/88B37
88B44	Rosedale/70056B	89B65	Rosedale/24415
88B45	Rosedale/70100	89B67	Rosedale/Clare
88B46	Rosedale/24417	89B68	Rosedale/88B37
88B47	Rosedale/3498	89B71	19451/24415
		89B72	19451/62325

The Turkish introductions CPI 70056B, CPI 70100 and the cultivar Rosedale (CPI 70124) were selected as parents due to good herbage and seed production in South Australian trials (5). Early flowering parents CPI 19451, CPI 62325, CPI 24415 and CPI 24417 are Israeli introductions. The Sardinian introduction 3498 was chosen for its vigour.

Seed increase and preliminary characterisation of 1988 and 1989 crosses was undertaken during 1991 and 1992 respectively at TRC, SA. Lines were scored for winter vigour, days to flowering from sowing date and spring production. All lines have been screened for low formononetin at the isoflavone laboratory in Perth, WA.

### *Evaluation in NSW*

A subset of F4 seed from 1988 crossbred material and early flowering Israeli introductions was sent to Greg Lodge, Tamworth, New South Wales in 1992 for regional selection in Tamworth, Walgett, Condobolin and Narrabri regions. Selection of crossbred lines for the subset was based on higher winter vigour score and spring production than Clare and Rosedale. A further subset of the 1989 crossbred material will be sent in 1993.

### *Selection and evaluation procedures in SA*

Three early generation selection procedures are being compared (F2-bulk, F2-progeny and pedigree) at TRC, trials commenced in 1992. Currently, the ASCALIP uses the pedigree method which is labour intensive and in addition plants are grown in undefoliated rows, hence there is no selection for grazing tolerance. In contrast natural selection is the major selection tool for F2-progeny and F2-bulk methods.

F2-bulk involves growing unselected crosses (i.e. a cross is made and the seed pooled) in swards in the target environment. Swards are subjected to selection pressures such as grazing and cereal cropping. Swards will be grown for eight years (F2 - F9). At the end of the eighth year, burrs will be sampled at random. Burrs will be then sown out into rows for the next 2 years for the selection of superior lines. The theory behind random burr sampling is that the most successful genotypes would have the highest proportion of burrs and therefore they will have a greater chance of being in the final selection stage.

F2-progeny is intermediate to pedigree and the F2-bulk method. Crossbred lines are identified from each cross (F2), they are then assessed in rows for one year (F3). For the next 3 to 4 years (F4 - F7) crossbred lines are sown in small sward plots (1.5x2 m). Similarly to the F2-bulk method, swards undergo natural

selection pressures. Burrs are sampled at random in the last year while lines are growing as swards. Burrs are then sown out into rows for the next 2 years (F7 - F8) for the selection of superior lines.

## Results and discussion

### *Seed increase and preliminary characterisation*

Caution is required when examining preliminary results from 1991 and 1992 because plants were grown on black plastic primarily for seed increase. However, the results do provide an indication of the relative performance between lines within those years. Future results from sward trials may support some of the preliminary characterisation data.

**Table 1: Average winter vigour score (measured 26.7.91 for 1988 crosses and 13.8.92 for 1989 crosses), spring production score (measured 18.10.91 for 1988 crosses and 22.10.92 for 1989 crosses) and days to flowering (from sowing date. 29.4.91 for 1988 crosses and 30.4.92 for 1989 crosses) for each cross.**

Cross	Winter vigour score (0-10)	Spring production score (1-5)	Days to flowering Average
<u>1988 Crosses</u>			
88B37	3.7	1.9	108
88B40	4.4	2.5	115
88B42	3.9	2.8	119
88B43	4.7	3.0	111
88B44	4.3	3.2	123
88B45	4.9	3.7	120
88B46	5.2	3.4	112
88B47	5.2	3.9	130
<u>1989 Crosses</u>			
89B60	3.1	2.9	124
89B61	3.5	3.0	116
89B63	3.9	3.3	118
89B64	4.1	3.6	116
89B65	3.6	2.8	117
89B67	3.9	3.1	133
89B68	3.9	3.1	119
89B71	4.8	3.3	107
89B72	5.3	3.6	110

1988 Crosses. Winter vigour score was measured on a scale 1 - 10, the greatest vigour = 10. Lines which had a winter vigour score 6 and greater represented 9.6% of the total number of lines (Clare and Rosedale scored 5.7 and 5 respectively). The most successful cross in terms of winter vigour was 88B46. Twenty-five percent of lines within this cross had winter vigour scores 6 and greater (Table 1).

Twenty-eight percent of the total number of lines had a spring production score 4 and greater (visual scale 1 - 5, the highest spring production = 5). Exceptional spring production was found within the crosses 88B45 and 88B47 (Table 1). The percentage of lines within these crosses which scored 4 and above were 67% and 63% respectively.

Early flowering was successfully incorporated into the lines. Sixty-two percent of all lines flowered before Rosedale. Rosedale is currently the earliest flowering cultivar of ssp. *brachycalycinum* (TRC, 1991, flowered 122 days after sowing in 1991). Ninety-c -ht percent of all lines flowered before Clare (TRC,

1991 = 136 days in 1991). The four earliest lines flowered 97 days after sowing. Three of the lines were from the cross between two early parents (19451/24417 = flowered 103 and 99 days after sowing respectively in 1991, TRC). The remaining line was tam. cross Rosedale and 19451. The mean days to flowering for the crosses ranged from 108 to 130 days (Table 1). In addition to recording the days to flowering for lines exceptionally early individuals within a line were identified and tagged. Seed from these individuals was harvested separately and grown on next season (1992) to ascertain whether they were early flowering.

1989 Crosses. Fourteen percent of the total number of lines scored 5 and greater for winter vigour (scale 1 - 10). Clare and Rosedale had only scored 4 and 3.5 respectively during the seed increase year of 1992. The cross 89B72 had the highest proportion (73%) of lines which scored 5 and greater. The parents of that cross are both early maturing (19451 and 62325 = flowered 108 and 110 days after sowing respectively in 1992, TRC).

Lines which scored 4 and greater for spring production represented 32.9% of the total number of lines (scale 1 - 5, Rosedale and Clare scored 4 and 4.5 respectively).

Early flowering was incorporated in the majority of lines; 98.9% flowered before Rosedale (136 days, TRC, 1992) and 99.7% flowered before Clare (144 days, TRC, 1992). The earliest line flowered at 94 days (89B65\*35). The parents of that line are Rosedale and 24415, and the next four earliest flowering lines have the same parentage. Twenty percent of the total number of lines flowered between 94 - 110 days. This maturity range includes all the early flowering parents which were also grown out on black plastic in the seed increase area at TRC in 1992; 24415 (103 days), 24417 (108 days), 19451 (108 days), 62325 (110 days) and the backcross 88B37 = 19451/24417 (110 days). Overall mean days to flowering for each of the nine 1989 crosses (107 - 133 days, Table 1) were very similar to the eight 1988 crosses. A total of 57 early flowering individuals have been identified and isolated from within lines, these will be checked next year (1993), to determine whether they are consistently early flowering.

#### *Selection and evaluation procedures*

Selection and comparison of the three selection procedures commenced in 1992 in cereal/sheep regions on neutral to slightly alkaline soils of central South Australia. Cropping and grazing of the swards by sheep will be an integral part of the selection process.

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