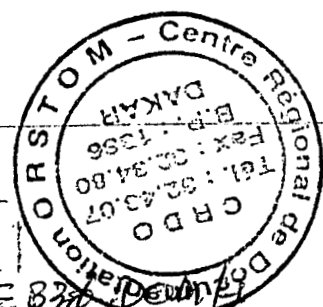


Vegetal
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Nodulation and N₂ Fixation in Nine *Casuarina* Clone-Frankia Strain Combinations

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Abstract

To answer the question whether the ranking of *Casuarina equisetifolia* clones according to their N₂-fixing potential is affected by the *Frankia* strains used, a variance analysis of four nitrogen fixation parameters was carried out using various combinations of *Casuarina equisetifolia* clones and *Frankia* strains.

Three *Casuarina equisetifolia* clones (α , β , and L) inoculated with three strains of *Frankia* (Cj, Br and CeF), were cultivated for 10 months on sand previously washed in hydrochloric acid and regularly irrigated with a N-free Hewitt nutrient solution. The four parameters analyzed were: the amount of N₂ fixed (=plant total N), total N of nodules, nodule dry weight and plant dry weight. The variance analysis showed: a highly significant (P<0.01) effect of clones (all strains combined, the ranking of the clone effect was: $\beta > \alpha > L$); and a highly significant effect (P<0.01) of strains (all clones combined, Cj strain was far more effective than CeF and Br strains).

The relative ranking of clones was not affected by the *Frankia* strain associated with the host plant.

Consequently it is suggested that a two-step selection procedure be adopted, the first step regarding the host-plant and the second one the *Frankia* strains.

Keywords: *Casuarina equisetifolia*, nitrogen fixation, *Frankia*, nodulation.

Résumé

Afin de déterminer si le classement de clones de *Casuarina equisetifolia* en fonction de l'aptitude à fixer l'azote est affecté par la souche de *Frankia* utilisée, une analyse de variance sur 4 paramètres de la fixation d'azote a été effectuée sur toutes les combinaisons possibles de clones et de souches.

Trois clones de *Casuarina equisetifolia* (α , β et L) inoculés avec 3 souches de *Frankia* (Cj, Br et CeF) sont cultivés pendant 10 mois sur sable lavé à l'acide chlorhydrique et régulièrement arrosés avec une solution nutritive de Hewitt sans azote. Les 4 paramètres mesurés sont : la quantité d'azote fixé (=azote total de la plante), azote total des nodules, poids sec des nodules et poids sec des plantes. L'analyse de variance montre un effet clone très significatif (P<0,01) dans l'ordre $\beta > \alpha > L$, et un effet souche très significatif (P<0,01), la souche Cj étant plus efficace que CeF et Br. L'ordre des clones n'est pas affecté par la souche de *Frankia* utilisée.

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En conséquence, il est suggéré d'adopter une procédure de sélection en deux étapes : d'abord sélectionner la plante-hôte puis la souche de *Frankia*.

INTRODUCTION

Earlier investigations have clearly shown that the N₂-fixing potential of *Casuarina equisetifolia* can be significantly improved through clonal selection (SOUGOUFARA *et al.*, 1987). However, it is not yet known whether the ranking of the clones with regard to their N₂ fixing potential is affected by the *Frankia* strain associated with the host plant. To elucidate this problem we designed a factorial experiment involving three clones of *Casuarina equisetifolia* associated with three *Frankia* strains, analyzing the following parameters: the amount of N₂ fixed (=plant total N), total N of nodules, nodule dry weight and plant dry weight.

MATERIAL AND METHODS

Dune soil (Cambérène soil) was thoroughly washed with hydrochloric acid (25% HCl, 75% soil, v/v), autoclaved for 1 hour at 120°C. This soil was mixed with 2 mm polystyrene beads (1/4 beads, 3/4 soil v/v) and used to fill up 81 10-liter plastic pots. When the cuttings of each of three clones of *Casuarina equisetifolia* (α , β and L) (SOUGOUFARA, 1990) were 3 months old, they were transplanted into the pots. Every 2 week, each plant received 50 ml of a N-free HEWITT (1966) nutrient solution.

The experiment involved nine treatments with nine replications:

- Treatment 1: clones α inoculated with Cj (syn., ORS 021001).
- Treatment 2: clones α inoculated with CeF (syn., ORS 020607).
- Treatment 3: clones α inoculated with Br (syn., ORS 020608).
- Treatment 4: clones β inoculated with Cj (syn., ORS 021001).
- Treatment 5: clones β inoculated with CeF (syn., ORS 020607).
- Treatment 6: clones β inoculated with Br (syn., ORS 020608).
- Treatment 7: clones L inoculated with Cj (syn., ORS 021001).
- Treatment 8: clones L inoculated with CeF (syn., ORS 020607).
- Treatment 9: clones L inoculated with Br (syn., ORS 020608).

Frankia strains ORS 021001, ORS 020607, ORS 020608 (DIEM *et al.*, 1982) had been cultivated on Qmod medium (LALONDE & CALVERT, 1979). Since uninoculated plants grown in the absence of combined N quickly died out, we did not include any uninoculated plant in the experimental design. At transplantation time each cutting was inoculated with an amount of 3-week-old *Frankia* culture equivalent to 20 μ g of protein (LOWRY *et al.*, 1951). All pots were placed on a table outside (height=1 m). Tap water was used to irrigate the plants regularly throughout the experiment. Seven months after being transplanted into the pots the plants were harvested.

Dry weight and total N content of shoots and roots (without nodules) and nodules were determined and expressed in terms of g dry weight of N plant⁻¹. Since soil or nutrient solution were devoid of N, the sole source of N for the plants was N₂ from the air. Thus all the plant N at the end of the experiment was derived from air (disregarding the minuscule amount of N contained in the seeds). In other words, the total N content of the shoots and roots (without nodules) at the end of the experiment was considered to represent the amount of N₂ fixed.

Investigations not reported here have indicated that the characteristics of clone α , especially N₂ fixation and nodule dry weight, are similar to the average characteristics of the non selected seedlings

usually used in Senegalese plantations. Therefore clone α could be held as a representative of the non selected *C. equisetifolia* (standard clone).

RESULTS

The amount of N_2 fixed (=plant total N), total N of nodules, nodule dry weight and plant dry weight were recorded for the nine *Casuarina* clone-Frankia strain combinations 7 months after inoculation. Data were log-transformed to homogenize the variances. Data were subjected to variance analysis to test the strain effect, the clone effect, and the strain clone interaction.

Strain effect

Whatever parameter was taken into account (amount of N_2 fixed (=plant total N), total N of nodules, nodul dry weight and plant dry weight), the strain effect averaged for the three clones was highly significant ($P < 0.01$) (table I). Thus N_2 fixed with Cj was 18% greater than with CeF and Br. Other parameters followed the same trend; compared with Br, Cj increased, total N of nodules by 40%, nodule dry weight by 17% and plant dry weight by 9%.

TABLE I. - Dry weight of whole plants, dry weight and total N of nodules, N_2 fixed per plant, in a factorial experiment comprising nine combinations of *Casuarina equisetifolia* clones-Frankia strains.

	Plant Dry weight (g plant ⁻¹)	Dry weight of nodule (g plant ⁻¹)	Total N of nodules (g plant ⁻¹)	N_2 fixed (g plant ⁻¹)
Strain effect (A)				
Cj	195.1a	6.62a	0.10a	1.70a
CeF	182.5b	5.98b	0.07b	1.45b
Br	178.2c	5.63b	0.07b	1.44b
Clone effect (B)				
β	239.6a	8.84a	0.12a	2.23a
α	200.0b	6.17b	0.08b	1.49b
L	116.2c	3.21c	0.03c	0.87c
Level of significance: *** $P < 0.01$, * $P < 0.05$, NS=not significant.				
— Strain effect	***	***	***	***
— Clone effect	***	***	***	***
— Interaction				
Clone-Strain	*	*	NS	*

The variance analysis was carried out on log transformed data.

Numbers followed by the same letter in a given column do not differ significantly at $P < 0.01$.

(A) Averaged for the three clones.

(B) Averaged for the three strains.

Clone effect

The clone effect averaged for the three strains was highly significant ($P < 0.01$), whatever parameter was taken into account (table I). Thus, N_2 fixed was 50% greater with clone β than with clone α and 156% greater than with clone L, indicating that the clone effect was stronger than the strain effect.

Clone-strain interaction

In the case of clone β \times strain Cj combination, the amount of N₂-fixed, the total N of nodules, the nodule dry weight and plant dry weight were 83%, 112%, 70%, 30% (fig. 1, 2, 3 et 4) higher than in the case of the standard clone α associated with strain Br.

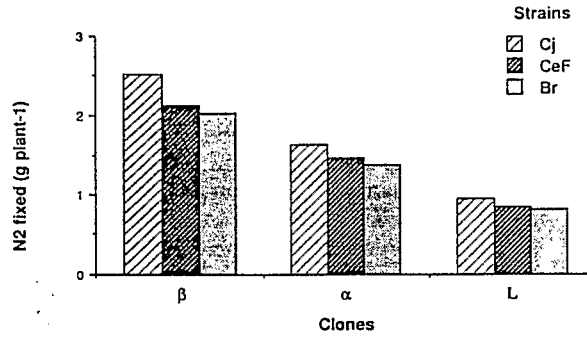


FIG. 1. - N₂ fixed by three 10 month-old clones of *Casuarina equisetifolia* (α , β and L) inoculated with three *Frankia* strains (Cj, CeF, Br).

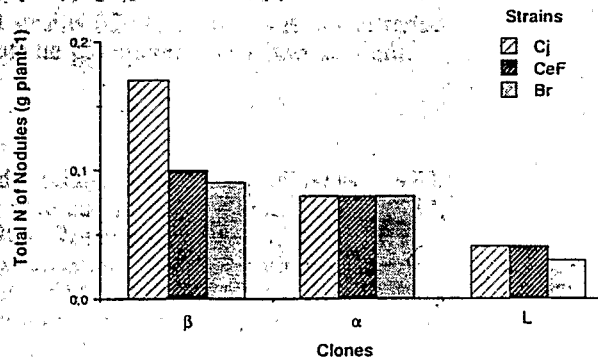


FIG. 2. - Total nitrogen of nodules of three 10 month-old clones of *Casuarina equisetifolia* (α , β and L) inoculated with three *Frankia* strains (Cj, CeF, Br).

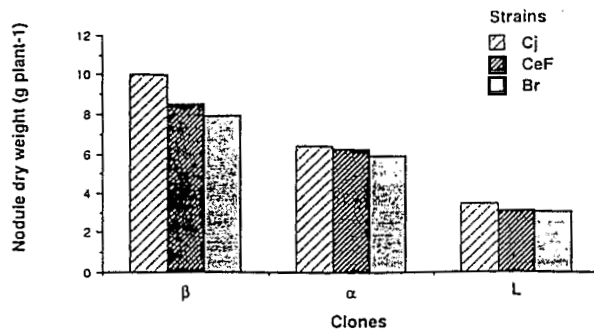


FIG. 3. - Nodules dry weight of three 10 month-old clones of *Casuarina equisetifolia* (α , β and L) inoculated with three *Frankia* strains (Cj, CeF, Br).

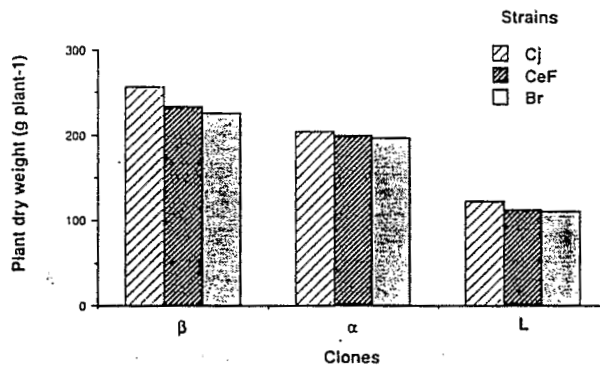


FIG. 4. - Plant dry weight of three 10 month-old clones of *Casuarina equisetifolia* (α , β and L) inoculated with three *Frankia* strains (Cj, CeF, Br).

The level of significance of the clone \times strain interaction ($P < 0.05$) was lower than that of strain or clone effects ($P < 0.01$) (table I).

The ranking of the clones ($\beta < \alpha < L$) and strains (Cj > CeF > Br) was not affected by the clone-strain interaction, irrespective of the parameter taken into account.

DISCUSSION AND CONCLUSION

Experiments carried out using crushed nodule suspensions (COYNES, 1973; GAUTHIER, 1984; REDDELL & BOWEN, 1986) or pure cultures (DIEM *et al.*, 1982, 1983; DILLON & BAKER, 1982; ZHANG & TORREY, 1985; ROSBROOK & BOWEN, 1987; TORREY & RACETTE, 1989; STEELE *et al.*, 1989; SOUGOUFARA *et al.*, 1989) or both pure cultures and crushed nodule suspensions (REDDELL *et al.*, 1988; SELSTEDT, 1988) have already shown that, to a certain extent, the *Frankia* strain governs the capacity of the system to nodulate and to fix N_2 . In all experiments the authors used seedlings (not clones) of casuarinas, which increased the experimental error.

Our experimental design clearly showed that the clone effect appeared to be more important than that of the strain effect. This can probably be explained by the large intraspecific variability of *C. equisetifolia*, which results from the allogamous,

monoecious (though sometimes dioecious) and anemophilous character of this tree which is shared with other casuarinas. In spite of the fact that there was a significant clone \times *Frankia* strain interaction for amount of N₂ fixed, total N of nodules, nodule and plant dry weight, the relative ranking of the clones was not affected by *Frankia* strains. Therefore it appears that the improvement of N₂-fixing ability in *Casuarina equisetifolia* can be achieved through a two-step procedure, starting by screening the best host plant (clone) and subsequently the *Frankia* strain associated with the best clone. Such an experimental approach should significantly facilitate the identification of the best clone \times *Frankia* strain combination.

This concept can be applied to other actinorhizal host plants exhibiting a large intraspecific variation, which is a potential source of genetic gain. Two promising candidates are *Alnus* (SIMON *et al.*, 1985; PRAT, 1989) and *Acacia mangium* (GALIANA *et al.*, 1991), in which the clone effect was found to be predominant. However, all actinorhizal plants do not exhibit any significant variation in their N₂-fixing ability; this is the case of *Ceanothus*, whose different accessions do not differ significantly in this respect (NELSON & LOPEZ, 1989).

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