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INTERSPECIFIC HYBRIDIZATION IN CENTROSEMA: HYBRIDS BETWEEN C. BRASILIANUM, C. VIRGINIANUM AND C. PUBESCENS

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SUMMARY

Steps taken to develop interspecific hybrids from the crosses *C. brasilianum* x *C. virginianum* and *C. brasilianum* x *C. pubescens* are described.

The close relationship between these species of *Centrosema* is indicated by the ease of hybridization and by the fact that the F_1 hybrids were viable and fully fertile.

The growth of the hybrids, particularly of *C. brasilianum* x *C. virginianum*, was superior to that of the parents and heterosis effects were evident in greater stolon development and in better disease and insect resistance.

The marked improvement shown by hybrid derivatives of the *C. brasilianum* x *C. virginianum* cross over commercial centro and other accessions of this legume justify efforts to develop it as a commercial variety. Approaches to achieve this objective are discussed.

I. INTRODUCTION

Centrosema pubescens Benth., commonly known in Australia as centro, was the first summer-growing perennial tropical legume grown to any extent in Queensland. It has become a persistent and productive pasture legume with guinea and other grasses in the high-rainfall areas of North Queensland.

Standard centro has a number of deficiencies. These are slow initial growth, poor production during the cool season and susceptibility to disease and insect attacks.

Hutton (1960) examined the breeding system of several tropical legumes and classed *C. pubescens* as cleistogamous. *C. brasilianum, C. virginianum* and the accession *C. pubescens* Q 8397 (Queensland Plant Introduction No.) included in this study were also found to be cleistogamous. Self-pollination as the normal mode of reproduction explains the marked intra-varietal uniformity observed in these three species of *Centrosema.*

Hutton suggested that, with autogamous tropical legumes, improvement may be achieved by examining a wide range of ecological races within the species and by combining desired characteristics by hybridization. Results of recent work have shown considerable variation between standard centro and new introductions in several important forage characters, and accessions with greater seasonal as well as greater total production have been isolated in North Queensland (Grof and Harding 1970).

This paper reports the results of a breeding investigation designed to study the possibility of hybridization between *C. brasilianum, C. virginianum* and *C. pubescens,* with the ultimate aim of combining the desirable characteristics of these closely related species in a stable hybrid.

The investigations were carried out at South Johnstone (latitude 17°36'S.) on the humid tropical coast of north Queensland under conditions which are typical of large areas of tropics in both hemispheres. This area has a mean annual temperature of 73.2°F. The hottest months are December, January and February, with temperature maxima of 87.4, 85.6 and 87.2°F respectively. Temperatures may fall as low as 41°F in winter. Temperature minima for the coldest months—June, July and August—are 60.0, 56.7 and 57.1°F respectively. The mean annual rainfall is 127 in.

II. MATERIALS AND METHODS

The parent species.—All species of *Centrosema* are exclusively tropical American in origin and they are prostrate, twining herbs or subshrubs. Full botanical descriptions of the species used in the hybridization programme were given by Bentham (1859).

Burkart (1943, p. 377) described five species, including *C. virginianum* and *C. brasilianum,* which occur in northern Argentina. *C. virginianum* also occurs in Uruguay and Brazil and it is adventive to the southern parts of North America, where it is occasionally cultivated as an ornamental creeper (Bailey 1935, p. 174).

C. pubescens is a variable species. It is widely distributed from tropical Brazil through Central America to South Mexico. The stems are twining, the young ones lightly pubescent. It differs from *C. virginianum* in having shorter calyx teeth. The leaflets are elliptic lanceolate in shape, often shortly acuminate and finally pubescent. The standard is 2.5 cm, roughly circular. Flower colour may vary from pure white to pale mauve, with a strip of cream and purple striae down the centre. The keel is approximately 1.8 cm in length and 12 mm in depth, pale mauve in colour. The stamens are enclosed in a whitish sheath with one free. Mature pods usually attain a length of 15-20 cm and a width of 5-6 mm, tapering to an acuminate style. They may carry as many as 20 seeds.

C. brasilianum (L.) Benth. is a vigorous plant with tuberous rhizomes and trailing stems. The leaflets are typically rounded. The standard is orbicular, 3-3.6 cm broad, white, bluish violet or pink in colour and very finely pubescent outside. The pods are 7·5-10 cm long, 5 mm broad, with longitudinal ribs about 1 mm from the sutures. Seeds are transversely oblong; fresh seeds are olive green in colour, turning light brown with age. Ducke (1949) mentioned that *C. brasilianum* is common in the interior regions of the Amazon. He described the typical form with pink corolla and recorded a white-flowered variety which occurs on the flood plains of the Amazon river. Both forms were introduced to Queensland from Brazil and were used in crosses at South Johnstone.

C. virginianum (L.) Benth. is a species easily distinguished from the preceding ones by its slender habit. The stems have a strong tendency to root at the nodes. The leaflets are 2.5-5 cm long, ovate lanceolate. The lobes of the calyx are usually at least twice as long as the tube. The standard is 2.5 cm, broad bluish-violet or white in colour, adpressed pubescent outside. The white-flowered Brazilian form was introduced under the Queensland introduction number of Q 8998 and it was used in the crossing programme.

Hybridization.—The flower buds of the three *Centrosema* species used in this study are large and hand emasculation and pollination were conveniently effected in the field. A technique similar to that described by Boling, Sander and Matlock (1961) for mung bean hybridization was used. Crossing was carried out at both 9 a.m. and 4 p.m. on overcast, humid days.

The standard of the fully expanded bud was split down the centre with a curved forceps by carefully withdrawing the forceps in a direction away from the stigma. The left side of the standard and the left wing were pushed outward, away from the bud, and held down with the thumb. A section of the keel around the stigma was cut off and the 10 anthers removed through this opening. The stigma was pollinated immediately with the desired pollen by brushing the excised stigma and anthers of the pollen parent against the stigma of the emasculated flower. The left wing and the standard were then closed to their original position on the bud. This served to maintain high humidity around the stigma and to prevent damage by insects. In the humid environment of South Johnstone (mean relative humidity 85% at 9 a.m.) no special precautions were necessary to obtain satisfactory crossing in the field.

Diallel crosses were made between *C. brasilianum, C. virginianum* and two accessions of *C. pubescens.*

Experimental.—Viable and fully fertile hybrids were obtained easily in crosses between *C. brasilianum* as the female parent and *C. virginianum. C. virginianum* appears unsuitable as a female parent, as all crosses in this direction aborted. When this species was used as the pollen parent it crossed with *C. pubescens* but the F_1's were often malformed and in general they were lacking vigour. The diallel crosses between *C. brasilianum* and *C. pubescens* accessions were also morphologically normal, with good seed yield and fertility. From the point of view of vegetative vigour, stolon development, disease resistance and cool-season productivity, the F_1's of *C. brasilianum* x *C. virginianum* appear to be the most successful.

Five F_1 hybrids of the cross *C. brasilianum* x *C. virginianum* and one of *C. brasilianum* x *C. pubescens* were compared with the parent species and standard centro in pot culture. All lines were grown from cuttings; three plants of each were established in 9 in. plastic pots containing 10 lb of soil, and were irrigated with a complete nutrient solution. The 10 treatments were arranged in five randomized blocks.

Wutoh, Hutton and Pritchard (1968) found that plant area and bulk rating were highly correlated with yield in F_1's of *Glycine wightii* and suggested that these parameters could provide an adequate estimate of yield in a plant-breeding programme. They also recorded positive correlations of a lower order between yield, stolon length and stolon number.

In the present study the following parameters were recorded.

Yield.—Plant tops were harvested after two successive growth periods of 4 weeks each by cutting the plants at 6 cm above soil level. Yields were expressed as dry matter in grams per pot. The experiment was carried out in July-August. This period coincides with the slow growth of standard centro.

Maximum stolon length.—The length of the longest stolon was measured in each pot prior to the first harvest.

Number of stolons.—The number of stolons exceeding 20 cm in length was counted.

Leaf damage score.—Leaf damage caused by pathogens or insects was estimated on an 0–5 scale. A score of 5 meant that 75% or more of the foliage was affected by Cercospora leaf spot or red spider (*Tetranychus* sp.)

A square root transformation was carried out on the data for stolon number and leaf damage score.

III. RESULTS

The results of the experiment are summarized in Table 1.

TABLE 1

MEAN YIELD OF DRY MATTER, NUMBER AND LENGTH OF STOLONS, AND LEAF DAMAGE SCORE FOR THREE PARENT SPECIES OF *Centrosema*, THEIR F_1 HYBRIDS AND STANDARD CENTRO

Parent Species and Control	Yield, 4 weeks' Growth (g/pot)		No. of Stolons*	Maximum Length of Stolon (cm)	Leaf Damage Score*
	1st Cut	2nd Cut			
C. pubescens Q8397–P_1 ..	2·50	1·84	2·03	53·8	1·96
C. virginianum Q8998–P_2	1·86	1·14	2·12	38·9	1·81
C. brasilianum Q8216–P_3	1·50	0·66	1·50	35·2	2·16
Standard centro	0·48	0·40	1·02	24·0	2·30
F_1's					
P_3 x P_2	5·04	3·78	3·07	88·2	1·02
	4·84	4·08	2·81	91·1	0·91
	4·74	2·82	3·38	85·5	1·27
	3·82	2·94	2·84	68·6	1·02
	3·60	2·90	2·60	87·2	1·50
P_3 x P_1	3·28	2·54	2·56	85·7	1·23
L.S.D.					
$P = 0·05$	1·31	1·16	0·55	27·57	0·31
$P = 0·01$	1·75	1·56	0·74	36·97	0·42

* Square root tranformations x + 0·5.

F_1's of the *C. brasilianum* x *C. virginianum* cross yielded significantly ($P \geqslant 0·01$) more than the parent species at both harvesting dates. The *C. brasilianum* x *C. pubescens* hybrid was better ($P \geqslant 0·01$) than the *C. brasilianum* parent at both the first and second harvests, but there was no significant yield difference between the hybrid mean and the parent species means at the second harvest.

In each case hybrid means for stolon length and number significantly ($P \geqslant 0·05$) exceeded the parent species means. The interspecific hybrids showed greater ($P \geqslant 0·05$) resistance to leaf-damaging pathogens and insects than the parents.

The hybrid means of all parameters recorded significantly exceeded those of the standard centro.

Heterosis was estimated as the percentage difference between the mean of the F_1 hybrid plants and the mean of the higher parents. Both interspecific hybrids showed positive heterotic effect for yield, stolon length and number, and resistance to disease and insects. These data are summarized in Table 2.

TABLE 2

PERCENTAGE INCREASE OF F_1 GENERATION HYBRIDS OF *C. brasilianum* x *C. virginianum* AND *C. brasilianum* x *C. pubescens* OVER THE HIGHER PARENT

F_1	Heterosis Effect: Percentage Increase over Higher Parent			
	Yield	Stolon Length	No. of Stolons > 20 cm	Insect/Disease Resistance
C. brasilianum x *C. virginianum*	51	54	28	36
C. brasilianum x *C. pubescens*	25	37	21	27

IV. DISCUSSION

The programme of interspecies hybridization, involving *C. brasilianum, C. virginianum* and *C. pubescens,* resulted in successful combinations of these species. The close relationship between *C. brasilianum* and *C. virginianum,* and *C. pubescens* and *C. brasilianum,* is indicated by the ease of hybridization and the resulting fertile progenies. Because of the potential value of the interspecific hybrid, efforts to develop it as a commercial variety seem justified. The various plant-breeding approaches at present under investigation are as follows:—

1. Selection of desirable characters from F_2 and F_3 material.
2. Back-crossing of *C. brasilianum* x *C. virginianum* F_1's onto *C. brasilianum.*
3. Double-crossing the F_1's of *C. brasilianum* x *C. virginianum* and *C. brasilianum* x *C. pubescens.*

The following stages have been reached in this programme.

Transgressive segregates with strongly stoloniferous habit and high dry-matter yield have been isolated from F_2 populations of *C. brasilianum* x *C. virginianum.*

The F_1 hybrids of *C. brasilianum* x *C. virginianum* have been successfully back-crossed on to *C. brasilianum.* These crosses provided new variations and recombination of several good forage characters.

Double-crosses have been made between *C. brasilianum* x *C. virginianum* and *C. brasilianum* x *C. pubescens.* The resulting hybrids exhibited a wide array of variation.

In this approach it will be necessary to select in each subsequent generation for types with desirable agronomic features such as cool-season growth, tolerance to grazing, resistance to insect and pathogen attack, and nitrogen contribution to the associated grass.

V. ACKNOWLEDGEMENTS

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CORRECTION—**CHARACTERIZATION OF INTRODUCTIONS OF** GLYCINE JAVANICA L. By J. E. FERGUSON

The author of this paper, which appeared in this journal Vol. 26 (pp. 517–28), did not have the opportunity to examine the final typescript or the proof. The following necessary amendments are pointed out:

page 518, para. 2, line 5. "grazed" = "defoliated"

page 519, para. 3, line 5. delete "then"

page 520. In cell 11 1966-67, arrowed vertical broken lines should descend from the right hand limits of the early and mid categories in the cell above.

page 524, col. 1. "F–8" = "F–3".

page 524, footnote to table. delete "as" after "number".

page 525, last line. "seed set"

page 526, Discussion, line 2. delete "7".

page 526, Discussion, line 6. delete "4".